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Screening and quantification of pharmaceuticals and their metabolites in municipal wastewater treatment facilities in Guangzhou, China

Zhineng Liu[a], Hongwei Rong[a,b,*], Zhaorui Chu[a], Huayong Luo[a], Meihua Zhao[a], Randeng Wang[a], Chaosheng Zhang[a]

[a]*School of Civil Engineering, Guangzhou University, Guangzhou 510006, China, emails: rhwcn@vip.163.com (H. Rong), liuzhineng@e.gzhu.edu.cn (Z. Liu), zrchu@gzhu.edu.cn (Z. Chu), lhy0909@gzhu.edu.cn (H. Luo), zmhua@gzhu.edu.cn (M. Zhao), wangrandeng@163.com (R. Wang), gdzcs@126.com (C. Zhang)*
[b]*Key Laboratory for Water Quality and Conservation of the Pearl River Delta, Ministry of Education, Institute of Environmental Research at Greater Bay, Guangzhou University, Guangzhou 510006, China*

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ABSTRACT

The combination of qualitative methods with quantitative analysis of pharmaceuticals and their metabolites in wastewater treatment plants using liquid chromatography coupled with quadrupole time-of-flight high-resolution mass spectrometry is presented. The selected pharmaceuticals were found at a low or medium level in raw wastewater, compared with previous studies around the world. The removal efficiencies of sulfamethoxazole in the four parallel biological treatment processes were similar. Data-independent and data-dependent mass spectrometry acquisition methods (All Ions MS/MS and auto MS/MS) were employed for qualitative analysis. Five of the 18 tentatively identified compounds resulted from data-independent acquisition mode were confirmed with standards. Thirty-nine pharmaceuticals and seven metabolites were tentatively identified using data-dependent acquisition mode. The parent pharmaceuticals and their metabolites, such as caffeine and its three metabolites (3-methylxanthine, 1,7-dimethyluric acid, and paraxanthine), were detected in wastewater. Irbesartan and valsartan were tentatively identified in both positive and negative modes. The present study demonstrates that both data-dependent and data-independent acquisitions are suitable and reliable for tentative determination of pharmaceuticals and their metabolites in municipal wastewater.

Keywords: Micropollutant; Municipal wastewater; Pharmaceutical; Metabolite; Suspect screening

1. Introduction

In recent years, as emerging aquatic pollutants pose more and more of a threat to humans and aquatic ecosystems, pharmaceuticals, and personal care products (PPCPs) and their metabolites have received increasing attention. Pharmaceuticals include many chemical categories including pharmaceuticals such as antibiotics, anti-inflammatory, anti-hypertensive, anti-allergy, antidepressant, and antineoplastic agents [1]. It was reported that in 2000–2013, Organization for Economic Cooperation and Development (OECD) member states had a two-fold increase in the daily defined doses of antihypertensive, cholesterol-lowering, antidiabetic, and antidepressant agents [2]. Most pharmaceuticals are only partly metabolized by human body [3,4]. Thus, both parents (pharmaceuticals) and metabolites can enter into municipal wastewater treatment plants (WWTPs) via feces and/or urine [5]. WWTPs with conventional activated sludge processes cannot effectively remove many pharmaceuticals

* Corresponding author.

in wastewater [6]. The pharmaceuticals are discharged to the aquatic environment with the treated wastewater. Pharmaceuticals' exposure may have chronic effects to aquatic organisms [2,7,8]. Not all metabolites of pharmaceuticals are non-toxic [9].

There were many kinds of pharmaceuticals and their concentrations were at ng/L–μg/L levels in wastewater [1,6,10–12]. An effective method is needed to obtain comprehensive information of a diversity of pharmaceuticals at ng/L levels. Liquid chromatography coupled with quadrupole time-of-flight high-resolution mass spectrometry (LC-QTOF) presented potential for identification of a large number of compounds and quantification of selected compounds [13,14]. It is not always practical to obtain the standards of all target pharmaceuticals since the number of pharmaceuticals was huge. Also, the standard of pharmaceuticals' transformation products/metabolites may not have commercial products [15]. LC-QTOF can tentatively identify pharmaceuticals and metabolites by using library MS/MS spectra without standards [16,17]. It is recently reported that LC-QTOF can quantify pharmaceuticals in wastewater [11].

Since dozens or hundreds of features (compounds) are usually extracted in one sample by the instrument, conducting manually qualitative analyses of the features one by one is not practical in suspect screening without preliminary screening by software. Specific software (mostly commercial) is the key in simplifying the data processing and final confirmation [15]. Acquisition and processing workflow of the data are not completely the same in QTOF produced by different companies. The instruments in the previous literature on pharmaceutical screening in surface water and wastewater were mostly from AB SCIEX [10,18–20] or Waters [11,14,21,22], while reports using QTOF from Agilent Technologies, (Santa Clara, CA, US) [23,24] were few. In addition, although the previous report showed that the total consumption of 100 pharmaceuticals in China was over 90 thousand tons in a year [4], there were few studies on wide-scope screening of pharmaceuticals and their metabolites using LC-QTOF in WWTPs in China [19].

As discharge from WWTPs is an essential way of pharmaceuticals entering into the environment and harmful to aquatic ecosystems [25], it is necessary to screen possible pharmaceuticals and metabolites in the wastewater and assess the removal efficiencies of typical pharmaceuticals by WWTPs. Specifically, this study (1) investigated the occurrence of pharmaceuticals in municipal WWTPs; (2) tentatively identified and confirm pharmaceuticals and their metabolites in the wastewater; (3) examined the removal of selected pharmaceuticals by four parallel treatment processes.

2. Materials and methods

2.1. Chemicals

Pharmaceutical standards were purchased from Aladdin (Shanghai, China), Cerilliant (Round Rock, TX, USA), and First Standard (Tianjin, China). The labeled internal standards of carbamazepine d-10 and trimethoprim d-3 were obtained from Cerilliant (Round Rock, TX, USA) and Dr. Ehrensorfer (Augsburg, Bavaria, Germany), respectively. Na_2EDTA was purchased from Tianjin Yongda Chemical Reagent Co., Ltd., (Tianjin, China). Methanol was obtained from Merck (Darmstadt, Germany). Formic acid was purchased from Aladdin (Shanghai, China). Ultrapure water was produced from water purification system (Milli-Q, MA, USA).

2.2. Sampling

Wastewater samples were collected from two large-scale municipal WWTPs (WWTP1 and WWTP2) which collected municipal sewage from combined sewage pipe network and canal. The designed treatment capacity of WWTP1 and WWTP2 were and 0.3×10^6 and 1.2×10^6 m^3/d, respectively. WWTP1 employed modified A/O process as the core biological treatment process. WWTP2 has four parallel biological treatment processes including A–B process (P1), UNITANK process (P2), and modified A^2/O process (P3 and P4). The samples were collected from WWTP1 and WWTP2 in April and July 2018, respectively. Influent, anaerobic (i.e., the effluent of the anaerobic tank in the modified A/O process), and secondary effluent samples from WWTP1, and influent and secondary effluent samples from WWTP2 were collected (as grab samples). The samples were stored in polypropylene bottles and transported to the laboratory for filtration as soon as possible.

2.3. Sample pretreatment

The pretreatment method was based on Zhang et al. [26]. Samples were filtrated with glass fiber filters (0.45 μm), spiked with Na_2EDTA, and adjusted to pH = 4 using hydrochloric acid, successively. After spiked with 50 uL 1 mg/L carbamazepine d-10, the samples (influent: 200 mL; secondary effluent: 400 mL) were processed by an automated extractor using SPE (Fotector-02HT, Reeko, Fujian, China). Methanol (12 mL), ultrapure water (6 mL), and ultrapure water (pH = 4; 6 mL) were used successively for conditioning of the SPE cartridges. The samples were loaded into the cartridges (10 mL/min). After being washed with ultrapure water (10 mL), the cartridges were dried with nitrogen. The analytes were eluted by methanol (8 mL) and evaporated by nitrogen to near dryness, spiked with 10 uL and 5 mg/L trimethoprim d-3, and diluted to 1 mL with methanol-water mixture (3:7 v/v) in brown glass vials.

2.4. Instrument analysis

LC-QTOF (1290-6545, Agilent Technologies, Palo Alto, CA, USA) was employed for both qualitative and quantitative analysis in this study. Chromatographic separations were carried out using an Agilent RRHD C18 column (2.1 mm × 50 mm, 1.8 μm) for the MS and All Ions MS/MS modes, and an Agilent XDB C18 column (2.1 mm × 150 mm, 3.5 μm) for the Auto MS/MS mode. The analytical columns were kept at 40°C. Two solvents were used in mobile phase. Solvent A was 0.1% formic acid spiked with 2 mM ammonium acetate, and solvent B was methanol. The flow rate was 0.3 mL/min. The gradient table was shown in Table S1.

The parameters of QTOF were based on an application note provided by Agilent [27] and shown in Table S1. A solution with reference masses was used to assure the

accuracy of the m/z in both positive and negative modes [23]. Three acquisition modes were employed for different purposes in this study. The full-scan MS mode was used to quantify the concentration of pharmaceuticals with standards, according to the application note provided by Agilent [27]. Simultaneous quantification and qualification of pharmaceuticals in WWTP1 were conducted in the All Ions MS/MS mode. In the All Ions MS/MS mode, QTOF acquires MS information (precursor ions), and MS/MS information (fragments from all precursor ions) in low (0 eV in this study) and high (20 and 40 eV in this study) collision energy, respectively [28]. The peak area of precursor ion with a collision energy of 0 eV (essentially equivalent to MS mode) was used in quantification for higher sensitivity in pharmaceuticals detection [18]. The mass spectra with both low and high collision energy were used in suspect screening with database. The Auto MS/MS mode was used for suspect screening of pharmaceuticals in WWTP2 with library spectra in the database. Details are shown in Table S2.

2.5. Data processing

The data acquired from QTOF were processed using software including quantitative analysis (for quantification) [27], qualitative workflows (for feature extraction and identification) [29], qualitative navigator (for double-check the identification) [23], and mass profiler professional (for alignment) [30]. For the auto MS/MS data, the Agilent Metlin database containing the MS/MS spectra of pharmaceuticals and their metabolites was used. The qualitative workflow was based on previous literature [23] and shown in Fig. 1. Briefly, the requirements in qualitative software were as follows: absolute abundance ≥ 5,000 counts, observed mass error ≤ 5 ppm, MS/MS spectra match score ≥ 80, and comprehensive score ≥ 80. The comprehensive score was based on the observed mass accuracy, profile of isotopes, and corresponding library MS/MS spectra. For the All Ions MS/MS data, an in-house database containing accuracy mass and formula of pharmaceuticals (~750 compounds) was used. The threshold for feature finding in qualitative software were as follows: absolute abundance ≥ 600 counts, observed mass error ≤ 5 ppm, fragment RT difference ≤ 0.1 min, S/N ≥ 5, coelution score ≥ 90, qualified fragment ion ≥ 1, and comprehensive score ≥ 80. Suspect compounds were excluded based on fragment ions using the Massbank database [31]. In compound alignment, the retention time window and the mass window were 0.15 min and 15 ppm + 2 m Da, respectively. The tentatively identified pharmaceutical was confirmed or excluded with standard if available. Information on the usage of the compound was obtained from the website such as Pubchem and Guidechem. The identification confidence levels in suspect screening refer to Schymanski et al. [32].

The removal efficiency of pharmaceutical in WWTP was calculated based on Eq. (1):

$$\text{Removal efficiency (\%)} = \frac{C_I - C_E}{C_I} \times 100\% \quad (1)$$

where C_I and C_E were the pharmaceutical concentrations in influent and secondary effluent, respectively (ng/L).

2.6. Quality assurance and quality control

For quantification, the calibration curves for pharmaceuticals all had correlation coefficients (R^2) higher than 0.99. A spiked blank (50 μg/L) was analyzed per ten samples to monitor the status of LC-QTOF. The instrument limits of detection ranged from 0.2 to 5 μg/L. Spiked matrices were analyzed alongside to assure the quality of the whole analysis process [33]. The recoveries of pharmaceuticals ranged from 71% to 99% (Table S3). For suspect screening, all wastewater samples were analyzed in triplicates. Only when the detection frequency of the compound was 100%, was the compound regarded as a suspect compound. A blank solvent was analyzed every six samples to check for cross-contamination between injections. Suspect compounds were excluded if they appeared in blanks [29].

3. Results and discussion

3.1. Concentrations and removal efficiencies of selected pharmaceuticals in wastewater

The concentrations of pharmaceuticals in wastewater are summarized in Table 1. In the influent, sulfamethoxazole was the most abundant pharmaceutical in the influent with a concentration of approximately 400 ng/L. The secondary abundant pharmaceutical was carbamazepine in WWTP1 and trimethoprim in WWTP2. In the secondary effluent, sulfamethoxazole was, again, the most abundant pharmaceutical. The concentrations of sulfamethoxazole in the influent in this study were at a medium level compared to those in EU-wide, France, Korea, Spain, Sweden, Switzerland, the United Kingdom, and Western Balkan Region (<3–980 ng/L) [34] and the United States (1,566 ng/L) [35]. The concentrations of carbamazepine in the influent in this study were at a low level compared to those in China, EU-wide, Greece, Korea, Spain, the United Kingdom, Western Balkan Region (<40–3,780 ng/L) [34], and France (33 ng/L) [36]. The concentrations of trimethoprim in the

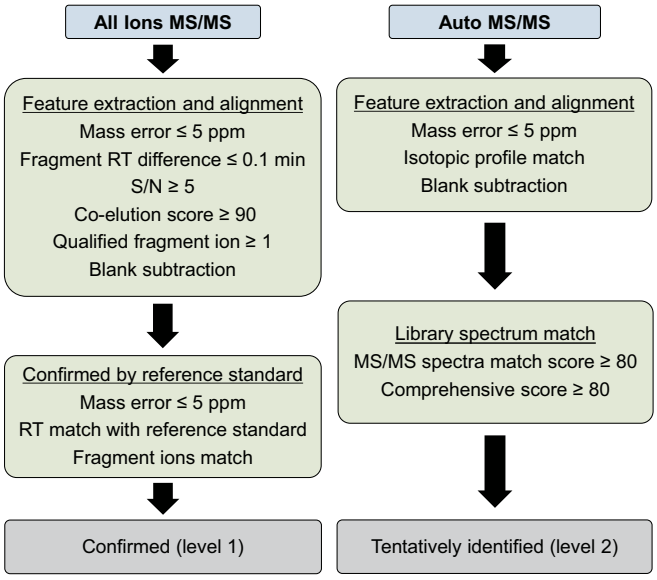

Fig. 1. Screening workflow.

Table 1
Concentrations of pharmaceuticals in wastewater (ng/L)

	CBZ	CIP	SMX	TMP
WWTP1				
Influent	92.6	ND	402	21.5
Anaerobic	55.7	18.2	227	19.1
Secondary effluent	29.6	8.0	79.0	20.1
WWTP2				
Influent	22.1	29.2	349	47.9
Secondary effluent-P1	13.8	17.8	83.7	18.4
Secondary effluent-P2	10.9	4.9	57.8	23.2
Secondary effluent-P3	12.3	ND	69.1	22.7
Secondary effluent-P4	12.5	10.5	50.5	9.4

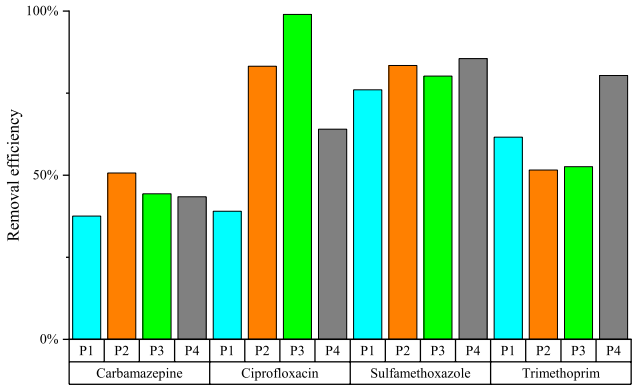

Fig. 2. Removal efficiencies of pharmaceuticals in the four parallel treatment processes of WWTP2.

influent in this study were at a low level compared to those in China, EU-wide, Korea, Spain, and the United Kingdom (60–6,800 ng/L) [34].

The removal efficiencies of sulfamethoxazole were generally higher than trimethoprim, ciprofloxacin, and carbamazepine (Fig. 2). In terms of treatment processes, the removal efficiencies of sulfamethoxazole were stable (coefficient of variation (CV) = 5%) among the four parallel biological treatment processes. It was reported that the main removal pathway of sulfamethoxazole in biological treatment was biodegradation and the effect of adsorption to remove sulfamethoxazole was limited [35]. Sulfamethoxazole can be anaerobic and aerobic biodegradation in activated sludge systems [37]. As for carbamazepine, the removal efficiency is lower in P1 (38%) than in the other three processes (43%–51%). The biological treatment process 1 (P1) did not have an anaerobic stage while the other three processes (P2–P4) had. An investigation showed that the removal efficiencies of carbamazepine in biological treatment processes were higher under anaerobic conditions than under aerobic conditions with the same HRT [37].

3.2. Screening and confirmation pharmaceuticals in wastewater by All Ions MS/MS

The data were acquired using All Ions MS/MS, with the fixed collision energy commonly used in mass spectrum library (20 and 40 eV), which made mass spectrometry matching easier. However, since precursor ions are fragmented without selection [38], interference of other co-eluted compound was inevitable. In this study, acetaminophen and phenethylamine have very similar retention time (Table 2). The fragment ion of acetaminophen (m/z 65.0391) was also regarded as the fragment ions of phenethylamine in the software. The tentative identification may be interfered if without standards or available MS/MS spectra. Reducing the elution intensity of the mobile phase can generally improve the chromatographic resolution, thereby reducing the occurrence of such interference.

The All Ions MS/MS workflow resulted in 18 suspects based on the formula, isotopic pattern, and coeluting fragment ions, of which 5 were confirmed with their reference standards (Table 2). Compounds in quantification were not included in Table 2. Cimetidine, irbesartan, and procaine were detected in the secondary effluent, which indicated the modified A/O process cannot completely remove them. Previous literature showed cimetidine, irbesartan, and procaine were found in the effluent of WWTPs with activated sludge process [39,40]. Cimetidine was also found in the treated wastewater of a WWTP with oxidation ditch process [41]. These results implied that WWTPs with conventional biological treatment technology cannot completely remove cimetidine, irbesartan, and procaine in wastewater.

3.3. Suspect screening of pharmaceuticals in wastewater by auto MS/MS

In total, 39 pharmaceuticals and 7 metabolites were tentatively identified with library MS/MS spectra. Among the 39 pharmaceuticals, 5 were anti-hypertensive agents; 5 were anti-inflammatory agents; 5 were antineoplastic

Table 2
Confirmed pharmaceuticals in WWTP1 (level 1)

Name	CAS #	RT (min)	Observed mass	Observed m/z	Fragment ion 1	Fragment ion 2
Acetaminophen	103-90-2	2.31	151.0634	152.0707	110.0604	65.0391
Cimetidine	51481-61-9	2.46	252.1157	253.1227	159.0689	95.0604
Irbesartan	138402-11-6	6.18	428.2332	429.2405	207.0922	195.1487
Phenethylamine	64-04-0	2.31	121.0894	122.0967	105.0700	79.0544
Procaine	59-46-1	2.48	236.1524	237.1597	164.0706	120.0443

Table 3
Tentatively identified pharmaceuticals and metabolites in WWTP2 (level 2)

Name	CAS #	Observed mass	Formula	RT (min)	Polarity	Category
Gallic acid	149-91-7	170.0215	$C_7H_6O_5$	2.82	Negative	Antineoplastic agent
3-Methylxanthine	1076-22-8	166.0495	$C_6H_6N_4O_2$	4.36	Negative	Metabolite of caffeine and theophylline
Chlorothiazide	58-94-6	294.9492	$C_7H_6ClN_3O_4S_2$	5.47	Negative	Diuretic
L-Amphetamine	156-34-3	135.1047	$C_9H_{13}N$	5.75	Positive	Psychotropic agent
Acetaminophen	103-90-2	151.0634	$C_8H_9NO_2$	6.12	Positive	Analgesic
Pyrocatechol	120-80-9	110.0368	$C_6H_6O_2$	6.85	Negative	Expectorant
Metronidazole	443-48-1	171.0646	$C_6H_9N_3O_3$	6.93	Positive	Antibiotic
Sulpiride	15676-16-1	341.1413	$C_{15}H_{23}N_3O_4S$	8.53	Positive	Antidepressant, antiemetic, antipsychotic agent, and dopaminergic antagonist
Cimetidine	51481-61-9	252.1161	$C_{10}H_{16}N_6S$	8.95	Positive	Anti-ulcer and analgesic agent
3,4-Dihydroxybenzoic acid	99-50-3	154.0265	$C_7H_6O_4$	9.45	Negative	Antineoplastic agent
Pseudoephedrine	90-82-4	165.1153	$C_{10}H_{15}NO$	9.55	Positive	Sympathomimetic agent
6-Methylthioguanine	1198-47-6	181.0425	$C_6H_7N_5S$	9.75	Positive	Cancer chemotherapy agent
1,7-Dimethyluric acid	33868-03-0	196.0598	$C_7H_8N_4O_3$	10.00	Negative	Major urinary metabolite of caffeine
Paraxanthine	611-59-6	180.0649	$C_7H_8N_4O_2$	10.29	Positive	Major metabolite of caffeine
Theophylline	58-55-9	180.0645	$C_7H_8N_4O_2$	10.55	Negative	Vasodilator, bronchodilator, anti-asthmatic, anti-inflammatory agent, muscle relaxant, immuno-modulator, and adenosine receptor antagonist
α-Hydroxymetoprolol	56392-16-6	283.1780	$C_{15}H_{25}NO_4$	11.28	Positive	Metabolite of metoprol
m-Coumaric acid	14755-02-3	164.0472	$C_9H_8O_3$	11.42	Negative	Metabolite of caffeic acid
Salicylic acid	69-72-7	138.0319	$C_7H_6O_3$	12.01	Negative	Anti-inflammatory and antibacterial agent
Caffeine	58-08-2	194.0807	$C_8H_{10}N_4O_2$	12.30	Positive	Stimulant
Lidocaine	137-58-6	234.1737	$C_{14}H_{22}N_2O$	12.32	Positive	Anesthetic
Ofloxacin	82419-36-1	361.1444	$C_{18}H_{20}FN_3O4$	12.50	Positive	Antibiotic
p-Cresol	106-44-5	108.0577	C_7H_8O	12.56	Negative	Germicide
Amantadine	768-94-5	151.1357	$C_{10}H_{17}N$	13.14	Positive	Antiviral and antiparkinsonian agent

(Continued)

Table 3 Continued

Name	CAS #	Observed mass	Formula	RT (min)	Polarity	Category
O-Desmethylvenlafaxine	93413-62-8	263.1890	$C_{16}H_{25}NO_2$	13.50	Positive	Metabolite of venlafaxine
Tramadol	27203-92-5	263.1887	$C_{16}H_{25}NO_2$	14.00	Positive	Analgesic
Caffeic acid	331-39-5	180.042	$C_9H_8O_4$	14.18	Negative	Anti-inflammatory and antineoplastic agent
Benzoic acid	65-85-0	122.0368	$C_7H_6O_2$	14.33	Negative	Antifungal agent
Alprenolol	13655-52-2	249.1731	$C_{15}H_{23}NO_2$	15.05	Positive	Anti-arrhythmia, anti-hypertensive, sympatholytic agent, and beta-adrenergic antagonist
Ifosfamide	3778-73-2	260.0253	$C_7H_{15}Cl_2N_2O_2P$	15.87	Positive	Anticancer and antineoplastic agent
Venlafaxine	93413-69-5	277.2044	$C_{17}H_{27}NO_2$	16.32	Positive	Antidepressant agent
Diphenhydramine	58-73-1	255.1627	$C_{17}H_{21}NO$	17.13	Positive	Anti-allergy agent
Oxcarbazepine	28721-07-5	252.0903	$C_{15}H_{12}N_2O_2$	17.38	Positive	Anticonvulsant
10-Hydroxycarbazepine	29331-92-8	254.1067	$C_{15}H_{14}N_2O_2$	17.45	Positive	Metabolite of oxcarbazepine
Chrysophanol	481-74-3	254.0581	$C_{15}H_{10}O_4$	17.62	Negative	Anti-inflammatory and antiviral agent
Fexofenadine	83799-24-0	501.2879	$C_{32}H_{39}NO_4$	19.10	Positive	Anti-allergy agent
Losartan	114798-26-4	422.1626	$C_{22}H_{23}ClN_6O$	20.30	Negative	Anti-hypertensive agent
Cetirizine	83881-51-0	388.1578	$C_{21}H_{25}ClN_2O_3$	20.33	Positive	Anti-allergy agent
Bicalutamide	90357-06-5	430.0615	$C_{18}H_{14}F_4N_2O_4S$	20.37	Negative	Anti-androgen agent
Gliclazide	21187-98-4	323.1312	$C_{15}H_{21}N_3O_3S$	20.70	Positive	Anti-hyperglycemic agent
Irbesartan	138402-11-6	428.2329	$C_{25}H_{28}N_6O$	21.29	Positive and negative	Anti-hypertensive agent
Telmisartan	144701-48-4	514.2381	$C_{33}H_{30}N_4O_2$	21.41	Positive	Anti-hypertensive agent
9-Dehydromethyltestosterone	1039-17-4	300.2099	$C_{20}H_{28}O_2$	21.61	Positive	Androgen
Valsartan	137862-53-4	435.2277	$C_{24}H_{29}N_5O_3$	21.72	Positive and negative	Anti-hypertensive agent
Nobiletin	478-01-3	402.1318	$C_{21}H_{22}O_8$	21.87	Positive	Antineoplastic agent
Diclofenac	15307-86-5	295.0167	$C_{14}H_{11}Cl_2NO_2$	23.06	Negative	Anti-inflammatory agent
Tapentadol	175591-23-8	221.1784	$C_{14}H_{23}NO$	23.24	Positive	Analgesic

agents; 4 were analgesic agents; 3 were anti-allergy agents; 2 were antidepressant agents; were antibiotics; and 2 were antiviral agents (Table 3). Some pharmaceuticals have multiple usages. It was reported that some pharmaceuticals such as flavonol glycosides and phenolics could be detected in both positive and negative modes [42]. Irbesartan had the same retention time and was tentatively identified in both positive and negative modes, which made its identification more reliable than identifications in only one mode. The same was true for valsartan.

It is not surprising that caffeine and its three metabolites (3-methylxanthine, 1,7-dimethyluric acid, and paraxanthine) were detected, due to the widespread presence of caffeine in drinks such as coffee and tea. Literature showed caffeine was widely detected in municipal WWTPs in Asia, North America, and Europe [43]. Caffeine can enter municipal wastewater with catering wastewater. 1,7-dimethyluric acid is a major urinary metabolite of caffeine and can enter municipal wastewater with urine.

The detected metabolite indicated their parent compounds were consumed by inhabitants. The metabolite of metoprolol (α-hydroxymetoprolol) was detected, which indicated that there may be inhabitants suffering from hypertension in the wastewater collection area. The metabolite of caffeic acid (m-coumaric acid) was detected, which indicated that there may be inhabitants receiving tumor-treatment in the wastewater collection area. The metabolite of venlafaxine (O-desmethylvenlafaxine) was detected, which indicated that there may be inhabitants experiencing depression in the wastewater collection area. The metabolite of oxcarbazepine (10-hydroxycarbazepine) was detected, which indicated that there may be inhabitants suffering from epilepsy and/or bipolar disorder in the wastewater collection area. In general, the pharmaceuticals' metabolite in municipal wastewater can reflect the usage of parent pharmaceuticals, and can be used to speculate on the health status of inhabitants to some extent.

Ofloxacin, irbesartan, and tapentadol were detected in both the influent and four secondary effluent samples, which indicated their removal was not complete in conventional biological treatment processes. Gallic acid, 3-methylxanthine, acetaminophen, pyrocatechol, 3,4-dihydroxybenzoic acid, 2,2-dimethyl succinic acid, pseudoephedrine, paraxanthine, theophylline, α-hydroxymetoprolol, salicylic acid, caffeine, caffeic acid, benzoic acid, chrysophanol, 9-dehydromethyltestosterone, and nobiletin were only detected in the influent samples, which indicated that all of the four parallel biological treatment processes have good removal efficiencies on these compounds.

4. Conclusions

This study investigated the occurrence of pharmaceuticals and their metabolites in WWTPs in Guangzhou, China. LC-QTOF was employed to suspect screening and quantification of pharmaceuticals and their metabolites. The concentrations of the selected pharmaceuticals in the influent were at a medium or low level compared with previous literature. Sulfamethoxazole was the most abundant pharmaceutical among the four selected pharmaceuticals in both the influent and effluent. The removal efficiencies of sulfamethoxazole were stable in the four parallel biological treatment processes. The All Ions MS/MS mode can quantify and tentatively identify pharmaceuticals in wastewater samples simultaneously. Five tentatively identified pharmaceuticals were confirmed with their reference standards. Thirty-nine pharmaceuticals and seven metabolites were tentatively identified using the Auto MS/MS mode. Four parallel biological treatments can nearly completely remove seventeen of the pharmaceuticals and metabolites, while they cannot completely remove ofloxacin, irbesartan, and tapentadol.

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Supplementary information

Table S1
Gradient table of LC

Agilent RRHD C18 (2.1 mm × 50 mm, 1.8 μm)		Agilent XDB C18 (2.1 mm × 150 mm, 3.5 μm)	
Time (min)	Methanol (%)	Time (min)	Methanol (%)
0	5	0	5
0.5	5	5	5
6	100	25	100
7	100	40	100
7.1	5	40.1	5

Table S2
QTOF parameters

	MS	All ions MS/MS	Auto MS/MS
General			
Ion polarity	Positive	Positive	Positive and negative
MS absolute data storage threshold	200	200	200
MS relative data storage threshold (%)	0.01	0.01	0.01
MS/MS absolute data storage threshold	–	–	5
MS/MS relative data storage threshold (%)	–	–	0.01
Dual AJS ESI			
Gas temperature (°C)	150	150	150
Drying gas (L/min)	10	10	10
Nebulizer (psig)	35	35	35
Sheath gas temperature (°C)	375	375	375
Sheath gas flow (L/min)	12	12	12
Capillary voltage (V)	3,500	3,500	3,500
Nozzle voltage expt (V)	200	200	200
MS TOF (expt)			
Fragmentor (V)	125	125	125
Oct RF Vpp (V)	750	750	750
Acquisition			
MS mass range (m/z)	100–1,100	50–1,100	100–1,100
MS acquisition rate (spectra/s)	3	5	5
MS acquisition time (ms/spectrum)	333.3	200	200
MS/MS mass range (m/z)	–	–	50–800
MS/MS acquisition rate (spectra/s)	–	–	5
MS/MS acquisition time (ms/spectrum)	–	–	200
Collision energy (eV)	0	0, 20, 40	10, 20, 40
Max precursor per cycle	–	–	2
Absolute precursor threshold (counts)	–	–	200
Relative precursor threshold (%)	–	–	0.01

Table S3
Quality control parameters of analytes

Name	CAS #	Abbreviation	R^2	Recovery (%)
Trimethoprim	738-70-5	TMP	0.996	71
Ciprofloxacin	85721-33-1	CIP	0.99	99
Sulfamethoxazole	723-46-6	SMX	0.998	98
Carbamazepine	298-46-4	CBZ	0.994	90