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Using liquid chromatography–ion trap mass spectrometry to determine pharmaceutical residues in Taiwanese rivers and wastewaters

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ABSTRACT

To establish their environmental concentrations and to support surface water protection programs, we have undertaken a preliminary study of the concentrations of selected acidic and neutral pharmaceutical residues (clofibric acid, ketoprofen, ibuprofen, diclofenac and carbamazepine) in Taiwanese river and wastewater samples. These pharmaceutical residues were extracted from the water samples through the Oasis HLB solid-phase extraction (SPE). The analytes were then identified and quantified using liquid chromatography–ion trap mass spectrometry with dual-polarity electrospray ionization in the product ion scan mode. The limits of quantification (LOQs) ranged between 0.5 and 20 ng l^{-1} for 250 ml samples of water. We investigated the intra- and interbatch precision and accuracy at two levels of concentration. The selected analytes were detected at concentrations ranging from <0.5 to 960 ng l^{-1} in wastewater treatment plant effluents and river water samples.

1. Introduction

Recently, the presence of pharmaceutical residues in aquatic environment has become an emerging environmental issue because of the potential risk to human life and wildlife (Daughton and Ternes, 1999; Kolpin et al., 2002; Kümmerer, 2004; Sarmah et al., 2006; Williams et al., 2006; Enick and Moore, 2007; Quinn et al., 2008). Unlike many industrial chemicals and pesticides, most drug residues are discharged into the environment continuously through domestic sewage systems. Pharmaceutical residues may also enter into surface runoff through the application of WWTP effluents and sewage sludge in agriculture (Pedersen et al., 2003, 2005). Many of these residues have been detected in samples of wastewater, surface water, groundwater and even in drinking water (Öllers et al., 2001; Heberer, 2002; Hilton and Thomas, 2003; Tixier et al., 2003; Zuehlke et al., 2004; Castiglioni et al., 2005; Gómez et al., 2006; Al-Rifai et al., 2007; Fatta et al., 2007; Kim et al., 2007; Kimura et al., 2007; Vieno et al., 2007). In Taiwan, the amount of medical and healthcare waste has increased since the national health insurance program began in 1995. This program increased the number and size of healthcare facilities and medical services and use of medical disposable products. Large quantities of the pharmaceutical residues are discharged directly into the rivers through the untreated wastewaters. The impact of pharmaceutical pollution on the aquatic environment is very significant because many pharmaceuticals are persistent and mobile in surface water, moreover, even surviving in drinking water treatment process (Togola and Budzinski, 2008). The large-scale usage of pharmaceutical and the increasing public concern over environmental issues have stimulated our interest in investigating their occurrence and behavior in the environment.

Extensive research in the USA and Europe regarding the concentrations of pharmaceutical residues in the environment has focused on their occurrence in surface water samples affected by WWTP effluents (Ternes, 1998; Westerhoff et al., 2005; Vieno et al., 2005, 2006, 2007; Haiß and Kümmerer, 2006; Gómez et al., 2007). There is few information available on the occurrence of pharmaceutical residues in untreated wastewaters directly discharged into the aquatic environment. This study would be significant because untreated wastewater discharged directly into the aquatic environment––as a result of inefficient wastewater treatment––is a significant source of surface water contamination throughout the emergent countries in the Asia–Pacific region due to the rapidly increasing in their population and large-scale usage of drugs.

High-performance liquid chromatography (HPLC) coupled with either triple-quadrupole mass spectrometry (QqQ-MS) or quadrupole time-of-flight mass spectrometry (Q-TOF-MS) is the most extensively applied method for analyzing pharmaceutical residues in various environmental samples (de Alda et al., 2003; Vanderford et al., 2003; Zuehlke et al., 2004; Castiglioni et al., 2005; Lin and Reinhard, 2005; Petrović et al., 2005, 2006; Gómez et al., 2006; Trenholm et al., 2006; Kim et al., 2007; Vieno et al., 2007). Tandem MS is generally more sensitive than single MS in measuring the

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contents of analytes in complicated matrices because it is associated with higher and more stable signal-to-noise (S/N) ratios as a result of the specific fragmentations of isolated precursor ions and the elimination of background noise. One of our goals for this study was to apply bench-top liquid chromatography–ion trap mass spectrometry utilizing dual-polarity electrospray ionization (ESI) and the product ion scan mode to determine pharmaceutical residues present in environmental samples at trace-levels. Therefore, it will be demonstrated that the high sensitivity and specificity could be achieved in quantifying pharmaceutical residues with the ion trap mass spectrometer coupled with a selective solid-phase extraction.

As part of a wider effort aimed at elucidating the impact of pharmaceutical residues in the environment in Taiwan, in this study we used the method presented to analyze the presence of three frequently used acidic analgesic/anti-inflammatory drugs (ibuprofen, ketoprofen and diclofenac), one acidic lipid regulator (clofibric acid), and one neutral antiepileptic (carbamazepine) in surface water and WWTP effluent samples. The accuracy and precision of our method were validated and calibrated using 2,4-dichlorobenzoic acid as an internal standard to compensate for the effect of the matrix, and the effectiveness of our method in determining of the contents of these pharmaceutical residues in river water and WWTP effluent samples was demonstrated and compared.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). 33% aqueous ammonium hydroxide solution was purchased from Riedel-de Haën (Seelze, Germany). Clofibric acid, ibuprofen, carbamazepine, ketoprofen, diclofenac and 2,4-dichlorobenzoic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and were used as received. Deionized water was further purified using a Millipore water purification device (Millipore, Bedford, MA, USA). Stock solutions of each drug (1000 $\mu g\ ml^{-1}$) were prepared in methanol. Mixtures of the drugs for working standard preparation and sample fortification were diluted with an appropriate volume of deionized water. All stock solutions and mixtures were stored at −10 °C in the dark.

2.2. Sample collection

River water samples were collected from Lao-Jie River (specific conductance 620 $\mu S\ cm^{-1}$) in the city of Chung-Li, which receives untreated municipal wastewater directly from the city; and Tour-Chyan River (specific conductance 470 $\mu S\ cm^{-1}$) in the city of Hsin-Chu, and Zen-Wen River (specific conductance 390 $\mu S\ cm^{-1}$) in Tainan county, which receive untreated municipal wastewater directly from their city and suburban areas. The river samples were collected at a 0.5-m depth from midstream using pre-rinsed glass bottles. Three WWTP effluents (average specific conductance 5,800 $\mu S\ cm^{-1}$) were collected at different months from the An-Ping community in the city of Tainan. This WWTP performs mechanical clarification, biological treatment, and flocculation filtration; population equivalent 380000. All samples were collected in duplicate (1000-ml for each) and shipped to the laboratory in ice-packed containers. Upon arrival, the samples were immediately passed through a 0.45-μm membrane filter (Advantec MFS, CA, USA), adjusted to pH 3.0 by adding concentrated HCl to depress microbial degradation, and then stored at 4 °C until analysis.

2.3. Sample extraction

Procedures for sample extraction of pharmaceutical residues using Oasis HLB-SPE cartridges (3-ml; 60 mg of resin weight; surface area 810 $m^2\ g^{-1}$; Waters, Milford, MA, USA) have been reported elsewhere (Öllers et al., 2001; Lin et al., 2005; Fatta et al., 2007). Briefly, each HLB-cartridge was pre-conditioned with 3 ml of methanol, and then rinsed by 3 ml of deionized water on an SPE manifold. The water sample 250-ml (pH 3.0) was passed through the HLB-cartridge at a flow-rate of ca. 4–6 $ml\ min^{-1}$. When the extraction was completed, the cartridge was washed with 1 ml of 5% aqueous methanol solution, and then air-dried under vacuum for at least 15 min. The analyte residues were then eluted from the cartridge using 2 ml of methanol as the eluent. The extract was evaporated to dryness under a gentle stream of nitrogen gas. The residues were redissolved in 100 μl of deionized water containing the internal standard (1 $\mu g\ ml^{-1}$), and made ready for LC–MS–MS analysis.

2.4. LC–MS–MS analysis

The analytes were separated using an Agilent 1100 series HPLC consisting of a G1311A quaternary pump and a G1313A autosampler, with a 15-cm narrow-bore (2.1-mm I.D.) Zorbax Extend-C_{18} column (5 μm particle size, Agilent, Palo Alto, CA, USA) fitted with a guard column containing the same stationary phase. The injection volume was 20 μl, and gradient elution was programmed at flow-rates from 0.1 to 0.4 $ml\ min^{-1}$ using solution of (A) 0.05% ammonium hydroxide in deionized water (pH 10.5) and (B) acetonitrile (ACN) as mobile phases. The linear gradient started at 1%, 5% or 10% ACN and followed the various linear gradient programs listed in Table 1. The initial gradient conditions were reestablished after 5 min, and then the column was equilibrated for another 3 min.

The analytes were detected using an Agilent LC-MSD Trap SL mass spectrometer (Palo Alto, CA, USA) equipped with an ESI interface aligned in an orthogonal geometry with a negative or positive ionization mode. The detection parameters for each analyte were optimized by estimating the signal intensity and fragmentation through a series of continuous-infusion experiments. Mass spectra were collected in the scan range m/z 100–350. The following optimized ESI parameters (see Section 3) were applied: capillary voltage 1000 (or −1000 V for carbamazepine) or 1500 V; nebulizing gas pressure 40 psi (1 psi = 6.895 kPa); drying gas flow-rate 10 $l\ min^{-1}$; drying gas temperature 350 °C. The helium background gas was maintained at a pressure of 6×10^{-6} torr for MS–MS measurement. Quantification of all target compounds was performed through product ion scan recording two transitions for clofibric acid and one transition for the others simultaneously. Table 2 provides an overview of the MS–MS transitions, fragmentation

Table 1
Gradient elution programs at various LC flow-rates

0.1 ml min^{-1}			0.2 ml min^{-1}			0.3 ml min^{-1}			0.4 ml min^{-1}		
Time (min)	A%	B%	Time (min)	A%	B%	Time (min)	A%	B%	Time (min)	A%	B%
0	90	10	0	95	5	0	99	1	0	99	1
5	65	35	5	80	20	5	75	25	4	88	12
8	40	60	10	75	25	16	70	30	8	88	12
13	55	45	15	65	35	20	60	40	10	74	26
18	55	45	20	40	60	25	45	55	12	30	70
20	40	60	25	30	70				13	15	85
25	15	85							16	15	85

A: 0.05% Ammonium hydroxide in deionized water (pH 10.5); and B: acetonitrile (ACN).

Table 2
ESI–MS–MS operating parameters with a LC flow-rate of 0.2 ml min^{-1}

Compound	t_R (min)	MRM transition m/z (%)[a]	Capillary voltage (V)	Fragmentation amplitude (V)
Internal standard	3.5	189 (7) $[M-H]^-$ > 145 (100)	1500	0.60
Clofibric acid	9.4	213 (7) $[M-H]^-$ > 127 (100), 85 (31)	1500	0.65
Ketoprofen	11.3	253 (9) $[M-H]^-$ > 209 (100)	1000	0.55
Ibuprofen-Na salt	12.6	205 (7) $[M-Na]^-$ > 161 (100)	1500	0.60
Diclofenac-Na salt	14.3	294 (13) $[M-Na]^-$ > 250 (100)	1500	0.50
Carbamazepine	23.7	237 (7) $[M+H]^+$ > 194 (100)	−1000	0.60

[a] Relative abundance is given in parentheses.

amplitude, capillary voltage and retention time for each target compound with a LC flow-rate of 0.2 ml min^{-1}.

3. Results and discussion

3.1. Optimization of LC–MS–MS analysis

In this study we used tandem-in-time mass spectrometric technique with ion trap mass analyzer to identify and quantify analytes due to its high selectivity and sensitivity. The selection of the ionization polarity is an important factor affecting the ionization efficiency when LC–MS is applied; it is based on the properties of the analytes and their functional groups. During the method development phase, we evaluated the behavior of five selected pharmaceuticals in both positive and negative ionization modes. The ionization efficiencies for the four acidic analytes (containing carboxy group) were optimized in the negative ESI mode forming the deprotonated ions $[M-H]^-$ or the ions $[M-Na]^-$, as described elsewhere (Farré et al., 2001; Hilton and Thomas, 2003; Vanderford et al., 2003; Lin and Reinhard, 2005). For carbamazepine, which contains an amino group, the intensity was optimized in the positive ESI mode forming the protonated ion $[M+H]^+$ (Vanderford et al., 2003). Although the ionization behavior of each of selected drugs was different, the combination of MS–MS and dual-polarity ionization techniques allowed us to detect all of the target analytes in a single LC–ESI–MS–MS run. We evaluated the effects of various nebulizer pressures, drying gas flow-rates, drying gas temperatures, and capillary voltages on the intensity of the LC peaks for each analyte under MS–MS conditions to obtain the highest and most stable S/N ratio from ESI–MS–MS signals. Under the optimized MS–MS conditions, the precursor ions $[M-H]^-$, $[M-Na]^-$ or $[M+H]^+$ were stored in the ion trap by adjusting the isolation segment window, and fragmented with an appropriate fragmentation amplitude to maximize the sensitivity of the characteristic MS and MS^2 signals of these pharmaceuticals. The nebulizer pressure was optimized at 40 psi (tested between 30 and 60 psi); the drying gas flow-rates and drying gas temperatures were optimized at 10 l min^{-1} (tested between 5 and 15 l min^{-1}) and 350 °C (tested between 300 and 365 °C), respectively; and the capillary voltage was optimized as listed in Table 2. Under these conditions, each of the acidic analytes underwent an $[M-H-COO]^-$ or $[M-Na-COO]^-$ fragmentation, except clofibric acid (Lin and Reinhard, 2005). For clofibric acid, the main product ion was observed at m/z 127 from the cleavage of the ether bond of the $[M-H]^-$ ion at m/z 213, with a minor fragment $[C_4H_5O_2]^-$ at m/z 85. The predominant fragmentation ion of carbamazepine $[M+H]^+$ at m/z 237 corresponded to the loss of an amide group $[M-CONH_2+H_2]^+$, as has been described elsewhere (Stolker et al., 2004). We used these predominant ions as quantifying ions to maximize the detection sensitivity of these analytes when present at trace-levels in complex environmental samples.

Because the properties of the mobile phase primarily govern the separation of the analytes and the degree of analyte ionization, optimizing the mobile phase composition is an important aspect of LC–ESI–MS–MS analysis. According to a previous report (Reza Anari et al., 2002), the ionization efficiency depends on both the apparent dissociation constant (pK_a) of the analyte and the pH value of the mobile phase. The pK_a values of the four acidic pharmaceuticals range from 3.18 to 4.41, and that of carbamazepine is 13.94 (SciFinder Scholar, 2006). Moreover, the ionization efficiency is inversely proportional to $[1+10^{(pKa-pH)}]$ and $[1+10^{(pH-pKa)}]$ for the acidic and basic functional groups, respectively (Reza Anari et al., 2002). Therefore, the presence of ammonium hydroxide would favor the deprotonation of analytes containing carboxy group; thus, we used 0.05% ammonium hydroxide in deionized water (pH 10.5) mixed with acetonitrile as the mobile phases to achieve the optimal separation and to enhance the ionization efficiency. However, the ionization efficiency of carbamazepine was about 99.96% with a basic eluent, according to the calculation with equation $[1+10^{(pH-pKa)}]$ (Reza Anari et al., 2002). Furthermore, we also considered the effect of the LC flow-rate on the degree of ionization in the ESI interface, evaluated after injection of 500 ng ml^{-1} of the five pharmaceuticals into the LC–ESI–MS– MS system. We studied LC flow-rate ranging from 0.1 to 0.4 ml min^{-1}. Fig. 1 shows that the highest degree of ionization of each acidic analyte was obtained at a low flow-rate of 0.2 ml min^{-1}, while the optimal ionization of basic compound carbamazepine was achieved at a flow-rate of 0.1 ml min^{-1}. For ESI, LC at a low flow-rate provides smaller-sized droplets during the nebulizing process, maximizing the surface area of each droplet, which is favorable for ion desorption into the gas phase and, hence, enhancing the ionization efficiency (Kloepfer et al., 2005). Although we observed the highest abundance of carbamazepine at a flow-rate of 0.1 ml min^{-1}, for subsequent experiments we chose a flow-rate of 0.2 ml min^{-1} (the second-highest sensitivity) to enhance the sensitivity of the acidic drugs.

3.2. Method validation

We investigated the analytical characteristics of developed method, such as its linear response range, reproducibility and limits of quantification (LOQs), to estimate the efficiency of the approach and the feasibility of applying it to the analysis of environmental samples. The range of linearity of the five pharmaceuticals were calculated and calibrated to the internal standard from five-level calibration curves. Table 3 summarizes the calibration results, detection limits, linear ranges, precisions, and the accuracies. The precisions of the curves, as indicated by the relative standard deviation (RSD) of the response factors (RFs), were 4.3–7.1%, with correlation coefficients (r^2) exceeding 0.994. The curves covered a range equivalent to the concentrations of the analytes in 250-ml environmental water samples after the extract has been concentrated to 100 μl. The limits of detection (LODs; $S/N \geqslant 3$) and the limits of quantification (LOQs; $S/N \geqslant 10$) were determined through SPE of spiked deionized water samples; their values ranged from 0.2 to 6 ng l^{-1} and from 0.5 to 20 ng l^{-1}, respectively. The intra- and interbatch precisions, as well as the accuracies, were evaluated at two levels of concentration, distributed equally over the low and high linear range. The intrabatch precision was determined by analyzing five spiked river samples (Tour-Chyan River) on the same day ($n = 5$). The interbatch precision was evaluated by determining five replicates per concentration level, on four consecutive days ($n = 20$). The accuracy was evaluated by comparing the mean recovery from 5 or 20 analyses to the nominal

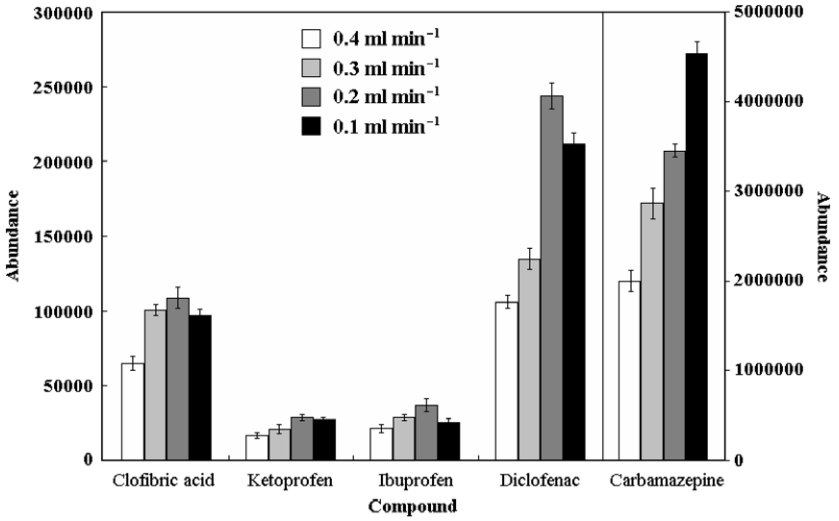

Fig. 1. ESI–MS–MS abundances of the five pharmaceuticals under different LC flow-rates; concentration of each analyte: 500 ng l^{-1}.

Table 3
Method Validation data in terms of calibration results, IDLs, LODs, LOQs, precision, and accuracy

Compound	Range (ng ml^{-1})	r^2	RF (RSD)	LOD (ng l^{-1})	LOQ (ng l^{-1})	Intrabatch (n = 5) recovery (%RSD)		Interbatch (n = 20) recovery (%RSD)	
						100 ng l^{-1}	1000 ng l^{-1}	100 ng l^{-1}	1000 ng l^{-1}
Clofibric acid	5–2000	0.999	6.7	1	3	77[a] (13)[b]	73 (13)	73 (7)	76 (7)
Ketoprofen	10–2000	0.994	5.5	6	20	82 (12)	82 (12)	80 (6)	81 (4)
Ibuprofen	5–2000	0.994	4.3	4	12	87 (8)	79 (8)	80 (5)	75 (5)
Diclofenac	5–2000	0.997	7.1	0.5	2	55 (14)	60 (14)	50 (4)	57 (4)
Carbamazepine	5–2000	0.994	6.3	0.2	0.5	95 (9)	96 (9)	90 (3)	93 (2)

[a] Mean recovery.
[b] Relative standard deviation (%RSD) is given in parentheses.

Table 4
Concentrations (ng l^{-1}) of selected pharmaceutical residues in various water samples and their spiked recoveries

	Compound				
	Clofibric acid	Ketoprofen	Ibuprofen	Diclofenac	Carbamazepine
Deionized water					
Spiked recovery (%)	86[a] (4)[b]	91 (4)	95 (5)	81 (4)	101 (4)
Lao-Jie River					
Background conc. (ng l^{-1})	<3[c]	110	<12[c]	24	120
Spiked recovery (%)	104 (4)	54 (6)	102 (4)	53 (7)	81 (4)
Tour-Chyan River					
Background conc. (ng l^{-1})	<3[c]	400	28	62	<0.5[c]
Spiked recovery (%)	99 (4)	79 (5)	83 (6)	57 (4)	115 (5)
Jen-Wen River					
Background conc. (ng l^{-1})	<3[c]	620	30	50	<0.5[c]
Spiked recovery (%)	117 (5)	56 (4)	98 (6)	54 (6)	113 (4)
WWTP effluent-1					
Background conc. (ng l^{-1})	<3[c]	680	<12[c]	30	400
Spiked recovery (%)	79 (3)	72 (8)	99 (6)	48 (7)	111 (8)
WWTP effluent-2					
Background conc. (ng l^{-1})	<3[c]	330	30	30	290
Spiked recovery (%)	51 (7)	55 (6)	68 (8)	55 (6)	77 (6)
WWTP effluent-3					
Background conc. (ng l^{-1})	90	700	34	<2[c]	960
Spiked recovery (%)	84 (8)	74 (5)	61 (8)	52 (7)	96 (5)

[a] Mean recovery (n = 3) at final concentration of 200 ng l^{-1} for each compound.
[b] Relative standard deviation (%RSD) is given in parentheses (n = 3).
[c] Concentrations were less than the LOQ.

concentration value. Table 3 indicates that the intra- and inter-batch precisions (RSD) for the selected pharmaceuticals were all less than 14% and 7%, respectively. The accuracies, determined as the mean recovery, ranged from 50% to 96%. These results reveal

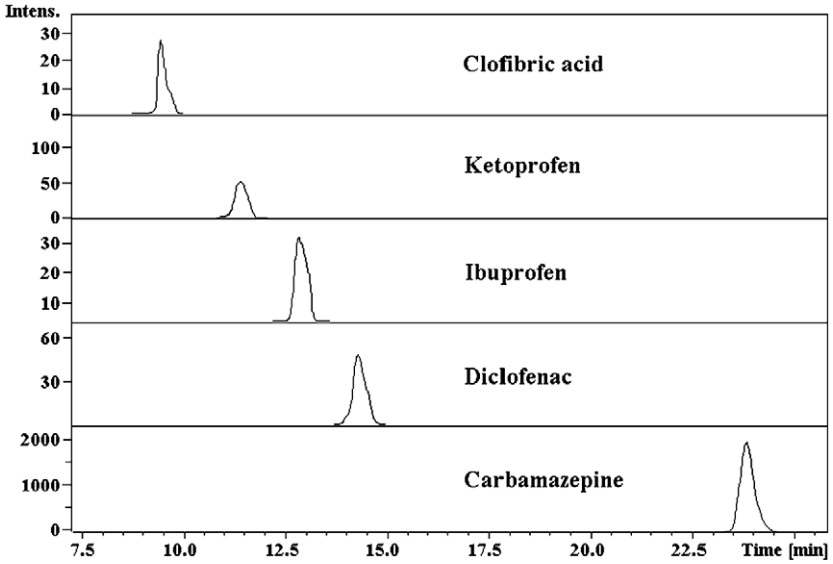

Fig. 2. LC–ESI–MS–MS extracted ion chromatograms of the pharmaceutical residue detected in the WWTP effluent-2 sample.

Table 5
Levels of the five selected pharmaceuticals in aquatic environments in various countries (ng l^{-1})

Location	Clofibric acid	Ketoprofen	Ibuprofen	Diclofenac	Carbamazepine	References
Surface waters						
Canada			142.6 ± 2.3	13.2 ± 6.9	98.9 ± 36.5	Yu et al. (2007)
Canada		<1.0	<0.8–9.5	<1.0		Verenitch et al. (2006)
Finland		8–28	12–69	10–55	21–80	Vieno et al. (2007)
Germany	up to 550	up to 120	up to 530	up to 1200	up to 1100	Ternes (1998)
Korea			11–38	1.1–6.8	4.5–61	Kim et al. (2007)
Romania			115.2 ± 20.7		75.1 ± 6.1	Moldovan (2006)
Spain	10–20	<30	<8–150	<2–60	<2–110	Gros et al. (2006)
Switzerland	0–30	0–10	0–80		10–320	Tixier et al. (2003)
UK	<0.5–36	<2.5–8	0.3–39	<0.5–14	<0.5–794	Kasprzyk-Hordern et al. (2007)
USA			up to 1000			Kolpin et al. (2002)
USA	3.2–26.7		0–34.0		42.9–113.7	Zhang et al. (2007)
Taiwan	<3	110–620	<12–30	24–62	<0.5–120	This study
WWTP effluents						
Austria			<20–2400	780–1680	465–1594	Clara et al. (2005)
Canada		8–268	2235–6718	32–457		Verenitch et al. (2006)
Germany	up to 1600	up to 380	up to 3400	up to 2100	up to 6300	Ternes (1998)
Italy	<0.36–82		<1.38		<1.3–1318	Castiglioni et al. (2005)
Japan	14 ± 4	445 ± 121	40 ± 32	145 ± 32		Kimura et al. (2007)
Korea			10–137	8.8–127	73–729	Kim et al. (2007)
Spain		200–750	<150–1050	<50–500	<100–600	Petrović et al. (2006)
Spain		330 ± 18	78 ± 5	155 ± 13		Quintana et al. (2007)
Spain	20–30	130–620	40–800	<10–390	<10–630	Gros et al. (2006)
Sweden		100–280	4.5–48	280–2320		Brown et al. (2007)
Switzerland	up to 60	up to 180	up to 1300	up to 990	up to 950	Tixier et al. (2003)
Taiwan	<3–90	330–700	<12–34	<2–30	290–960	This study

that the LC–ESI–MS–MS technique with ion trap analyzer provides high reproducibility with excellent linearity and sensitivity for the five selected pharmaceuticals.

3.3. Application

We employed LC–ESI–MS–MS to quantify the five pharmaceutical residues in river and WWTP samples, using internal standard calibration to compensate for the effect of the matrix. Table 4, which lists the recovery rates of the matrix-spiked samples and the concentrations of the selected pharmaceuticals detected in environmental water samples, indicates the versatility of developed method. Qualification of the compound peaks was performed through comparison of the retention times and the MS–MS spectra of the standard solutions; the quantities were calculated using response factor (RF). For deionized water samples, the average recoveries of the pharmaceuticals ranged from 81% to 101%, with the RSD ranging from 4% to 5%; for river water samples, these ranges were 53–117% and 4–7%, respectively; for WWTP effluents, these ranges were 48–111% and 3–8%, respectively. The recoveries of the river samples and WWTP effluents might have been affected by the presence of co-extracted matrix components, which typically suppress or, less frequently, enhance, the analyte signals. Although the average recoveries of the spiked river water samples and WWTP effluents differed (ranging from 2% to 36%) from those observed for the spiked deionized water sample because of matrix effects, this method remains acceptable for application to environmental samples when LC–ESI–MS–MS is performed in the

dual-polarity ionization mode. Fig. 2 displays the LC–ESI–MS–MS extracted ion chromatograms of selected pharmaceutical residues detected in the WWTP effluent-2. The concentrations of pharmaceutical residues in the river samples ranged from <0.5 to 620 ng l^{-1}. The high levels of these selected pharmaceutical residues were expected because of their high usage (up to 90.6 tons annually) in Taiwan (www.nhi.gov.tw). For the WWTP effluents, the concentrations of pharmaceutical residues ranged from <2 to 960 ng l^{-1}. These compounds have been observed at various levels in WWTP effluents and surface water samples in various countries. Table 5 summarizes the concentrations of five selected pharmaceuticals found in this study and those reported in several previous publications. The concentrations of pharmaceutical residues in surface waters have been detected in ranges from 8.0 to 80 ng l^{-1} in Finland (Vieno et al., 2007), from 1.1 to 61 ng l^{-1} in Korea (Kim et al., 2007) and from 75.1 to 115.2 ng l^{-1} in Romania (Moldovan, 2006). The concentrations of pharmaceutical residues in WWTP effluents ranged from n.d. to 2400 ng l^{-1} in Austria (Clara et al., 2005), from 8.8 to 729 ng l^{-1} in Korea (Kim et al., 2007) and from 10 to 566 ng l^{-1} in Japan (Kimura et al., 2007). Several of these drugs have been detected at very high concentrations in various bodies of water. Ibuprofen has been detected at concentrations up to 1000 ng l^{-1} in surface waters (Kolpin et al., 2002) and 6718 ng l^{-1} in Canadian WWTP effluents (Verenitch et al., 2006); and diclofenac at concentration up to 1200 ng l^{-1} in German surface waters (Ternes, 1998) and 2320 ng l^{-1} in a Sweden WWTP effluent (Brown et al., 2007). In this study we found that the concentrations of pharmaceutical residues in Taiwanese WWTP effluents ranged from <2 to 960 ng l^{-1}, similar to the concentrations present in other countries, although the concentrations in the Taiwanese surface waters were higher than those found in Canada, USA and the European countries, possibly due to the direct discharge of wastewater into the rivers without proceeding by a WWTP.

4. Conclusion

We have developed a reliable and sensitive analytical procedure utilizing Oasis HLB-SPE and LC–ESI-ion trap MS–MS system with dual-polarity ionization mode for the unequivocal identification and quantification of five pharmaceutical residues in environmental water samples at trace-levels. Qualification through MS–MS spectral comparison confirmed the presence of these pharmaceutical residues; the selectivity and sensitivity were improved using the product ion scan technique. This method satisfied analytical validation criteria, allowing precise measurement of the levels of pharmaceutical residues in river water samples. Our preliminary results reveal that the five pharmaceutical residues are present in various aquatic environment in Taiwan, and that their concentrations in Taiwanese rivers are higher than those found in other developed countries. This method might be of use for surface water protection programs and further environmental studies of the pharmaceutical residues burden in Taiwan.

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