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Rapid determination of pharmaceuticals from multiple therapeutic classes in wastewater by solid-phase extraction and ultra-performance liquid chromatography tandem mass spectrometry

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A new analytical method utilizing ultra-performance liquid chromatography (UPLC) tandem mass spectrometry (MS/MS) has been developed to determine 16 pharmaceuticals from 8 therapeutic classes in wastewater: bezafibrate, clofibric acid, carbamazepine, caffeine, chloramphenicol, diclofenac, gemfibrozil, indomethacin, ketoprofen, mefenamic acid, metoprolol, nalidixic acid, N,N-diethyl-meta- toluamide, propranolol, sulpiride and trimethoprim. Key parameters of MS/MS, UPLC and solid phase extraction (SPE) were optimized. In general, recovery of target pharmaceuticals was over 70% for the wastewater effluent samples and 50% for the influent samples. The effects of matrix suppression, loss during the pretreatment as well as instrument variability were successfully corrected by two internal standards, and acceptable relative recovery was obtained. Target compounds were quantitatively analyzed using multiple reaction monitoring (MRM) mode, and the detection limits ranged from 0.3 to 20 ng/L. A detailed study, matrix effect in effluent wastewater was also present. The method was applied to detecting pharmaceuticals in the wastewater from three wastewater treatment plants (WWTPs) in Beijing, China and the results demonstrated that most target compounds were detectable in both the influent and effluent, with the mean concentrations ranging from 20.5 to 5775.6 ng/L and 4.6 to 418.6 ng/L, respectively.

pharmaceuticals, solid phase extraction, tandem mass spectrometry, UPLC, wastewater

In recent years, pharmaceuticals have been frequently detected in the aquatic environment, which is thought to be a new environmental problem and received global concern[1–4]. Every year, a large number of pharmaceuticals, unchanged or as active metabolites, are continuously carried to wastewater treatment plants (WWTPs). However, due to ineffective removal, they remained in treated wastewater and are released to the aquatic environment. Results of toxicology studies have revealed that some pharmaceuticals are suspected to have direct toxicity to certain aquatic organisms[5,6]. Besides, their continual but undetectable effects could accumulate slowly, and finally lead to irreversible change on wildlife and human beings[2]. Therefore, more details are required about the occurrence, fate and transformation of various pharmaceuticals in the WWTPs, which have been identified as the main source of these emerging pollutants[1]. So far, concentrations of pharmaceuticals from multiple therapeutic classes in the WWTPs have been well documented in USA[3,4,7], Japan[8], and some European countries[1,9–11]. Reported species and concentrations of pharmaceuticals varied from country to country,

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and plant to plant, owing to the different usage patterns. However, very few studies about the situation in China have been reported. Generally, previous studies only focused on one specific therapeutic class, which was antibiotics[12–14], probably due to the challenges for simultaneous analysis of broad-spectrum pharmaceuticals with different physicochemical characteristics.

To analyze environmental samples, liquid chromatography tandem mass spectrometry (LC/MS/MS) is preferred because of its specificity and selectivity[16]. In recent years, a novel approach for chromatographic separation named ultra-performance liquid chromatography (UPLC) has been developed. UPLC, affording high pressure required to pump the mobile phase through the small-diameter package (< 2 μm), can increase the separation efficiency, and consequently reduce the run time and ion suppression[17,18]. Until now, the UPLC-based methods have been quite commonly used for pharmaceuticals determination in environmental samples. Petrovic et al. developed a multi-residue analytical method for pharmaceuticals from different therapeutic classes in wastewater by UPLC quadrupole-time-of-flight mass spectrometry (Q-TOF), however, this method showed some disadvantages concerning sensitivity compared to the methods for those in wastewater by UPLC-MS/MS[10]. Huerta-Fontela et al. described a sensitive and rapid method for stimulatory drugs of abuse in wastewater and surface waters by UPLC-MS/MS[18]. Recently, the UPLC-MS/MS method for multiple classes of pharmaceuticals and personal care products (PPCPs) has been studied and applied to surface water and wastewater samples[19–22].

In this study, we used UPLC-MS/MS to determine pharmaceuticals from eight therapeutic classes, namely, antilipidemics, anticonvulsant, stimulant, antibiotics, insect repellent, anti-inflammatories, anti-hypertensive, and antipsychotic (Details information are provided in Appendix on electronic edition). Compared with previous studies, the list of target pharmaceuticals did not duplicate the existing ones, and more therapeutic classes were involved. The compounds were selected due to their frequent detection reported in literature[4,7,23,24], as well as the large consumption amount in China[25]. Key analytical parameters of MS/MS, UPLC, and solid phase extraction (SPE) were optimized, and the developed method was applied to determining target pharmaceuticals in the influents and effluents of three WWTPs in Beijing, China.

1 Materials and methods

1.1 Chemicals and material

All pharmaceutical standards (Appendix) were of analytical grade (>90%), and purchased from Sigma- Aldrich(Steinheim, Germany). Isotopically labeled compounds, used as internal standards (IS), were ^{13}C-phenacetin obtained from Sigma-Aldrich and ^{3}D-mecoprop from Dr. Ehrenstorfer (Augsburg, Germany). HPLC grade methanol, acetone, dichloromethane, hexane, as well as formic acid were provided by Dikma (USA). Ultra-pure water was produced by a Milli-Q unit (Millipore, USA). Stock solutions of individual compounds were prepared in methanol and mixture standards with different concentrations were prepared by diluting the stock solutions before each analytical run. All the solutions were stored at 4℃ in the dark.

The cartridges tested for solid phase extraction were: Oasis HLB (200 mg, 6 mL) from Waters Corporation (USA), Supelclean ENVI-Chrom P (500 mg, 6 mL) and Supelclean ENVI-18 (500 mg, 6 mL) from Supelco (USA).

1.2 Sample preparation

Wastewater samples were collected in amber glass bottles prewashed by methanol and ultra-pure water. As soon as delivered to the laboratory, they were filtered through prebaked (400℃, >4 h) glass microfiber filters (GF/F, Whatman). After adjusting the pH of wastewater samples to 7 with 1 mol/L HCl and 1 mol/L NaOH solutions, 25 μL of the mixture of internal standards (400 ng/L for each IS) was added. Solid phase extraction was conducted on a Supelco Visipre SPE Vacuum Manifold (USA). First, the SPE cartridge was conditioned with 5 mL methanol and 3×5 mL ultra-pure water. Then, wastewater sample(200 mL effluent or 100 mL influent) was introduced to the cartridge via a PTFE tube, at a flow rate of 5－10 mL/min. After being washed with 5 mL of 5.0% methanol solution, the cartridge was dried under vacuum for 2 h and eluted with 5 mL methanol at a flow rate of 1－2 mL/min. The extract was concentrated to 0.4 mL under a gentle nitrogen stream and stored at 4℃ for analysis.

1.3 UPLC/MS/MS

Liquid chromatography analysis was carried out on a Waters Acquity UPLC system (Waters Corporation,

USA) equipped with Acquity UPLC BEH C18 column (50 mm×2.1 mm, particle size 1.7 μm, Waters Corporation). The mobile phase consisted of a binary mixture of solvents A (0.01% formic acid in methanol) and B (0.01% formic acid in ultra-pure water) at a flow rate of 0.35 mL/min. The gradient started with 20% A and 80% B, and linearly ramped to 90% A and 10% B in 10 min. The column was allowed to equilibrate for 2 min, and the total run time was 12 min. The injection volume was 5 μL. The tandem MS analysis was performed on a Quattro Premier XE tandem quadrupole mass spectrometer (Waters Corp., USA) equipped with an electrospray ionization source. The analysis was conducted using multiple reaction monitoring (MRM) mode and the parameters, such as cone voltage and collision energy, were optimized by continuous injection of individual standard solution to the mass spectrometry. Optimized parameters for each compound are listed in Table 1, and were used to determine target pharmaceuticals in real wastewater samples.

2 Results and discussion

2.1 Optimization of mass spectrometry

Perfect ionization of carbamazepine, caffeine, DEET, metoprolol, nalidixic acid, propranolol, and sulpiride was realized in positive ion mode, producing an intense protonated molecular ion $[M+H]^+$. While for anti-inflammatory drugs, antilipidemic drugs and chloramphenicol, negative ion mode was adopted and deprotonated molecular ion $[M-H]^-$ was selected as the precursor ion. Cone voltage was optimized in the range of 0 to 70 V in order to obtain maximum response of each precursor ion.

Table 1 Retention time and optimized MS-MS parameters corresponding to each target pharmaceutical

Compound	Retention time (min)	Cone voltage (V)	Collision energy (eV)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	*Q*/*q*	*Q*/*q* ratio*
Positive ionization mode							
CBZ	4.04	40	20 20	236.9	193.7 191.7	Q q	4.5 (2)
CF	0.97	15	20 20	194.9	137.7 109.6	Q q	4.9 (4)
DEET	4.71	40	20	191.8	118.6	Q	–
MTP	1.51	30	20 20	268.0	158.7 132.6	Q q	1.7 (4)
NA	3.27	20	20 40	232.9	186.7 103.7	Q q	1.4 (2)
PPN	2.68	30	15 20	260.1	115.7 182.7	Q q	1.3 (2)
SP	0.45	50	25 35	342.0	111.6 213.8	Q q	3.3 (1)
TP	0.78	50	25 20	290.9	122.7 229.8	Q q	1.2 (4)
Negative ionization mode							
BF	5.65	30	15 30	360.0	273.8 153.6	Q q	2.2 (5)
CA	5.08	20	15 10	212.8	126.5 84.5	Q q	3.8 (7)
CP	2.04	30	15 10	320.8	151.5 256.7	Q q	1.3 (5)
DF	6.77	20	10 20	293.8	249.7 213.9	Q q	20.9 (14)
GF	7.87	20	15 10	248.9	120.5 126.6	Q q	13.6 (33)
IM	6.85	20	10 20	356.2	311.9 296.7	Q q	3.09 (6)
KP	5.14	20	10	253.0	208.7	Q	–
MA	7.66	30	20 30	239.9	195.8 179.7	Q q	19.7 (26)

* Average value (n=9; 3 concentration levels, 3 replications of each) and RSD in parenthesis.

The most abundant fragment ion produced by precursor ion was selected as the primary product ion. The intensity as a function of collision energy is present in Figure 1. As the target compounds were probably mis-identified in the matrix, a secondary product ion of each compound except ketoprofen and DEET was selected for confirmation. For ketoprofen and DEET, there is no stable secondary product ion regardless of the collision energy. The theoretical intensity ratio of quantitative (Q) to confirmative (q) transition was calculated from analytes in standard solution at the concentrations of 10, 50 and 200 μg/L, injected in triplicate (n=9). Confirmation of analytes in the following experiments was in compliance with the requirement of European regulation[34].

Very similar fragmentation was observed for pharmaceuticals from the same therapeutic class. The primary product ions of all anti-inflammatory drugs investigated were generated by expulsion of CO_2 from the precursor ion. For the antilipidemic drugs, $C_7H_{12}O_2$ or $C_4H_6O_2$ resulting from the cleavage of the ether bond was the most abundant fragment ion.

2.2 UPLC optimization

The particle size of HPLC column normally ranged from 3.5 to 5 μm, while the UPLC column was packed with 1.7 μm particles. Therefore, theoretically speaking, the UPLC system could increase linear velocity while maintaining satisfactory operating efficiency. As a result, significant reduction in run time, as well as sensitivity improvement can be achieved by UPLC[17]. The results in this study confirmed such advantages. All the target pharmaceuticals were analyzed within 8 min, much shorter than previously reported HPLC-MS/MS methods[9,15,35–38].

A mixed standard solution of target pharmaceuticals and internal standards at the concentration of 200 μg/L was used to optimize the parameters of UPLC. Severe tailings were observed for all the compounds except caffeine, ^{13}C-phenacetin, carbamazepine and DEET when methanol and ultra-pure water without formic acid were used as mobile phases. The addition of formic acid significantly decreased peak tailings and resulted in good peak shapes for all the target compounds. Then, the amount of formic acid in the mobile phase was optimized. Generally, for pharmaceuticals analyzed in positive mode, a reasonable high concentration of formic acid should enhance the ionization, thus increasing the sensitivity of the method. However, in the present study, 0.01% formic acid was found to be more suitable than 0.1% formic acid, which was suggested in some previous studies[11,35], because 0.01% formic acid resulted in the decreased retention time and higher intensity of most pharmaceuticals regardless of their different pK_a values (Figure 2). Chromatogram for 16 target compounds spiked in the wastewater effluent is shown in Figure 3.

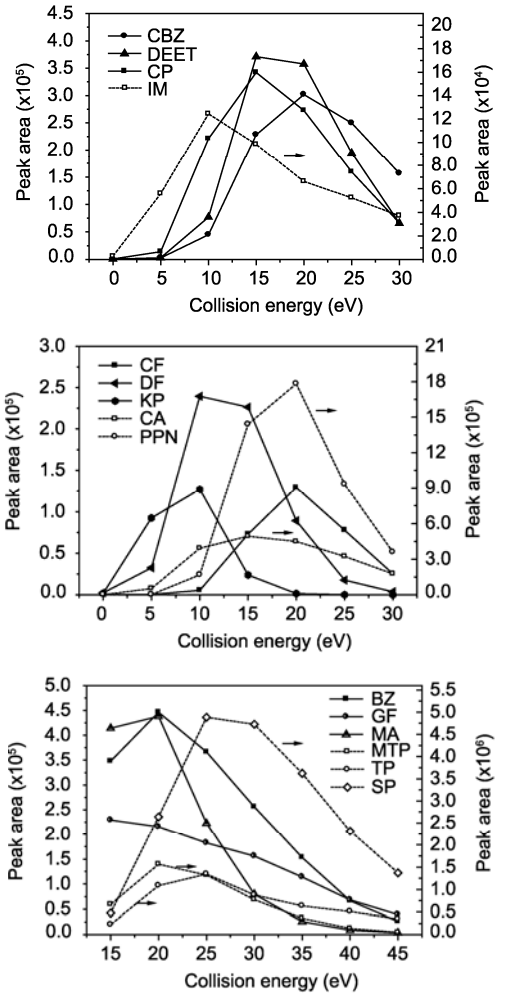

Figure 1 Optimization of collision energy for individual pharmaceutical.

2.3 Optimization of solid phase extraction

In this study, three types of SPE cartridges ENVI-18, ENVI-Chrom P and Oasis HLB were tested. Except for Bezafibrate, carbamazepine, diclofenac, gemfibrozil and ketoprofen, the recovery of other pharmaceuticals varied markedly while using different cartridges (Figure 4). Oasis HLB cartridge displayed good recovery (75%—121%) for all the pharmaceuticals except DEET,

mefenamic acid and nalidixic acid. ENVI-18 cartridge showed very poor recovery for clofibric acid, caffeine, chloramphenicol, metoprolol, propranolol, sulpiride and trimethoprim, all of which had the recovery less than 35%. ENVI-Chrom P cartridge showed poor recovery for metoprolol, propranolol, sulpiride and trimethoprim with their recovery less than 35%.

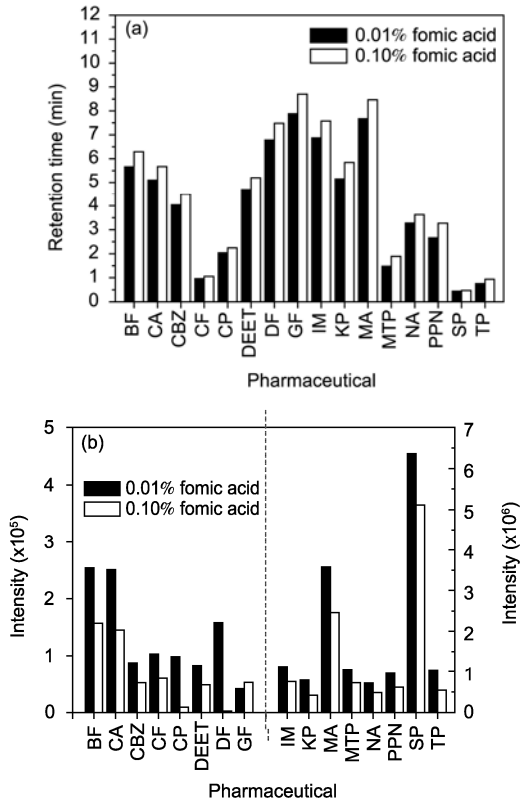

Figure 2 Effect of formic acid concentration in the mobile phase on the retention time (a) and intensity (b).

Four organic solvents of different polarities were evaluated. In this set of experiments, Oasis HLB SPE cartridge was employed and the samples were adjusted to pH 7 before loading. As shown in Figure 4, when dichloromethane was used, poor recovery was obtained for some pharmaceuticals, probably due to the high polarity of the target compounds. Either methanol or acetone gave high recovery for most compounds, and methanol was chosen as the elution solvent finally.

Duplicate samples were prepared at pH 3, 7 and 9 before extraction in order to investigate the effect of pH on the recovery of target compounds. Methanol was used as the elution solvent, and Oasis HLB SPE cartridge was employed. Although higher recovery would

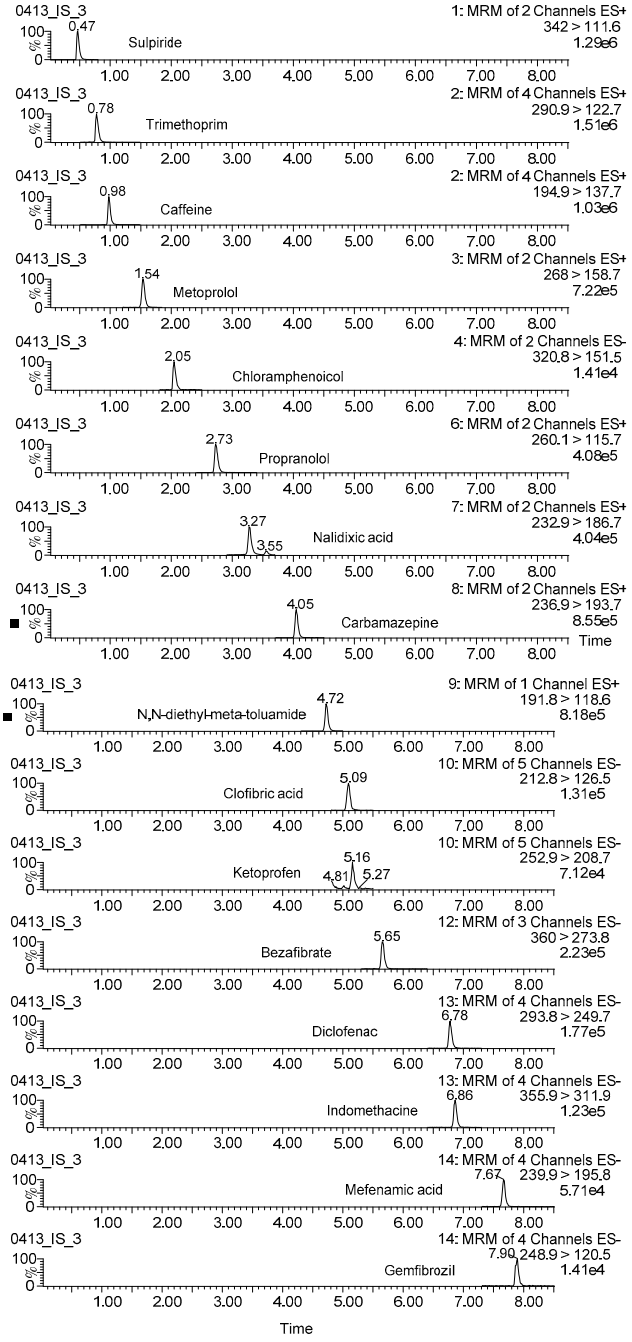

Figure 3 Chromatograms for pharmaceuticals spiked at the concentration of 200 ng/L in the sewage effluent. Only one of the two ion transitions is shown.

be expected for acidic pharmaceuticals in their neutral form at pH 3, the results (Table 2) showed that the recovery at pH 3, 7 and 9 was similar for most pharmaceuticals, which was consistent with some previous studies[21,22,35]. This result could be ascribed to the Oasis HLB cartridge. The sorbent of this cartridge consists of the hydrophilic N-vinylpyrrolidone units and the lipo-

philic divinylbenzene units. The two units can be interacted with different moieties of most target compounds. Therefore, the anion form of most acidic pharmaceuticals could be retained in the HLB SPE cartridge, too. At pH 9, low recovery of clofibric acid was observed. The poor retention due to molecular ionization might be the explanation. At pH 3, sulpiride displayed low recovery, most likely because of the significant matrix effects. At low pH, more matrix components (mainly humic and fulvic acids) might be extracted[35], and resulted in more severe ion suppression. At pH 7, no compound displayed significantly adverse effect. For this reason, pH 7 was chosen for sample preparation.

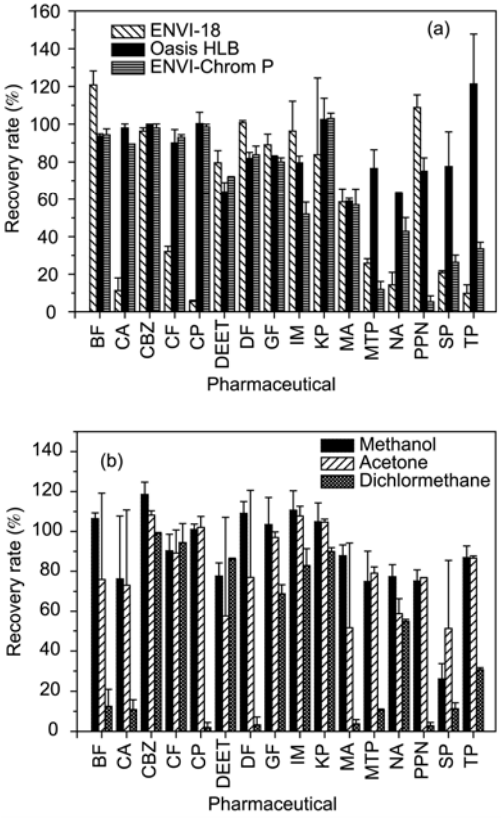

Figure 4 Recovery of pharmaceuticals using different types of SPE cartridges (a) and elution solvents (b).

2.4 Method performance

The repeatability of the instrumental analysis was evaluated by three replicate injections of standard solution every 6 h (n=30). The short-term and long-term relative standard deviations (RSDs) of each analyte were less than 7.9% and 10.4%, respectively. The variability of the retention time was lower than 1% for all the cases.

Table 2 Effect of pH on the recovery (%) of pharmaceuticals

	pH=3	pH=7	pH=9
BF	103±3	105±1	111±4
CA	90±6	93±1	19±10
CBZ	105±22	90±1	91±2
CF	94±4	86±3	97±6
CP	96±6	100±1	99±1
DEET	64±31	45±10	50±20
DF	105±4	110±6	121±23
GF	108±2	114±4	126±13
IM	93±6	114±5	119±34
KP	101±4	106±2	105±5
MA	60±1	75±10	94±43
MTP	89±2	83±2	80±3
NA	64±12	55±9	86±19
PPN	90±2	83±1	77±5
SP	4±2	74±8	46±16
TP	86±0	85±4	85±0

Seven calibration standards (0.1, 0.5, 2, 10, 50, 200, 800 μg/L) for bezafibrate and sulpiride, and six calibration standards (0.5, 2, 10, 50, 200, 800 μg/L) for the other pharmaceuticals were used to establish the non-weighted and linear internal standard calibration curves. The curves were drawn by calculating the ratio of the peak area of analyte to the relative IS, and analyzed at the beginning and the end of each sample batch. Linear regression coefficients (R^2) were higher than 0.99 for all the investigated compounds.

The instrumental quantification limit (IQL) was defined as the concentration that yielded a signal-to-noise ratio of 10:1 and varied from 0.5 to 10 pg (Table 3). The limit of quantification of the method (LOQ) for each matrix was calculated considering IQL, recovery, concentration factor, and matrix effect[37], as shown in eq. (1):

$$\mathrm{LOQ} = \frac{\mathrm{IQL} \times 100}{R(\%) \times C}, \quad (1)$$

where R is the absolute recovery of the analyte in the corresponding matrix (%), and C is the concentration factor (1000, 500 and 250 for the ultra-pure water, effluents and influents, respectively). In ultra-pure water, where there was no matrix effect, the LOQ ranged from 0.1 to 2.8 ng/L. For the wastewater samples, the LOQ ranged from 0.3 to 5.5 ng/L in the effluent and 0.7 to 18.1 ng/L in the influent. This method is more sensitive than techniques previously reported (Table 3). Most published HPLC-MS/MS methods have LOQs of >10 ng/L for investigated pharmaceuticals[36,37,39,40], while in the present study,

LOQs were about 1.0 ng/L for bezafibrate, metoprolol, propranolol and trimethoprim. Reported UPLC/TOF method and HPLC/UV method obtained even higher LOQs than HPLC-MS/MS methods, i.e., normally 20—300 ng/L in the wastewater influent[10,41].

To determine the recovery from different matrices, six replications of ultra-pure water, wastewater influents and effluents were spiked with a stock solution containing all the target pharmaceuticals, then extracted and analyzed by the developed method. The absolute recovery was determined as the ratio of the peak area in the spiked samples (the peak area in the non-spiked sample was subtracted) to the peak area in the standard solution. The relative recovery was defined as the ratio of the absolute recovery of the analyte to the corresponding internal standard.

The mean absolute recovery was 85%, 81% and 70% in the ultra-pure water, wastewater effluent and influent, respectively (Table 4). The decreased recovery was probably due to increased matrix components. However, ^{13}C-phenacetin and ^{3}D-mecoprop, the internal standards for positive and negative ion mode respectively, were able to compensate for the loss of most analytes, and good relative recovery was achieved for all types of samples. The RSD of each analyte were within 20%, with only two exceptions in the wastewater influent. Therefore, the analytical procedure was considered to be reliable for the quantification of selected pharmaceuticals in various matrices.

2.5 Matrix effects

As aforementioned, the mean recovery decreased from 85% in ultra-pure water to 81% in wastewater effluent and 70% in wastewater influent, which might be ascribed to matrix effects, a significant drawback of electrospray mass spectrometry[15,35,39]. To assess this effect, six wastewater effluent samples were extracted. Then three of the extracts were spiked with standard solution, and the others were not. Signal suppression in the wastewater effluent sample was calculated as the difference of signal intensity between the two groups of extracts versus that of standard solution[35,41], expressed as eq. (2):

Table 3 IQL and LOQ of target pharmaceuticals in different matrices

Analyte	IQL (pg)	LOQ in this study (ng/L)			LOQ in previous studies (ng/L)		
		Ultra-pure water	WWTP effluent	WWTP influent	Value*	Analytical method	Ref.
BF	0.5	0.1	0.3	0.7	2–150	HPLC-MS/MS	[8, 40]
					50	UPLC-TOF	[10]
CA	10	2.3	5.4	16.0	48–1118	HPLC-MS/MS	[36, 40]
					25	UPLC-TOF	[10]
CBZ	2.5	0.5	1.0	2.8	1.4–34	HPLC-MS/MS	[37, 40]
					100	UPLC-TOF	[10]
CF	2.5	0.6	1.7	3.3	50–934	HPLC-MS/MS	[8, 40]
CP	2.5	0.5	1.0	2.3	80–250	HPLC/UV	[41]
					0.3–50	HPLC-MS/MS	[8]
DEET	2.5	1.2	1.3	3.2	10–40	HPLC-MS/MS	[8]
DF	10	1.9	4.7	9.4	15–2850	HPLC-MS/MS	[36, 39, 40]
					50	UPLC-TOF	[10]
GF	10	1.8	4.2	20.2	50	UPLC-TOF	[10]
					32–86	HPLC-MS/MS	[40]
IM	2.5	0.5	1.3	2.8	80	UPLC-TOF	[10]
					5–877	HPLC-MS/MS	[8, 40]
KP	10	2.0	5.5	18.1	150	UPLC-TOF	[10]
					20–100	HPLC-MS/MS	[8, 36]
MA	10	2.8	5.5	5.2	20	UPLC-TOF	[10]
					4–950	HPLC-MS/MS	[8, 40]
MTP	2.5	0.6	1.1	3.3	15	UPLC-TOF	[10]
					9.1–2111	HPLC-MS/MS	[37, 40]
NA	2.5	0.9	1.1	2.1	2–80	HPLC-MS/MS	[8]
PPN	2.5	0.7	1.4	3.2	100	UPLC-TOF	[10]
					9–57	HPLC-MS/MS	[8, 40]
SP	0.5	0.2	0.4	1.0	0.6–4	HPLC-MS/MS	[8]
TP	2.5	0.7	1.0	2.7	10	UPLC-TOF	[10]
					100–300	HPLC-UV	[41]
					6–570	HPLC-MS/MS	[8, 39, 40]

* LOQs in the previous studies include the ones for wastewater influents, effluents and surface waters.

Table 4 Recovery of individual pharmaceutical from different types of samples spiked at the concentration of 200 ng/L

	Absolute recovery (%; *n*=6)			Relative recoveries (%; *n*=6)		
	Ultra-pure water	Effluent	Influent	Ultra-pure water	Effluent	Influent
BF	102 (2)	74 (3)	58 (5)	103 (3)	94 (4)	94 (10)
CA	88 (2)	74 (4)	50 (4)	88 (3)	94 (6)	82 (8)
CBZ	92 (2)	100 (4)	71 (12)	107 (6)	130 (6)	133 (23)
CF	88 (6)	60 (3)	61 (37)	103 (8)	77 (4)	114 (70)
CP	105 (2)	98 (7)	86 (9)	106 (3)	124 (10)	140 (17)
DEET	41 (5)	76 (3)	63 (6)	48 (6)	99 (5)	118 (14)
DF	108 (4)	86 (12)	85 (19)	109 (4)	109 (16)	139 (32)
GF	113 (3)	95 (12)	40 (11)	114 (4)	120 (16)	65 (18)
IM	111 (3)	80 (12)	73 (13)	112 (4)	101 (16)	119 (22)
KP	102 (1)	69 (9)	44 (3)	103 (2)	87 (12)	72 (7)
MA	73 (4)	73 (11)	154 (24)	74 (4)	92 (15)	251 (41)
MTP	78 (2)	88 (3)	60 (3)	91 (5)	115 (5)	113 (9)
NA	57 (9)	91 (9)	95 (9)	66 (10)	118 (12)	178 (20)
PPN	77 (2)	73 (3)	63 (10)	90 (5)	95 (4)	118 (20)
SP	51 (6)	51 (5)	42 (3)	60 (8)	67 (7)	79 (7)
TP	73 (2)	102 (7)	74 (16)	85 (5)	132 (9)	139 (31)

$$\text{Signal suppression}\ (\%) = [1-(I_{s+x}-I_x)/I_s]\times 100, \quad (2)$$

where I_{s+x} is the peak area of target analyte spiked in the wastewater effluent extract, I_x is the peak area of that in the unspiked effluent extract, and I_s is the analyte peak area in ultra-pure water spiked at the same level.

All the compounds except diclofenac showed various degrees of matrix suppression (Table 5). More severe signal suppression was observed for some early eluting compounds, sulpiride in particular, signal suppression of which was more than 60%, probably because many polar matrix components were extracted by methanol during the SPE procedure. Similar results were reported by Hilton[9] and Hernando[36], who found that compounds eluting at the beginning of LC gradient was more affected by the matrix components. In the present study, ion suppressions of most compounds were less than 30%, much lower than those previously reported[15,35], probably due to the efficient UPLC separation.

In addition, it should be noted that the severe signal suppression, rather than the pretreatment losses, seemed to be the main reason for the poor absolute recovery of sulpiride, caffeine and ketoprofen in the wastewater effluent.

2.6 Application of method

To validate the applicability of the developed method, pharmaceuticals in samples collected in the winter of 2008, from three WWTPs, referred to as Q, G, and X, located in Beijing, China were determined. These WWTPs employ similar conventional treatment process: primary treatment to remove particles coupled with secondary biological treatment. The removal of COD and SS in all the three WWTPs was effective, with the removal rates of 85.4%—89.3% and 94.9%—96.3%, respectively.

Ketoprofen was the only non-detectable pharmaceutical in all the wastewater samples. Propranolol was detected at low concentrations in all the effluent samples, and not detected in any influent sample. Nalidixic acid could be found only in the effluent samples from one WWTP. By contrast, the other 13 compounds were found in all the samples at ng/L-μg/L level. Caffeine was the most abundant compounds in the influent, with the mean concentration of 5775.6 ng/L. It was in agreement with the fact that large amounts of them were consumed in everyday life (i.e. drinking coffee and tea), not only from the consumption of pharmaceuticals. Besides, concentrations of trimethoprim, diclofenac, sulpiride, indomethacin, gemfibrozil, chloramphenicol and DEET were over 100 ng/L in the influent. Relatively low levels were found in the effluent (Table 6).

Table 5 Matrix suppression of target pharmaceuticals

Compound	RT (min)	Ion mode	Matrix effect (%)
SP	0.45	positive	63.3
TP	0.78	positive	4.9
CF	0.97	positive	49.3
MTP	1.51	positive	15.3
CP	2.04	negative	38.6
PPN	2.68	positive	27.1
NA	3.27	positive	3.4
CBZ	4.04	positive	6.3
DEET	4.71	positive	24.1
CA	5.08	negative	30.1
BF	5.65	negative	26.7
KP	5.14	negative	46.0
DF	6.77	negative	-3.5
IM	6.85	negative	1.3
MA	7.66	negative	13.2
GF	7.87	negative	28.0

In both the influent and effluent, concentrations of most target pharmaceuticals were lower than those reported in the European countries, where concentrations of pharmaceuticals were usually higher than 100 ng/L, or even higher than 500 ng/L in some wastewater effluent samples[10,16,27,36,37]. While in the present study, 9 out of 16 compounds were detected at the mean concentrations less than 100 ng/L, and none of them were found to exceed 500 ng/L in the effluent sample.

As mentioned above, very few papers reported the levels of pharmaceuticals in the wastewater in China and most of them only focused on antibiotics[12–14]. The concentrations of chloramphenicol in the influents (<LOQ-31 ng/L) and effluents (<LOQ-17 ng/L) of 4 WWTPs in Guangzhou[12] were similar to our results. Trimethoprim was detected in five WWTPs of Hong Kong and Shenzhen, with the concentrations of 120－320 ng/L and 120－230 ng/L in the influents and effluents, respectively[14], which were slightly lower than those in the wastewaters in Beijing. It was noteworthy that although antibiotics were the most frequent pharmaceuticals in the wastewater samples, some pharmaceuticals from other therapeutic classes were detected at very high concentrations, such as caffeine, DEET, diclofenac, indomethacin, sulpiride and carbamazepine. Therefore, it has been proved to be important to conduct the survey on the pharmaceuticals from different therapeutic classes in the wastewaters in China.

Table 6 Concentrations of target pharmaceuticals in wastewater of three WWTPs

WWTP	Analyte	BF	CA	CBZ	CF	CP	DEET	DF	GF
Q	IF(ng/L)	34.6	19.8	49.4	4046.0	71.2	157.4	147.4	133.6
	EF(ng/L)	13.5	21.0	96.8	12.7	16.9	24.3	210.3	111.6
	RR(%)	61	–6	–96	100	76	85	–43	16
G	IF(ng/L)	82.8	26.4	70.0	6740.4	64.0	93.2	270.4	108.8
	EF(ng/L)	26.0	31.1	107.8	284.4	43.1	44.5	290.3	125.6
	RR(%)	69	–18	–54	96	33	52	–7	–15
X	IF(ng/L)	28.8	32.6	68.6	6540.4	191.0	179.8	282.6	216.0
	EF(ng/L)	11.1	19.9	105.6	93.6	9.6	57.6	269.9	39.2
	RR(%)	62	39	–54	99	95	68	5	82
WWTP	Analyte	IM	KP	MA	MTP	NA	PPN	SP	TP
Q	IF(ng/L)	117.4	<LOQ	16.0	63.6	<LOQ	<LOQ	92.6	248.8
	EF(ng/L)	123.6	<LOQ	12.8	87.8	<LOQ	4.5	97.3	360.8
	RR(%)	–5	–	20	–38	–	–	–5	–45
G	IF(ng/L)	157.2	<LOQ	26.6	72.6	<LOQ	<LOQ	75.6	225.8
	EF(ng/L)	118.4	<LOQ	9.9	124.5	<LOQ	4.8	134.0	535.2
	RR(%)	25	–	63	–71	–	–	–77	–137
X	IF(ng/L)	149.6	<LOQ	19.0	96.6	<LOQ	<LOQ	136.8	453.4
	EF(ng/L)	125.4	<LOQ	20.5	99.4	8.8	4.6	141.2	359.9
	RR(%)	16	–	–8	–3	–	–	–3	21

IF-Influent; EF; effluent; RR; removal rate.

3 Conclusions

A simple and rapid method has been developed for the simultaneous extraction and determination of 16 pharmaceuticals from 8 therapeutic classes. The application of UPLC allowed a reduction of total run time and matrix effect, as well as an enhancement of sensitivity. Acceptable recovery and low LOQs ranging from 0.3 to 20.0 ng/L were obtained. To our best knowledge, it is the first study on the occurrence of pharmaceuticals from multiple therapeutic classes in the wastewaters in China. 13 and 15 out of 16 target pharmaceuticals were detected in the influents and effluents of three WWTPs, with the mean concentrations of 20.5— 5775.6 ng/L and 4.6—418.6 ng/L, respectively. Particularly, some pharmaceuticals such as caffeine, DEET, diclofenac, indomethacin, sulpiride and carbamazepine were found at high levels, which revealed the potential risk and the necessity for further investigation.

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