Analytical and Bioanalytical Chemistry (2020) 412:203–222
https://doi.org/10.1007/s00216-019-02229-8

RESEARCH PAPER

Simultaneous determination of 44 pharmaceutically active compounds in water samples using solid-phase extraction coupled with ultra-performance liquid chromatography-tandem mass spectrometry

Ming Xue[1] · Haocheng Wu[2] · Shaoying Liu[1] · Xihui Huang[1] · Quan Jin[1] · Ren Ren[1]

Received: 5 September 2019 / Accepted: 21 October 2019 / Published online: 4 December 2019

Abstract

This study examines an improved and simplified method for solid-phase extraction (SPE), which offers rapid and accurate determination and identification of 44 pharmaceutically active compounds using ultra-performance liquid chromatography (UPLC) and tandem mass spectrometry (MS/MS). The common active compounds include four macrolides, seventeen sulfonamides, four quinolones, chloramphenicol, eight β-lactams, four tetracyclines, lincomycin, amantadine, 4-acetamidophenol, phenylbutazone, trimethoprim, clenbuterol, and hydrocortisone in water samples. We optimized crucial parameters of MS/MS, UPLC, and SPE and studied the matrix effect related to the modified analytical process from water samples. The matrix-matched calibration curves were accomplished at seven concentration levels and a satisfactory linear relationship ($r^2 > 0.994$) was observed within the range of 0.1–500 ng/mL. Results show varying limits of detection (0.0111–0.966 ng/L for different analytes based on signal-to-noise (S/N) = 3) and limits of quantitation (0.0382–3.26 ng/L). Recoveries of the spiked samples ranged from 75.7 to 108% with relative standard deviation lower than 9.6%. The proposed method was successfully applied to the analysis of real samples.

Keywords Pharmaceutically active compounds · Ultra-performance liquid chromatography-tandem mass spectrometry · Water · Solid-phase extraction

Introduction

Pharmaceutically active compounds (PhACs) are monomer compounds with medical efficacy or physiological activity. They are widely used as a common treatment for diseases in human and veterinary medicine [1]. Besides, the preventive use of antibiotic feed additives still has been existed in livestock [2]. A 2013 surveillance report by the European Centre for Disease Prevention and Control stated that β-lactams, macrolides, lincosamides and streptogramins, and tetracyclines accounted for 83.5% of the total human antibiotic sales in all 30 European countries [3]. As exogenous environmental contaminants, an increasing amount of PhACs has been released into aquatic environment with discharged human and animal excretions, causing a worldwide PhACs' pollution [1]. Contaminated water can be introduced back into the human body through food chain. Therefore, increasing concerns have been raised for PhACs' bioaccumulation and biomagnification in the aquatic organisms, which can lead to various health and environmental risks [4, 5]. Previous literature has demonstrated that excess PhACs might cause diseases such as prostate and breast cancer [6]. Notably, the misuse of PhACs such as antibiotics has permeated our lives through a variety of channels. Research found that nearly 60% of the residue found comes from environmental and food residues rather than drugs [7]. A 2016 report from a urine sample of 586 children from Shanghai (city located in Southeast China) supported this finding and detected 21 antibiotic residues with the overall detection frequency of 79.6% [8].

✉ Ming Xue
xmxueming@126.com

[1] Hangzhou Center for Disease Control and Prevention, 568 Mingshi Rd., Zhejiang 310021, Hangzhou, China

[2] Zhejiang Provincial Center for Disease Control and Prevention, Zhejiang 310051, Hangzhou, China

Prior research investigated the residues of PhACs in the aquatic environment. Recent studies demonstrated that compounds including sulfadiazine, sulfamethoxazole, oxytetracycline, tetracycline, trimethoprim, amoxicillin, quinolones, analgesics, and antianxietics are frequently detected at trace levels (nanograms to low micrograms per liter) in waste water [9–11], surface water [12–15], raw water [16, 17] and drinking water [18, 19]. For example, Tamtam et al. detected maximum contents of sulfamethoxazole at 544 ng/L in Seine River [20]. Similarly, Shen et al. [21] found that concentrations of sulfonamides and tetracyclines were up to 2680 ng/L and 1470 ng/L in Huangpu River (Shanghai City, China). The investigation conducted by Grujic et al. showed that the maximum content of azithromycin in ground water was 140 ng/L [22]. Moreover, residues of multiple pharmaceuticals had been detected in tap water in Germany [23] and China [9]. Table 1 shows a summary of LC-MS and LC-MS/MS methods for determination of relevant PhACs in water samples.

Despite the extent evidence, the previous studies mostly focused on a particular class or several classes of pharmaceuticals in water. Little has been done to simultaneously detect frequently used PhACs. It is essential to develop accurate methods for simultaneous analyses of human and veterinary PhACs at trace levels in a wide range of aquatic environmental matrices. Research shows that simultaneous analysis allows fast, accurate, and reliable data collection on the sources of target analytes from the environment [29]. Furthermore, tests based on a limited number of PhACs classes lack scientific basis, which prohibits proper evaluation of environmental contamination and implementation of safety measures. In order to overcome this methodological drawback in research, we determine 44 PhACs including four macrolides, seventeen sulfonamides, four quinolones, chloramphenicol, eight β-lactams, four tetracyclines, lincomycin, amantadine, 4-acetamidophenol, phenylbutazone, trimethoprim, clenbuterol, and hydrocortisone. The choice of these 44 PhACs was based on the commonly consumed compounds [13, 16] and prescription data in China [30–32]. Furthermore, to our best knowledge this study is the first to test amantadine in aquatic environment, thus expanding the understanding of PhACs' pollution status.

Due to the varying physicochemical properties of these chosen 44 analytes, appropriate sample pretreatment techniques must be employed. To date, a number of techniques

Table 1 Summary of LC-MS and LC-MS/MS methods for quantitative determination of PhACs in environmental samples

Matrix	Total number of different PhACs determined	Sample preparation	Ionization and detection	Detection limits (ng/L)	Ref.
Bottled water, tap water, seawater, river water, wastewaters	33 (APAP, CFC, TMP, SMD, SPD, SMT, SDM, SDZ, AZM + 24 additional drugs)	SPE	UPLC-MS/MS	0.02–185	[24]
Effluent wastewater, surface water	40 (APAP, SDZ, SDX, SMZ, ROXY, TMP + 34 additional drugs)	On-line SPE	UPLC-MS/MS	≤ 3–7	[25]
Drinking water, tap water, river water, influent wastewater, effluent wastewater	10 (SDZ, SMZ, DOXY, CFC, OTC, TET, CAP + 3 additional drugs)	SPE and DLLME	UPLC-MS/MS	1.67–5.57	[10]
Ground water, surface water, wastewater	74 (APAP, PHE, DOXY, CTC, OTC, TET, AZM, ROX, TYL, SDZ, SMZ, SMR, TMP, EFC, CFC, DFC + 58 additional drugs)	On-line SPE	LC-MS/MS	0.01–5	[26]
River water	77 (PHE, AZM, SDZ, SMZ, EFC, CFC, DFC, TMP, CAP + 68 additional drugs)	On-line purification technology (TurboFlow™)	LC-MS/MS	0.04–15.7	[27]
Surface water, effluent wastewater	50 (APAP, TYL, ROX, CFC, EFC, SFC, SMZ, SDZ, STZ, MY, TMP, CAP + 38 additional drugs)	SPE	UPLC-MS/MS	0.1–86	[28]
Surface water, treated water	18 (CFC, EFC, DFC, STZ, SMZ, SMR, OTC, TET + 10 additional drugs)	HFLPME	LC-MS/MS	0.02–0.25	[13]
Influent wastewater	10 (CFC, OTC, TET, SDZ, DOXY, SMZ, CAP + 3 additional drugs)	SPE and DLLME	UPLC-MS/MS	0.08–1.67	[9]
Surface water	8 (AMOX, CFC, SMZ, TET, TMP + 3 additional drugs)	SPE	LC-MS/MS	0.13–0.76	[18]
Waste water	7 (AMOX, PEN G, TET, DOXY + 3 additional drugs)	SPE	LC-DAD-MS	70–920	[16]
Surface water, ground water	19 (AMOX, SMZ, TMP, AZM, DOXY + 14 additional drugs)	SPE	LC-MS	0.15–12.46	[22]

SPE, solid-phase extraction; *DLLME*, dispersive liquid-liquid microextraction; *HFLPME*, hollow fiber liquid-phase microextraction

have been used in sample pretreatment procedure to detect PhACs in aqueous environment matrices, such as liquid-liquid extraction [10], hollow fiber liquid-phase microextraction [13], and dispersive solid-phase microextraction [9]. However, solid-phase extraction (SPE) has been frequently reported as a more efficient method to concentrate, separate, and screen target analytes from aqueous samples [15, 33, 34]. Moreover, new extraction materials have been invented including Oasis HLB (styrenedivinylbenzene-vinylpyrrolidone copolymer) [35] and Oasis MCX (styrenedivinylbenzene-vinylpyrrolidone copolymer-SO_3H) [36], which are proven to have achieved improved degree of precision and recoveries. Further, research has also suggested advantages of residue analytical technologies such as ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), including improved resolution, shortened time for analysis, and direct detection of target compounds [37–39]. Although these methods (i.e., SPE-UPLC-MS/MS) have been widely employed in various fields, hitherto the approach of detecting 44 PhACs by UPLC-MS/MS remained to be explored.

In this paper, we aimed to develop a rapid, accurate, and sensitive analytical method to extract the 44 target compounds belonging to different therapeutical classes, which greatly facilitates the simultaneous UPLC-MS/MS determination process. We demonstrated a robust and efficient analytical method that is capable of determining 44 PhACs. This method is proven to improve extraction efficiency and minimize interferences, which raises the detection sensitivities and facilitates the studies of residue analysis in water samples. The optimized method was successfully applied to fifteen samples including raw water, treated water, and tap water, offering important evidence for distribution of selected human and veterinary drug pollution through water environment in China.

Experimental

Reagents and samples

A total of 44 PhACs and 10 isotopically labeled compounds were selected as the model analytes and surrogates and/or internal standards. The abbreviations, CAS numbers, purities, sources, and usage are displayed in Table 2. HPLC-grade methanol (MEOH), acetonitrile (MECN), and formic acid were supplied by Merck (Darmstadt, Germany). Phosphoric acid (H_3PO_4) was purchased from Titan (Shanghai, China). Na_2EDTA was obtained from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained by means of a Milli-Q apparatus by Millipore (Milford, MA, USA). The solid extraction columns filled with Oasis HLB (200 mg, 6 mL), MCX (200 mg, 6 mL), and WAX (200 mg, 6 mL) were supplied by Waters (Wexford, Ireland). Stocked solutions for each standard were prepared at a level of 1000 μg/mL in MEOH except for ceftiofur dissolved in MEOH/water (1:1, v/v) to get uniform solution. A calibration curve of standard solutions (0.1, 1, 5, 10, 50, 100, 500 ng/mL) was prepared from stock solutions by serial dilution with MECN/water (1:9, v/v). The internal standards mentioned before were prepared at 200 ng/mL in MEOH/water (1:1, v/v). By the means of adding blank water extracts to each serially diluted standard solution, the matrix-matched standard solutions were similarly arranged (0.1, 1, 5, 10, 50, 100, and 500 ng/mL). All standard solutions were retained in the dark at 4 °C.

Raw water and treated water samples were obtained from five water companies in Hangzhou City in China. The samples of tap water were attained from five different households. All samples were collected in March 2019. Permissions for collecting the water samples were obtained.

Apparatus

The Acquity Ultra Performance LC system (Waters, Milford, USA) consisted of a degasser, a binary gradient pump, an autosampler (10 °C), and a column oven (40 °C). The analytes were detected using a Xevo TQD with Masslynx™ software (version 4.1). A 150 mm × 2.1 mm i.d. ACQUITY UPLC® BEH C_{18} column with 1.7-μm particles (Waters) was used for separation of 44 PhACs.

Chromatographic conditions

A mixture of (A) ultrapure water with 0.1% formic acid and (B) MECN with 0.1% formic acid was chosen as a mobile phase. The flow rate of the mobile phase was 0.3 mL/min. The following gradient program was used for the analysis: 90% A (initial), 90–80% A (1.0–1.5 min), 80–60% A (1.5–3.5 min), 60% A (3.5–6.0 min), 60–40% A (6.0–6.5 min) , 40% A (6.5–7.0 min), 40%–0% A (7.0–7.5 min), 0% A (7.5–10.5 min), 0–90% A (10.5–11.0 min). 2.5 min of equilibration was executed before the next injection. The injection volume was 10 μL, and the column temperature was maintained at 40 °C.

Mass spectrometry

Compounds were detected by multiple reaction monitoring (MRM) using a Micromass Quattro Ultima triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) source. The parameters were attained for the mass spectrometry in positive and negative ion modes. In order to achieve maximum sensitivity for identification and detection of 44 PhACs, we set the typical ESI parameters as follows: the source temperature was 150

Table 2 Major information of 44 target analytes and 10 isotopically labeled standards

Analytes	Abbreviations	CAS numbers	Purities (%)	Concentration (mg/L)	Sources	Usage
Amantadine	AMA	768-94-5	99.5	-	Dr.Ehrenstorfer (Augsburg, Germany)	VP
Paracetamol	APAP	103-90-2	99.9	-	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Phenylbutazone	PHE	50-33-9	99.7	100	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Clenbuterol hydrochloride	ClEN	21898-19-1	-	100	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Hydrocortisone	HC	50-23-7	-	100	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Sulfapyridine	SPD	144-83-2	-	100	BePure (Beijing, China)	Preferred as VP
Sulfadiazine	SDZ	68-35-9	-	100	BePure (Beijing, China)	Preferred as VP
Sulfamethoxazole	SMZ	723-46-6	-	100	BePure (Beijing, China)	Preferred as VP
Sulfathiazole	STZ	72-14-0	-	100	BePure (Beijing, China)	Preferred as VP
Sulfamerazine	SMR	127-79-7	-	100	BePure (Beijing, China)	Preferred as VP
Sulfamoxole	SMO	729-99-7	-	100	BePure (Beijing, China)	Preferred as VP
Sulfisoxazole	SIZ	127-69-5	-	100	BePure (Beijing, China)	Preferred as VP
Sulfamethizole	SMT	144-82-1	-	100	BePure (Beijing, China)	Preferred as VP
Sulfaquinoxaline	SQX	59-40-5	-	100	BePure (Beijing, China)	Preferred as VP
Sulfaphenazole	SPA	526-08-9	-	100	BePure (Beijing, China)	Preferred as VP
Sulfadixine	SDX	2447-57-6	-	100	BePure (Beijing, China)	Preferred as VP
Sulfadimethoxine	SDM	122-11-2	-	100	BePure (Beijing, China)	Preferred as VP
Sulfacetamide	SAA	144-80-9	-	100	BePure (Beijing, China)	Preferred as VP
Sulfabenzamide	SBA	127-71-9	-	100	BePure (Beijing, China)	Preferred as VP
Sulfadimidine	SM2	57-68-1	-	100	BePure (Beijing, China)	Preferred as VP
Sulfamonomethoxine	SMM	1220-83-3	-	100	BePure (Beijing, China)	Preferred as VP
Sulfametoxydiazine	SMD	80-35-3	-	100	BePure (Beijing, China)	Preferred as VP
Trimethoprim	TMP	738-70-5	-	100	BePure (Beijing, China)	Preferred as VP
Penicillin G	PEN G	61-33-6	99.46	-	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Penicillin V	PEN V	87-08-1	98.8	-	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Amoxicillin trihydrate	AMOX	61336-70-7	98.74	-	Dr.Ehrenstorfer (Augsburg, Germany)	Preferred as HP
Azlocillin sodium salt	AZ	37091-65-9	96	-	BePure (Beijing, China)	HP
Cloxacillin sodium salt monohydrate	CLX	7081-44-9	98.95	-	BePure (Beijing, China)	Preferred as VP
Piperacillin	PIP	61477-96-1	98	-	Dr.Ehrenstorfer (Augsburg, Germany)	Preferred as VP
Cephalexin monohydrate	CN	23325-78-2	98.3	-	Dr.Ehrenstorfer (Augsburg, Germany)	Preferred as VP
Ceftiofur	EFT	80370-57-6	97.54	-	Dr.Ehrenstorfer (Augsburg, Germany)	

Table 2 (continued)

Analytes	Abbreviations	CAS numbers	Purities (%)	Concentration (mg/L)	Sources	Usage
						Preferred as VP
Sarafloxacin hydrochloride	SFC	91296-87-6	-	100	TMRM (Beijing, China)	VP
Enrofloxacin	EFC	93106-60-6	-	100	TMRM (Beijing, China)	VP
Ciprofloxacin hydrochloride	CFC	93107-08-5	94	-	Dr.Ehrenstorfer (Augsburg, Germany)	Preferred as VP
Danofloxacin mesylate	DFC	119478-55-6	94.2	-	Dr.Ehrenstorfer (Augsburg, Germany)	VP
Doxycycline hydrochloride	DOXY	10592-13-9	98.4	-	Dr.Ehrenstorfer (Augsburg, Germany)	VP
Chlorotetracycline hydrochloride	CTC	64-72-2	94.6	-	Dr.Ehrenstorfer (Augsburg, Germany)	VP
Oxytetracycline dehydrate	OTC	6153-64-6	98	-	Dr.Ehrenstorfer (Augsburg, Germany)	Preferred as VP
Tetracycline hydrochloride	TET	64-75-5	97.8	-	Dr.Ehrenstorfer (Augsburg, Germany)	Preferred as VP
Azithromycin dehydrate	AZM	117772-70-0	99	-	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Roxithromycin	ROX	80214-83-1)	96.2	-	Dr.Ehrenstorfer (Augsburg, Germany)	HP
Tylosin tartrate	TYL	74610-55-2	92.1	-	Dr.Ehrenstorfer (Augsburg, Germany)	VP
Chlormycetin	CAP	56-75-7	99.24	-	Dr. Ehrenstorfer (Augsburg, Germany)	VP
Lincomycin	MY	7179-49-9	98.2	-	Dr. Ehrenstorfer (Augsburg, Germany)	VP
1-Aminoadamantane-d15	-	33830-10-3	99.6	-	CDN Isotope Laboratories (Quebec, Canada)	-
Danofloxacine-d13	-	1217683-55-0	99.5	-	TMstandard (Beijing, China)	-
Threo-Chloramphenicol-d5	-	202480-68-0	99.7	-	Be Pure (Beijing, China)	-
Trimethoprim-d3	-	1189923-38-3	98.5	-	Dr.Ehrenstorfer (Augsburg, Germany)	-
Benzylpenicilline-d7 N-Ethylpiperidinium salt	-	1217445-37-8	98.9	-	WITEGA (Berlin, Germany)	-
Sulfapyridine-d4	-	1189863-2	98	-	Toronto Research Chemicals (Toronto, Canada)	-
Sulfadiazine-^{13}C6	-	1189426-16-1	99.6	-	Toronto Research Chemicals (Toronto, Canada)	-
Doxycycline-d3 Hyclate	-	-	97	-	Toronto Research Chemicals (Toronto, Canada)	-
Roxithromycin-d7	-	-	96	-	Toronto Research Chemicals (Toronto, Canada)	-
Lincomycin-d3	-	-	97	-	Toronto Research Chemicals (Toronto, Canada)	-

HP, human pharmaceutical; VP, veterinary pharmaceutical

°C, while the capillary voltage was set at 3.5 kV and − 3.0 kV for positive ions and negative ions; the cone gas was 50 L/h; desolvation temperature and desolvation gas were held at 500 °C and 800 L/h respectively; nitrogen was preformed both as nebulizing and desolvation gas.

Sample pretreatment and extraction

2.5-L amber glass bottles were used for sampling, which were previously cleaned in the laboratory. All samples were immediately acidified with H_3PO_4 to pH 2.0 and stored at 4 °C. One-liter samples were filtered through 0.45-μm MCM membrane filters (Agela, Tianjing, China). Each water sample was spiked with 200 μL of the internal standard (200 ng/mL), then added 0.2 g Na_2EDTA to prevent PhACs from complexation with metal ions before solid-phase extraction (SPE) [40]. Extraction of 44 PhACs was performed using 200-mg Oasis HLB SPE cartridges. The columns were set up in series and preconditioned successively with 6 mL of MEOH, 6 mL of Milli-Q water, and 6 mL of H_3PO_4 solution at pH 2.0. The samples were loaded at a flow rate of 5 mL/min. After loading, the HLB cartridge was rinsed with 6 mL deionized water. The cartridge was dried for 15 min under vacuum, and elution of the retained targets was performed with 10 mL of 2% formic acid solution in MEOH/MECN (4:1, v/v). This volume was evaporated until dryness under a nitrogen stream and redissolved in 1.0 mL of water/MECN (9:1, v/v). The extracts were centrifuged for

10.0 min at 12,000 rpm before the UPLC-MS/MS analysis. Each test corresponds at least to three individual experiments, which was executed in triplicate in each experiment.

Validation study

To evaluate the performance of the established techniques, method validation criteria such as linear dynamic range, recovery, precision, and limits of detection and quantification were determined. Matrix-matched calibration curves were obtained by the ratios of peak areas for standard and internal standard solutions at seven concentrations, ranging from 0.1 to 500 ng/mL for three runs. A total of six blank water samples were executed to verify interference from the matrix. We followed Niessen et al.'s equation ($n = 6$) [41] and calculated the matrix effect in order to assess the level of matrix-induced signal suppression/enhancement (ME) caused by water matrix, and the ion suppression/enhancement due to matrix effects was determined:

$$\text{Matrix effect}\ (\%) = (C-B)/A \times 100\%$$

where A is the responses of the 44 PhAC standards in solvent, B is the responses of target analytes in unspiked water effluent extracts, and C is the responses of that spiked in water effluent extract. The LOD and the LOQ were detected as the lowest injected concentrations that produced the signal-to-noise (S/N) ratios of 3 and 10, respectively.

The extraction recoveries were determined for three replicates by analyzing spiked samples which consisted of three different concentrations of standard mixture. The precision of the analytical method was evaluated by calculating intra- and inter-day precision and accuracy, expressed as relative standard deviation (RSD %) values, as well as recoveries achieved from the spiked samples [42]. Intra-day ($n = 6$) and inter-day ($n = 9$) precision were obtained by analyzing spiked samples at different times on the same day and on the consecutive days at the concentrations of 5, 50, and 200 ng/L.

Results and discussion

Optimization of MS/MS conditions

To evaluate the mass spectral fragmentation pattern of each compound, individual standard solution (500 ng/mL) of each compound was optimized by direct injection in the spectrometer with MEOH/water (1:1, v/v) as the solvent [43]. In both positive and negative ion modes, the precursor ion with the best relative intensity was obtained by full-scan data acquisition, and its daughter ions were selected with the help of collision energy. The multiple reaction monitoring (MRM) mode was used to heighten the sensitivity and selectivity of the detection in order to monitor for each analyte [44]. The most abundant and stable daughter ion was selected as the quantitative ion and another is the qualitative ion for each target. Experimental data showed that higher precursor ion signal intensities and better fragmentation patterns were derived for PEN G, PEN V, CLX, and CAP in negative mode, which deprotonated molecular ion $[M\text{-}H]^-$. The rest of targets were determinated in positive mode and generated an intense protonated molecular ion $[M+H]^+$. Table 3 lists an overview of the data obtained for the 44 PhACs under the various ionization conditions.

Optimization of chromatographic separation

Four chromatographic columns including Waters Acquity UPLC BEH C18 (150 mm × 2.1 mm, 1.7 μm), Waters Acquity UPLC CSH C18 (100 mm × 2.1 mm, 1.7 μm), Waters Acquity UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm), and Agilent ZORBAX Eclipse XDB-C18 (100 mm × 2.1 mm, 1.8 μm) were tested. Ultimately, BEH C18 column was selected due to good separation and retention behavior of the compounds with high sensitivity and good resolution. All the analytes were eluted at less than 10.5 min with a 12 min runtime. Consistent with literature [11, 16], MEOH and MECN with formic acid at various concentrations were investigated as mobile phases. The results indicated that MECN led to superior elution strength and decreased retention time. It is suggested that formic acid can enhance ionization efficiency in mobile phases [17]. Therefore, we eventually selected a solvent system as the mobile phases using gradient elution. Such solvent system consists of 0.1% formic acid in MECN and 0.1% formic acid aqueous solution, which afforded the most satisfied chromatographic response and promoted the ionization efficiency of mass spectrometry. The chromatograms for each target compound under optimized condition are displayed in Fig. 1.

Optimization of SPE cartridges

When analyzing water samples, it was highly desirable to concentrate and generate efficient extracts using SPE. Feng et al. [45] and Barbara et al. [36] had successfully applied HLB and MCX columns to extract some kinds of antibiotics. In the test, we analyzed the 44 PhACs simultaneously. Three different commercially SPE cartridges including Oasis HLB, MCX, and WAX were used. The 44 compounds investigated were from different types, such as sulfonamides, quinolones, tetracyclines, macrolides, β-lactam antibiotics, lincomides, adrenomimetics, antipyretic analgesics, and glucocorticoids. The reported values of p*K*a for sulfonamides, quinolones, tetracyclines, macrolides, and β-lactam antibiotics were in range of 1.4–8.4, 5.5–8.5, 3.3–9.3, 7.1–8.8, and 2.7–7.1. The p*K*a

Table 3 MRM conditions for 44 PhACs under study

Analytes	ESI mode	Transition (*m/z*)	Cone (V)	Collision (eV)	t_R (min)
AMA	+	152 > 135[a] 152 > 107	25	15 20	3.49
APAP	+	152.1 > 110.3[a] 152.1 > 65	30	15 24	2.33
PHE	+	309.1 > 119.9[a] 309.1 > 160	29	20 19	8.71
ClEN	+	277.2 > 167.8[a] 277.2 > 131.8	22	26 24	3.92
HC	+	363.1 > 121.1[a] 363.1 > 327.2	31	24 15	5.01
SPD	+	250.1 > 92.1[a] 250.1 > 156.1	26	28 16	3.09
SDZ	+	251.1 > 156[a] 251.1 > 92.1	22	14 27	2.86
SMZ	+	254 > 92.1[a] 254 > 108.1	18	26 22	4.39
STZ	+	256.1 > 92.1[a] 256.1 > 156.1	18	26 14	3.05
SMR	+	265.1 > 92.1[a] 265.1 > 156	14	26 14	3.24
SMO	+	268 > 156.1 [a] 268 > 113	26	17 14	3.45
SIZ	+	268 > 92.1[a] 268 > 156.1	22	28 12	4.55
SMT	+	271.1 > 92.1[a] 271.1 > 108.1	24	28 26	3.61
SQX	+	301.1 > 156.1[a] 301.1 > 208.1	32	16 14	4.91
SPA	+	315 > 158[a] 315 > 222	32	28 19	5.00
SDX	+	311.2 > 92.1[a] 311.2 > 156.1	42	30 18	4.34
SDM	+	311 > 156[a] 311 > 108	28	19 25	4.94
SAA	+	215.1 > 156.1[a] 215.1 > 108	14	18 10	2.69
SBA	+	277 > 156[a] 277 > 108	25	12 24	4.84
SM2	+	279 > 186[a] 279 > 124	29	18 21	3.50
SMM	+	281.2 > 92.1[a] 281.2108.1	26	30 26	3.64
SMD	+	281.2 > 92.1[a] 281.2 > 156.1	28	28 16	3.95
TMP	+	291.1 > 110.1[a] 291.1 > 230	39	34 25	3.32
PEN G	-	333 > 191.9[a] 333 > 73.7	20	12 25	5.45
PEN V	-	349.2 > 208[a] 349.2 > 92.9	15	11 21	6.13
AMOX	+	366.6 > 113.9[a] 366.6 > 159.9	20	35 12	2.13
AZ	+	461.9 > 217.9[a] 461.9 > 175	25	23 38	4.65
CLX	-	433.8 > 293[a] 433.8 > 257	15	21 12	7.60
PIP	+	517.9 > 142.9[a] 517.9 > 159.9	20	31 9	5.01
CN	+	348 > 174.1[a] 348 > 157.9	15	19 20	3.32
EFT	+	524 > 241[a] 524 > 125.7	30	15 34	4.53
SFC	+	386.2 > 299.1[a] 386.2 > 348.2	30	26 30	3.79
EFC	+	360.2 > 203.3[a] 360.2 > 245.1	34	36 28	3.54
CFC	+	333.2 > 231.1[a] 271.1 > 203.2	32	36 36	3.34
DFC	+	358.3 > 82.1[a] 358.3 > 255.3	32	40 38	3.41
DOXY	+	445.2 > 428.1[a] 445.2 > 321.5	25	20 30	4.41
CTC	+	478.8 > 153.9[a] 478.8 > 444.1	25	30 20	4.23
OTC	+	461.2 > 425.8[a] 461.2 > 201.2	25	20 35	3.42
TET	+	445.1 > 409.8[a] 445.1 > 427.1	25	20 15	3.60
AZM	+	749.6 > 116.2[a] 749.6 > 82.9	50	49 50	4.37
ROX	+	837.8 > 158.1[a] 837.8 > 116	29	35 45	6.71
TYL	+	916.7 > 174.1[a] 916.7 > 101	48	41 45	5.20
CAP	-	320.8 > 151.9[a] 320.8 > 256.9	25	20 10	4.72
MY	+	407.2 > 126.2[a] 407.2 > 359.2	32	24 18	3.11

[a] Quantification ions

values of CAP were 9.5 and 11.0, while TMP were at 1.32 and 7.12 [36]. The known pK_1 values of APAP and CLEN were 9.5 and 9.6. These target analytes existed as cationic, zwitterionic, and anionic groups at acidic, neutral, and basic pH, correspondingly [44]. Therefore, the 2.0–10 pH range was covered in the present research. One hundred milliliters of tap water was spiked with the mixed standard solution containing all 44 targets at 50 ng/mL. It then was adjusted to pH 2.0 with H_3PO_4 and passed through the three widely available SPE cartridges. The performance data of the study are summarized in Fig. 2. Excellent repeatabilities had been attained for the three SPE columns, whose RSDs for all analysis were between 0.4 and 9.6%. By comparing the contents of individual components, we found that except for APAP, CLEN, SFC, EFC, DOXY, CTC, and ROX, the recoveries of other

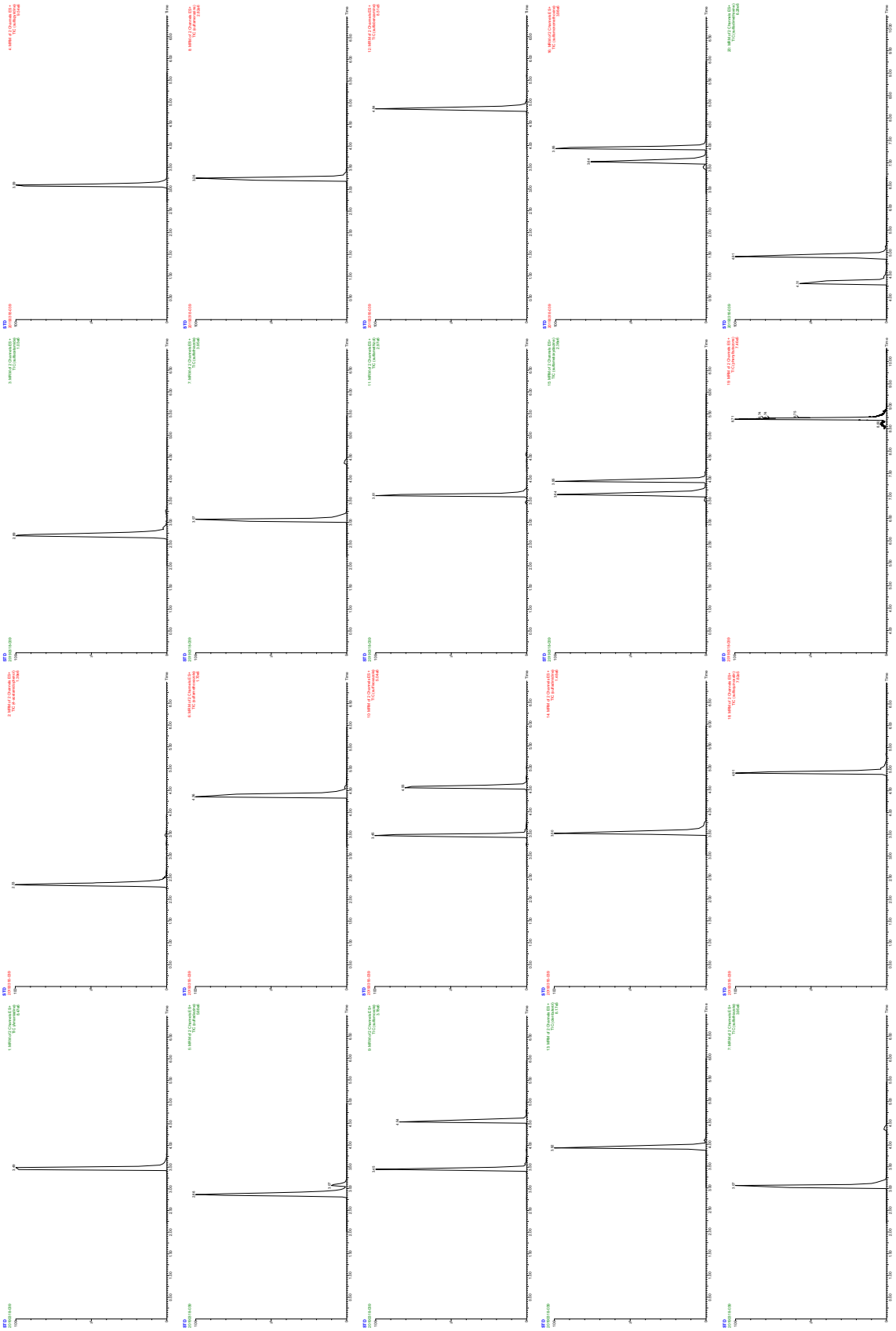

Fig. 1 MRM chromatogram for the 44 PhACs standards under optimum UPLC-MS/MS conditions

Fig. 1 (continued)

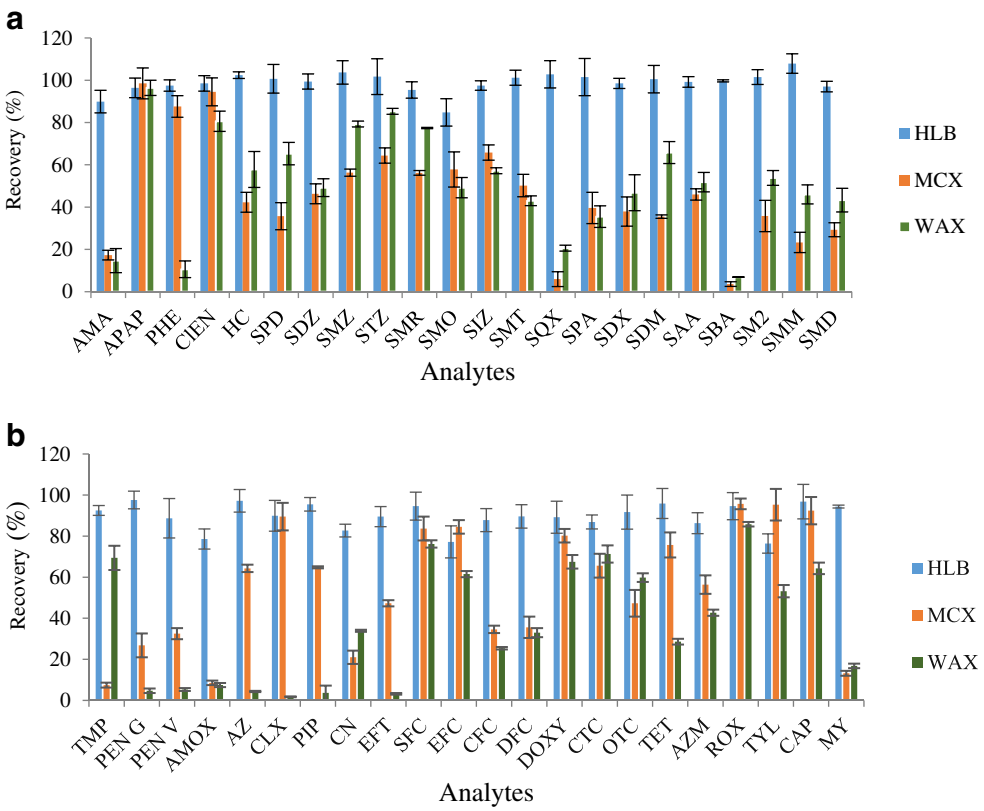

Fig. 2 Effect of different SPE materials on the recoveries of 44 PhACs (spiked at 50 ng/L) from water samples at pH 2.0 (n = 3)

compounds varied markedly using different cartridges. The recoveries of MCX and WAX cartridges ranged from 3.6 to 98.5% and from 3.7 to 96.4% respectively, while those for HLB cartridge varied from 76.4 to 108%. The uppermost extraction rates were gained by means of HLB cartridges, which might attribute to its sorbent. It is combined with hydrophilic-lipophilic polymer, which provided reversed-phase capacity with a special polar hook for superior capture of polar compounds [45, 46]. Guo et al. also reported the HLB column displayed upper adsorption capacity for antibiotics [47]. The recoveries obtained with WAX columns were very low as these were inferior to 20% for AMA, PHE, SBA, PEN G, PEN V, AMOX, AZ, CLX, PIP, EFT, MY, and ERY. On the contrary, MCX provided good recoveries for the majority of those mentioned before (up to 50%) but failed in recovering SDX, SBA, TMP, AMOX, and MY. In view of the best recoveries for all targets, HLB column was opted for further study.

Eluent optimization

A series of solvents were employed to evaluate the appropriate eluent efficiency. Target analytes were extracted as described previously in the "Sample pretreatment and extraction" section. After loading the water samples onto HLB cartridges, 10 mL of several solvents was eluted, including (A) MEOH, (B) MECN, (C) MEOH/MECN (1:1, v/v), (D) MEOH/MECN (2:1, v/v), (E) MEOH/MECN (4:1, v/v), and (F) 2% formic acid solution in MEOH/MECN (4:1, v/v). Recoveries of 44 targets attained by different eluents are presented in Table 4. Initial trials MEOH and MECN (A and B) were conducted as individual eluent. Both showed poor extraction results for most compounds (less than 60%). Accordingly, different eluents (C, D, and E) were tested. Results showed that the recoveries of these three eluted targets had increased significantly, but did not vary greatly except for AMA, PHE, and TYL. As the proportion of MECN increased, the recoveries of AMA, PHE, and TYL improved steadily, especially the ones of TYL, which went from 12.0 to 57.3%. Although the extraction results attained by eluent E for the majority of the 44 compounds exceeded 70%, the extraction rates of some compounds (i.e., PHE, AMOX, AZ, PIP, CN, SFC, EFC, CFC, DFC, OTC, TET AZM, ROX, TYL, and MY) only increased significantly after adding 2% formic acid solution. In summary, excellent recoveries collected from eluent F (76.4% ± 5.8% to 102% ± 6.6%) were found to be superior to the other five solvents for all the analytes (n = 3).

Method validation

Matrix effect

The ESI source is greatly impressionable to ingredients in the matrix, which may lead to ion suppression or

Table 4 Recoveries of 44 target compounds attained by different eluents

Analytes	MEOH (%)	MECN (%)	MEOH/MECN (1:1, v/v) (%)	MEOH/MECN (2:1, v/v) (%)	MEOH/MECN (4:1, v/v) (%)	2% formic acid solution in MEOH/MECN (4:1, v/v) (%)
AMA	86.1	64.7	74.3	69.7	92.5	89.9
APAP	50.7	59.4	89.1	93.1	90.5	96.4
PHE	61.6	21.4	44.3	60.5	68.5	97.5
ClEN	72.2	88.4	92.6	90.5	82.5	98.5
HC	14.4	77.1	76.4	70.3	71.8	102
SPD	57.4	58.5	97.2	87.4	80.8	100
SDZ	58.9	59.2	97.9	84.4	89.6	99.4
SMZ	67.5	47.9	101	92.9	97.8	103
STZ	58.9	50.2	102	96.3	95.3	101
SMR	60.3	50.3	93.5	98.4	95.6	95.4
SMO	58.2	68.7	102	101	97.3	97.8
SIZ	57.3	47.2	101	76.7	94.5	97.5
SMT	51.8	51.2	102	95.5	97.1	101
SQX	53.7	60.4	101	87.1	99.1	102
SPA	47.8	57.2	95.2	86.9	97.1	101
SDX	59.6	59.4	97.7	90.9	91.5	98.5
SDM	48.2	57.1	101	90.3	95.1	100
SAA	66.4	40	99.9	80.3	102	99.2
SBA	39.4	60.7	75.5	90.7	85.6	99.7
SM2	44.2	54.6	73.1	99.1	93.3	101
SMM	40.3	60.7	67.6	73.4	81.2	102
SMD	40.5	61.1	62.4	90.5	89.8	97
TMP	65.6	70.8	82.4	78.9	76.3	92.5
PEN G	30.4	80.4	87.4	76.5	79.8	97.6
PEN V	27.5	84.1	95.4	83.2	86.7	88.7
AMOX	12.4	66.7	68.2	64.1	59.5	78.6
AZ	15.9	68.7	68	54.9	49.7	97.2
CLX	12.4	90.8	92.9	91.1	82.6	89.9
PIP	11.7	77.4	73.2	63.2	62.8	95.5
CN	14.6	49.7	33.9	29.5	33.3	82.7
EFT	14.8	81.4	85.6	79.7	71.7	89.5
SFC	47.9	59.9	36.9	40.9	39.8	94.6
EFC	30.4	65.7	40.4	35.7	46.6	77.2
CFC	24.5	44.5	24.9	26.8	23.8	87.8
DFC	14.5	45.3	58.6	35.3	34.8	89.6
DOXY	43.7	69.4	96.8	88	82.9	89.2
CTC	32.3	67.1	91.8	75.7	75	86.9
OTC	19.6	51.7	92.3	86	68	91.7
TET	12.4	57	59.9	47.9	45.4	95.9
AZM	14.6	34.6	28.3	32.3	29.3	86.3
ROX	58.4	25.7	30.3	31.5	32.4	94.6
TYL	64.7	24.4	12	15.8	57.3	76.4
CAP	13.4	83.9	95.3	90.2	87.4	96.8
MY	66.6	49.3	60.4	65.6	70.3	94.4

enhancement. The results listed in Fig. 3 show that signal suppression or enhancement (ME) did exist in real water samples. The matrix effects of major compounds were from − 56.8 to 53.3% (except for PEN G, CTC,

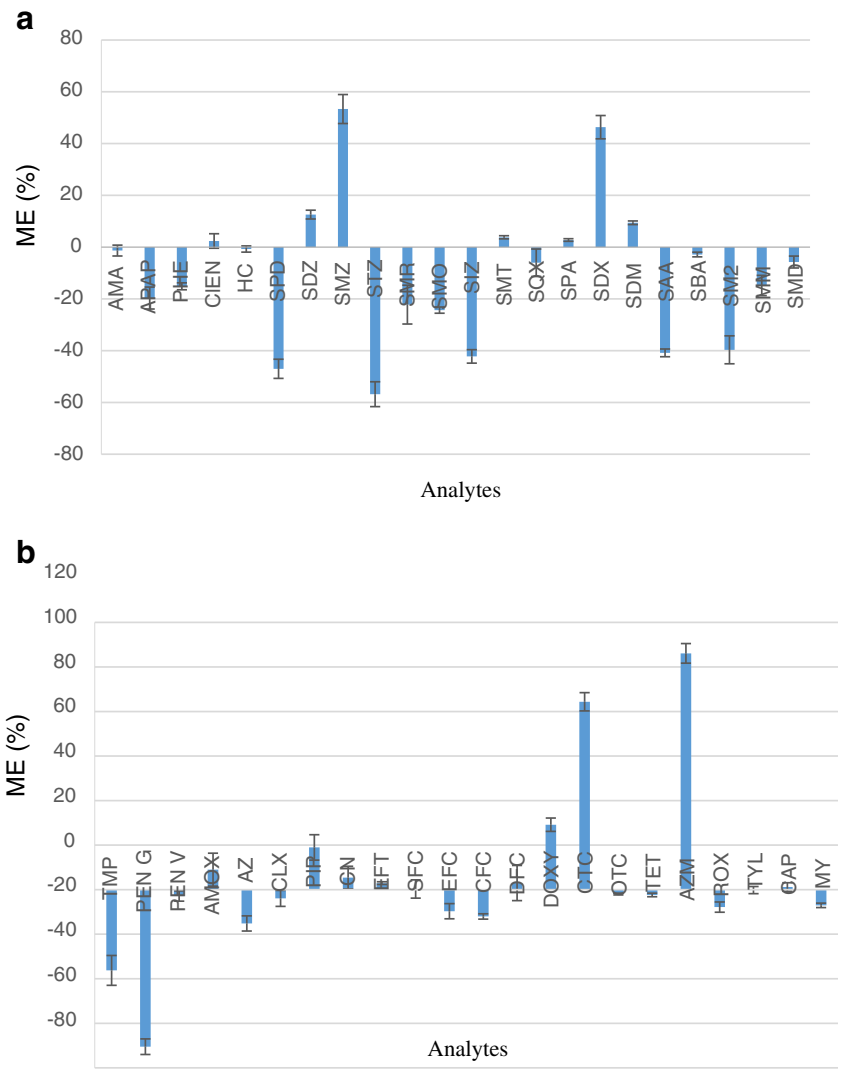

Fig. 3 Matrix effect of 44 PhACs in water samples (n = 6)

and AZM), and approximately 70% analytes were interfered by weak matrix effects (− 20 to 20%) [35]. To compensate for ME of compounds and low SPE recoveries, ten internal/surrogate standards were utilized. The choice of standards above was made on the basis of similar structure and performance in the established method. In addition, the standards of APAP, PHE, CLEN, and HC were diluted directly, for which IS/SS did not compensate for ME [1].

Linearity of calibration and limits of detection and quantification

The matrix-matched calibration curves were for all targets. The results of linearity, linear range, the LOD, and the LOQ are reported in Table 5. Satisfactory linearity and coefficients of determination (r^2 > 0.99) were attained over the concentration range of 0.1–500 ng/mL for AMA, PHE, SPD, SDZ, SMZ, STZ, SMR, SMT, SQX, SAA, SBA, SM2, SMM, SMD, YMP, PEN G, PIP, DOXY, CTC, OTC, ROX, TYL, and MY. The rest of compounds were achieved over the range of 1.0–500 ng/mL. The results indicated approved sensitivity for the proposed method. The LOD and LOQ, determined as the minimum concentration of compounds in the spiked blank samples with a signal-to-noise ratio (S/N) of 3 and 10, ranged from 0.0111 to 0.966 ng/L and from 0.0382 to 3.26 ng/L, respectively. These results are consistent with the findings from previous studies which tested 10 and 11 antibiotics simultanously (e.g., Sergiane et al. [12]; Soparat et al. [13]).

Recoveries and precision

Extraction recoveries and precision assays were conducted at three different concentrations (5, 50, 200 ng/L) of the 44 target compounds. The results of these assays are reported in Table 6. RSD values lower than

Table 5 Calibration parameters of 44 PhACs

Analytes	Linearity (r^2)	Linear range (ng/mL)	LOD (ng/L)	LOQ (ng/L)	IS used
AMA	0.9981	0.1~500.0	0.0111	0.0382	1-Aminoadamantane-d15
APAP	0.9998	1.0~500.0	0.0842	0.255	-
PHE	0.9979	0.1~500.0	0.0368	0.123	-
ClEN	0.9989	1.0~500.0	0.121	0.369	-
HC	0.9953	1.0~500.0	0.348	1.13	-
SPD	0.9997	0.1~500.0	0.0142	0.0564	Sulfapyridine-d4
SDZ	0.9996	0.1~500.0	0.0491	0.163	Sulfadiazine-^{13}C6
SMZ	0.9978	0.1~500.0	0.0369	0.123	Sulfapyridine-d4
STZ	0.9995	0.1~500.0	0.0492	0.163	Sulfapyridine-d4
SMR	0.9995	0.1~500.0	0.0237	0.0804	Sulfadiazine-^{13}C6
SMO	0.9977	1.0~500.0	0.256	0.849	Sulfapyridine-d4
SIZ	0.9994	1.0~500.0	0.221	0.769	Sulfapyridine-d4
SMT	0.9995	0.1~500.0	0.0638	0.205	Sulfapyridine-d4
SQX	0.9972	0.1~500.0	0.0541	0.173	Sulfapyridine-d4
SPA	0.9989	1.0~500.0	0.293	0.993	Sulfapyridine-d4
SDX	0.9986	1.0~500.0	0.130	0.433	Sulfadiazine-^{13}C6
SDM	0.9991	1.0~500.0	0.173	0.584	Sulfadiazine-^{13}C6
SAA	0.9992	0.1~500.0	0.0990	0.330	Sulfapyridine-d4
SBA	0.9997	0.1~500.0	0.0432	0.143	Sulfapyridine-d4
SM2	0.9995	0.1~500.0	0.0534	0.176	Sulfadiazine-^{13}C6
SMM	0.9999	0.1~500.0	0.0222	0.133	Sulfadiazine-^{13}C6
SMD	0.9992	0.1~500.0	0.0300	0.121	Sulfadiazine-^{13}C6
TMP	0.9995	0.1~500.0	0.0269	0.0932	Trimethoprim-d3
PEN G	0.9970	0.1~500.0	0.0542	0.165	benzylpenicilline-D7 N-ethylpiperidinium salt
PEN V	0.9942	1.0~500.0	0.481	1.61	benzylpenicilline-D7 N-ethylpiperidinium salt
AMOX	0.9994	1.0~500.0	0.966	3.26	benzylpenicilline-D7 N-ethylpiperidinium salt
AZ	0.9967	1.0~500.0	0.230	0.775	benzylpenicilline-D7 N-ethylpiperidinium salt
CLX	0.9964	1.0~500.0	0.215	0.727	benzylpenicilline-D7 N-ethylpiperidinium salt
PIP	0.9953	0.1~500.0	0.0183	0.0685	benzylpenicilline-D7 N-ethylpiperidinium salt
CN	0.9983	1.0~500.0	0.0958	0.347	benzylpenicilline-D7 N-ethylpiperidinium salt
EFT	0.9997	1.0~500.0	0.210	0.727	benzylpenicilline-D7 N-ethylpiperidinium salt
SFC	0.9981	1.0~500.0	0.176	0.589	Danofloxacine-d13
EFC	0.9977	1.0~500.0	0.256	0.858	Danofloxacine-d13
CFC	0.9985	1.0~500.0	0.150	0.564	Danofloxacine-d13
DFC	0.9987	1.0~500.0	0.251	0.867	Danofloxacine-d13
DOXY	0.9956	0.1~500.0	0.0183	0.0688	Doxycycline-d3 Hyclate
CTC	0.9974	0.1~500.0	0.0553	0.187	Doxycycline-d3 Hyclate
OTC	0.9984	0.1~500.0	0.0411	0.137	Doxycycline-d3 Hyclate
TET	0.9993	1.0~500.0	0.108	0.369	Doxycycline-d3 Hyclate
AZM	0.9973	1.0~500.0	0.178	0.597	Roxithromycin-d7
ROX	0.9983	0.1~500.0	0.0590	0.200	Roxithromycin-d7
TYL	0.9965	0.1~500.0	0.0132	0.045	Roxithromycin-d7
CAP	0.9956	1.0~500.0	0.231	0.777	Threo-Chloramphenicol-d5
MY	0.9996	0.1~500.0	0.0134	0.0496	Lincomycin-d3

Table 6 Validation parameters of target analytes

Compound	Added (ng/L)	Extraction recoveries (%)	Repeatability (RSD (%))	Intra-day precision (RSD (%))
AMA	5	86.8	5.3	3.5
	50	94.2	4.6	6.4
	200	89.9	2.6	8.8
APAP	5	88.4	3.6	2.4
	50	86.8	1.5	6.5
	200	96.4	6.7	2.5
PHE	5	93.8	3.5	5.6
	50	92.5	5.5	3.5
	200	97.5	8.4	4.6
ClEN	5	90.1	3.8	2.5
	50	96.0	6.4	0.4
	200	98.5	2.2	4.3
SPD	5	102	8.6	9.6
	50	99.8	2.4	4.9
	200	101	4.6	5.5
SDZ	5	99.4	0.8	7.5
	50	101	1.6	3.3
	200	103	7.3	3.1
SMZ	5	93.7	7.7	4.9
	50	103	4.8	6.8
	200	103	3.4	7.8
STZ	5	101	1.3	5.6
	50	101	4.6	5.7
	200	99.4	3.0	7.8
SMR	5	102	4.4	3.4
	50	107	6.7	8.3
	200	103	2.6	7.3
SMO	5	84.8	8.0	5.4
	50	97.9	3.9	3.5
	200	97.2	2.7	6.7
SIZ	5	97.5	1.9	2.8
	50	91.3	4.8	3.0
	200	104	0.5	5.3
SMT	5	101	1.5	4.9
	50	103	6.2	4.6
	200	97.8	3.6	6.0
SQX	5	103	1.1	5.5
	50	98.7	1.0	2.0
	200	97.7	3.4	4.3
SPA	5	101	5.7	4.2
	50	98.3	3.6	5.8
	200	94.6	3.9	4.7
SDX	5	98.5	4.8	3.8
	50	106	8.5	6.4
	200	95.9	5.3	7.1
SDM	5	100	4.2	4.7
	50	108	9.4	4.3
	200	100	7.3	5.9
SAA	5	99.2	4.6	3.8
	50	103	4.8	9.0
	200	99.2	3.6	6.0
SBA	5	101	2.3	5.3
	50	99.7	5.4	5.5
	200	106	5.1	4.1
SM2	5	101	8.5	7.4
	50	104	5.2	4.9
	200	103	4.6	4.4
SMM	5	102	3.9	8.2
	50	96.8	3.5	6.5
	200	107	7.8	4.3
SMD	5	96.3	5.6	5.8
	50	98.4	5.9	5.8
	200	96.7	8.6	3.2
TMP	5	90.3	2.6	6.4
	50	99.5	5.4	1.9
	200	92.5	7.4	5.8
PEN G	5	86.5	9.0	8.3
	50	92.4	2.3	6.8
	200	97.6	7.3	6.2
PEN V	5	83.2	4.0	2.7
	50	95.4	3.5	1.9
	200	88.7	5.0	3.5
AMOX	5	79.5	8.2	4.1
	50	76.3	7.9	7.0
	200	78.6	4.6	0.9
AZ	5	98.0	1.6	2.6
	50	98.3	8.3	8.4
	200	97.2	5.7	5.0
CLX	5	89.9	4.1	4.4
	50	86.5	4.6	8.5
	200	92.9	3.5	1.4
PIP	5	90.0	5.4	7.5
	50	98.1	3.6	8.4
	200	95.5	7.7	2.3
CN	5	83.9	7.5	8.3
	50	88.6	5.4	7.3
	200	82.7	5.4	1.9
EFT	5	87.9	8.0	1.3
	50	95.4	7.9	7.8
	200	89.5	5.4	9.1
SFC	5	86.9	5.4	7.5
	50	95.3	6.9	5.7
	200	94.6	5.4	1.3
EFC	5	75.7	4.3	1.2

Table 6 (continued)

Compound	Added (ng/L)	Extraction recoveries (%)	Repeatability (RSD (%))	Intra-day precision (RSD (%))
	50	80.4	3.7	0.3
	200	77.2	3.7	2.5
CFC	5	74.9	8.4	4.6
	50	86.9	6.5	3.4
	200	87.8	9.3	7.5
DFC	5	88.6	5.6	2.4
	50	95.8	8.4	5.9
	200	89.6	7.4	1.4
DOXY	5	88.0	6.4	1.7
	50	94.1	0.2	4.6
	200	89.2	5.1	4.8
CTC	5	80.7	6.2	4.2
	50	81.8	8.8	3.4
	200	86.9	7.5	4.5
OTC	5	86.0	3.5	1.5
	50	92.3	7.7	4.7
	200	91.7	1.4	3.3
TET	5	88.8	5.7	9.6
	50	95.9	2.5	1.4
	200	92.1	5.3	4.6
AZM	5	88.3	6.3	1.9
	50	86.3	3.9	5.5
	200	94.7	7.2	5.6
ROX	5	84.2	4.8	3.9
	50	90.3	3.7	5.3
	200	94.6	2.8	3.6
TYL	5	69.8	1.7	8.2
	50	76.4	1.2	7.0
	200	77.9	5.0	7.7
CAP	5	90.2	9.8	3.6
	50	96.8	4.5	3.9
	200	95.3	7.3	4.6
MY	5	95.4	7.7	3.9
	50	99.8	3.8	7.9
	200	93.9	2.1	5.7

10% were attained for all the samples. Generally, good recoveries (> 70%) were achieved. Recoveries ranged from 75.7 ± 4.3% (SFC) to 108 ± 9.4% (SDM). The developed methodology presented acceptable reproducibility and less interferences and background noise (n = 3). Similar recoveries of SMZ, TMP, DOXY, CTC, OTC, TET, AZM, CAP, and MY were reported by Boix et al., Liang et al., and Vergeynst et al. [8, 25, 48]. The intra- and inter-day precision were also satisfactory with RSDs being always lower than 9.6% for all compounds.

Application of the method

The fully optimized and validated experimental procedure was later applied to the analysis of the 44 targets from the fifteen real samples containing raw water, treated water, and tap water in Hangzhou, China. The model analytes were confirmed by the retention time, accurate mass, and MS/MS spectrum following the criteria described in European Commission Decision 2002/657/EC [49]. The levels of the 44 PhACs were quantified by the matrix-matched calibration curves. The chromatograms for a real water sample are presented in Fig. 4. It was possible to detect the 44 PhACs in all the considered aquatic environmental matrices. In fact, the results demonstrated the presence of the 17 PhACs in the water samples. There were remarkable differences among the three types of samples. Thirteen of them (AMA, SPD, SMZ, SQX, SMM, SMD, TMP, AZ, PIP, EFT, DOXY, ROX, and MY) were found in raw waters (Table 7). It was clear that all the examined samples in raw water contained one or more target contaminants, in which AMA, SMZ, ROX, and MY were more representative. The highest contents of those analytes reached 374.72, 8.86, and 3.53 ng/L, respectively. Meanwhile, the concentrations of other compounds were almost lower than 9 ng/L in raw water samples. Results show that the concentrations of sulfonamides in raw water are close to the ones found by Li et al. [10], Tang et al. [50], and Cahill et al. [51], but less than those in a study from Batt et al. [43]. Although high consumption of tetracyclines played significant role in human and veterinary medicine, we only detected trace concentration of DOXY in raw water samples, while the highest level was 1.97 ng/L. Similar findings were reported by a number of publications [2]. This phenomenon can be attributed to the formation of stable complexes by tetracyclines and bivalent or trivalent cations. Therefore, tetracyclines are more likely to remain in soil surface or combine with suspended matter or sewage slugged during the wastewater treatment. In relation to treated water and tap water, up to 13 compounds were detected in the samples analyzed. The data collected from treated water were similar to those from tap water. AMA and ROX were the compounds most frequently detected, being present in 100% and 60% of the treated water samples, respectively, while AMA was present in 80% of tap water samples analyzed. Overall, most compounds detected are veterinary PhACs, in which AMA is the most representative, which may be explained by the fact that veterinary drugs have been more extensively used for treating diseases and as

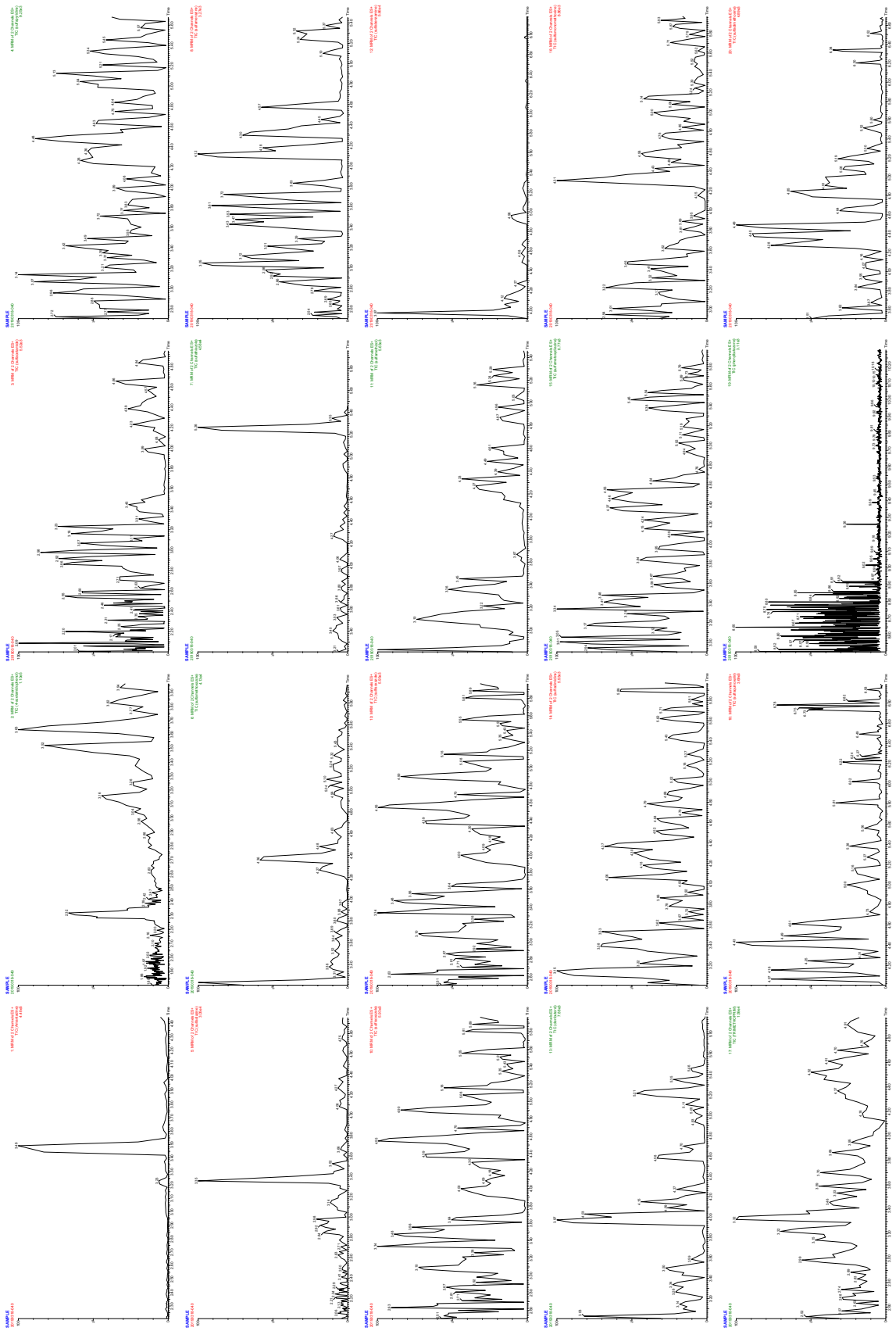

Fig. 4 MRM chromatogram for the 44 PhACs in a real water sample

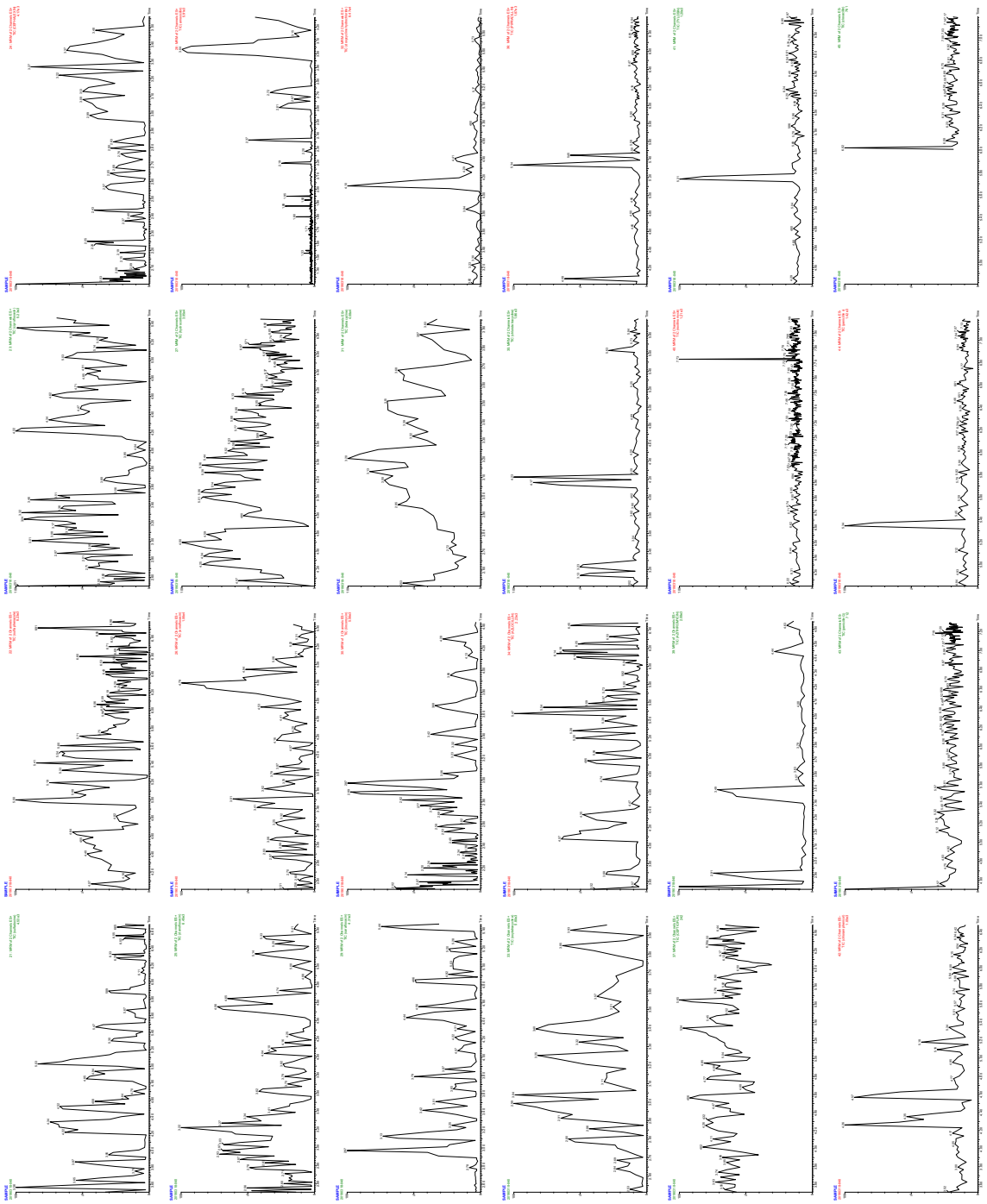

Fig. 4 (continued)

Table 7 Concentration (ng/L) of 17 target analytes detected in water samples

Samples	AMA	SPD	SMZ	SQX	SMM	SMD	TMP	AZ	PIP	CN	EFT	SFC	DOXY	AZM	ROX	TYL	MY
Raw water 1	103.48	0.62	8.86	2.68	-	-	-	2.80	-	-	-	-	1.97	-	3.53	-	0.35
Raw water 2	342.82	2.03	3.35	-	-	-	-	6.51	1.31	-	1.20	-	1.92	-	0.27	-	0.72
Raw water 3	23.70	-	2.34	-	-	1.95	0.20	8.33	-	-	-	-	+	-	-	-	0.65
Raw water 4	374.72	+	-	-	6.75	9.29	-	+	-	-	-	-	0.25	-	0.47	-	+
Raw water 5	34.73	-	0.72	-	-	-	-	+	-	-	-	-	+	-	0.53	-	0.53
Treated water 1	33.03	-	-	-	-	-	-	-	-	-	-	-	-	2.47	3.48	-	+
Treated water 2	24.12	-	6.35	-	-	-	-	-	1.61	0.96	-	-	+	-	0.71	0.53	0.72
Treated water 3	10.17	-	9.46		-	-	-	-	-	-	-	-	-	-	-	-	-
Treated water 4	23.81	2.05	-	-	3.19	3.72	-	-	-	-	-	-	+	-	1.44	-	-
Treated water 5	5.84	+	+		-	0.32	-	-	-	-	-	-	-	-	-	-	-
Tap water 1	4.84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tap water 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Tap water 3	15.58	-	-	-	-	-	-	-	-	-	-	0.95	-	-	-	-	-
Tap water 4	21.91	+	5.47	1.15	-	-	-	+	-	-	-		+	-	+	-	-
Tap water 5	38.06	-	+	-	-	-	-	-	-	-	-	1.33	-	-	2.45	-	0.46

"-" represented "not detected"

"+" represented that the targets were detected in samples, but the contents were less than the LOQ

feedstuff additives in the livestock industry [52]. These representations implied current status of the use of pharmaceuticals.

Conclusions

This study makes notable contributions to knowledge in the areas of methodology in pharmaceutically active compounds in water environment. To the best of our knowledge, this is the first paper to implement an efficient method for the simultaneous determination of the 44 PhACs. In particular, amantadine was detected in aquatic environment for the first time, thus expanding the understanding of PhACs' pollution status. The whole optimized SPE-UPLC-ESI/MS process for the extraction, separation, and determination of various target analytes was further verified in water samples, with low limits of detection, satisfactory linearity, and good recoveries and reproducibility. Crucially, compared with previous studies that detected one or limited numbers of classes of pharmaceutical, we have also obtained the similar detection limits for simultaneous determination of the 44 targets. Furthermore, the proposed method was successfully applied to the analysis of the 44 PhACs in water samples, which presented great potential in the analysis of the target compounds detected. Overall, most compounds detected are veterinary PhACs, in which AMA is the most representative. This technique can be employed as a large-scale tool for monitoring exposure of the water population to the 44 PhACs. Although some PhACs found in water environment are a result of clinical or veterinary use, more epidemiological research should explore further exposure assessment of pharmaceuticals detected in water samples. This is particularly important for consumers in areas where water quality is deemed poor.

Acknowledgments The authors would like to thank Fanxu Yang and Tao Xue for their kind help and useful scientific discussions.

Funding information This work has been supported by Zhejiang Medical and Health Technology Project (2017KY131) and Hangzhou Science and Technology Development Project (20170533B72).

Compliance with ethical standards

The authors declare that they have no conflict of interest.

References

1. Venkata R, Panditi SRB, Piero RG. Online solid-phase extraction-liquid chromatography-electrospray-tandem mass spectrometry determination of multiple classes of antibiotics in environmental and treated waters. Anal Bioanal Chem. 2013;405:5953–64.
2. Carvalho IT, Santos L. Antibiotics in the aquatic environments: a review of the European scenario. Environ Int. 2016;94:736–57.
3. ECDC. Antimicrobial consumption interactive database (ESAC-Net). http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/esac-net-database/Pages/database.aspx. Accessed 16 Nov 2015.
4. Kim S, Aga DS. Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. J Toxicol Environ Health B. 2007;10:559–73.

5. Sui Q, Huang J, Deng SB, Yu G. Rapid determination of pharmaceuticals from multiple therapeutic classes in wastewater by solid-phase extraction and ultra-performance liquid chromatography tandem mass spectrometry. Chin Sci Bull. 2009;54:4633–43.
6. Omar TFT, Aris AZ, Yusoff FM, Mustafa S. Risk assessment of pharmaceutically active compounds (PhACs) in the Klang River estuary, Malaysia. Environ Geochem Health. 2019;41:211–23.
7. Liao D. Residue Characteristics and harmful effects of typical veterinary antibiotics in aquatic environment. Trop Agric Eng. 2013;37:11–4.
8. Wang H, Wang N, Wang B, Fang H. Antibiotics detected in urines and adipogenesis in school children. Environ Int. 2016;89-90:204–11.
9. Liang N, Huang P, Hou X, Li Z, Lei T, Zhao L. Solid-phase extraction in combination with dispersive liquid-liquid microextraction and ultra-high performance liquid chromatography-tandem mass spectrometry analysis: the ultra-trace determination of 10 antibiotics in water samples. Anal Bioanal Chem. 2016;408:1701–13.
10. Li Y, Dong F, Liu X, Xu J. Miniaturized liquid–liquid extraction coupled with ultra-performance liquid chromatography/tandem mass spectrometry for determination of topramezone in soil, corn, wheat, and water. Anal Bioanal Chem. 2011;400:3097–107.
11. Huerta B, Rodriguez-Mozaz S, Barcelo D. Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. Anal Bioanal Chem. 2012;404:2611–24.
12. Sergiane SC, Catia MB, Juliana RG, Maria AKS, Ana LVE, Ednei GP. Determination of Pharmaceuticals, personal care products, and pesticides in surface and treated waters: method development and survey. Environ Sci Pollut Res. 2013;20:5855–63.
13. Soparat Y, Chayada C, Natchanun L. Simultaneous determination of multi-class antibiotic residues in water using carrier-mediated hollow-fiber liquid-phase microextraction coupled with ultra-high performance liquid chromatography tandem mass spectrometry. Microchim Acta. 2011;172:39–49.
14. Yin YM, Shen YQ, Zhu YF, Qing HB. Simultaneous determination of sulfonamides, quinolones and chloramphenicols in water and sediment samples by ultra performance liquid chromatography-tandem mass spectrometry. Anal Sci. 2015;31:228–32.
15. Anekwe JE, Mohamed AA, Stuart H. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg Contam. 2017;3:1–16.
16. Ocsana O, Maria-Loredana S, Virginia C, Florina C, Dumitru R. Determination of some frequently used antibiotics in waste waters using solid phase extraction followed by high performance liquid chromatography with diode array and mass spectrometry detection. Cent Eur J Chem. 2013;11:1343–51.
17. Zhang J, ZONG DL, Chang AM. Determination of common antibiotics in aquatic environment by solid-phase extraction and ultra pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Environ Chem. 2015;34:1446–52.
18. Marco AFL, Fernando FS, Wilson FJ. Determination of antibiotics in Brazilian surface waters using liquid chromatography–electrospray tandem mass spectrometry. Arch Environ Contam Toxicol. 2011;60:385–93.
19. Errayess SA, Lahcen AA, Idrissi L. A sensitive method for the determination of Sulfonamides in seawater samples by solid phase extraction and UV-visible spectrophotometry. Spectrochim Acta A. 2017;181:276–85.
20. Tamtam F, Mercier F, Lebot B, et al. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci Total Environ. 2008;393:84–95.
21. Shen QH, Ji XL, Fu SJ, Liu YY. Preliminary studies on the pollution levels of antibiotic and antibiotic resistance genes in Huangou River, China. Ecol Environ Sci. 2012;21(10):1717–23.
22. Grujic S, Vasiljevic T, Lausevic M. Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography-ion trap-tandem mass spectrometry. J Chromatogr A. 2009;1216(25):4989–5000.
23. Jemba PK. The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown arable land: a review. Agric Ecosyst Environ. 2002;93(5):267–78.
24. Paíga P, Santos LH, Delerue MC. Development of a multi-residue method for the determination of human and veterinary pharmaceuticals and some of their metabolites in aqueous environmental matrices by SPE-UHPLC–MS/MS. J Pharmaceut Biomed. 2017;135:75–86.
25. Boix C, María I, Sancho JV, Rambla J. Fast determination of 40 drugs in water using large volume direct injection liquid chromatography-tandem mass spectrometry. Talanta. 2015;131:719–27.
26. López SR, Pérez S, Ginebreda A, Petrovic M. Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry. Talanta. 2010;83:410–24.
27. López SR, Petrovic M, Barceló D. Direct analysis of pharmaceuticals, their metabolites and transformation products in environmental waters using on-line TurboFlowTM chromatography–liquid chromatography–tandem mass spectrometry. J Chromatogr A. 2012;1252:115–29.
28. Gracia E, Sancho JV, Hernández F. Multi-class determination of around 50 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by ultra-high performance liquid chromatography–tandem mass spectrometry. J Chromatogr A. 2011;1218:2264–75.
29. Perez FV, Rocca LM, Tomai P, Fanali S. Recent advancements and future trends in environmental analysis: sample preparation, liquid chromatography and mass spectrometry. Anal Chim Acta. 2017;983:9–41.
30. Zeng YW, Wu KW. Analysis of prescriptions of antimicrobial drugs in Pediatric outpatient department of our hospital. Eval Anal Drug Use Hosp China. 2010;10:117–8.
31. Sun JY, Liu HF. Analysis of antibiotic prescription in outpatient and emergency department. Eval Anal Drug Use Hosp China. 2012;12(6):548–50.
32. Tian XD. Analysis of medication in 300 pediatric patients with fever. China Pharm. 2013;22:91–2.
33. Li LY, Fan H, Fan L, Huang Z, Li FX. Analysis on glucocorticosteroid use in 69 cases of child bronchopneumonia. Chem Anal Meterage. 2017;26:38–40.
34. Garcia-Galan MJ, Diaz-Cruz MS, Barcelo D. Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC-MS/MS). Talanta. 2010;81:355–66.
35. Liang Y, Liu J, Zhong Q, Yu D. A fully automatic cross used solid-phase extraction online coupled with ultra-high performance liquid chromatography–tandem mass spectrometry system for the trace analysis of multi-class pharmaceuticals in water samples. J Pharm Biomed. 2019;174:330–9.
36. Barbara KH, Richard MD. Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry Alan JG. Anal Bioanal Chem. 2008;391:1293–308.
37. Omar TFT, Ahmad A, Aris AZ, Yusoff FM. Endocrine disrupting compounds (EDCs) in environmental matrices: review of analytical strategies for pharmaceuticals, estrogenic hormones, and alkylphenol compounds. TrAC Trends Anal Chem. 2016;85:241–59.
38. Lu Y, Cheng Z, Liu C, Cao X. Determination of sulfonamides in fish using a modified QuEChERS extraction coupled with ultra-performance liquid chromatography-tandem mass spectrometry. Food Anal Methods. 2016;9:1857–66.

39. Gros M, Rodriguez-Mozaz S, Barcelo D. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J Chromatogr A. 2013;1292:173–88.
40. Lindsey ME, Meyer M, Thurman EM. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. Anal Chem. 2001;73:4640–6.
41. Niessen WMA, Manini P, Andreoli R. Matrix effects in quantitative pesticide analysis using liquid chromatography-mass spectrometry. Mass Spectrom Rev. 2006;25:881–99.
42. Zhang H, Xue M, Lu Y, Dai Z, Wang H. Microwave-assisted extraction for the simultaneous determination of Novolac glycidyl ethers, bisphenol A diglycidyl ether, and its derivatives in canned food using HPLC with fluorescence detection. J Sep Sci. 2010;33:235–43.
43. Cristina PF, Maria CB, Antia SD, Pilar BB. Phthalates determination in pharmaceutical formulae used in parenteral nutrition by LC-ES-MS: importance in public health. Anal Bioanal Chem. 2010;397:529–35.
44. Liu SY, He H, Huang XH, Jin Q, Zhu G. Comparison of extraction solvents and sorbents in the quick, easy, cheap, effective, rugged, and safe method for the determination of pesticide multiresidue in fruits by ultra high performance liquid chromatography with tandem mass spectrometry. J Sep Sci. 2015;38:3525–32.
45. Feng Y, Wei C, Zhang W, Liu Y. A simple and economic method for simultaneous determination of 11 antibiotics in manure by solid-phase extraction and high-performance liquid chromatography. J Soils Sediments. 2016;16:2242–51.
46. Rao RN, Venkateswarlu N, Narsimha R. Determination of antibiotics in aquatic environment by solid-phase extraction followed by liquid chromatography–electrospray ionization mass spectrometry. J Chromatogr A. 2008;1187:151–64.
47. Guo M, Shi LL, Shan ZJ, Kong DY. Determination and the photolytic characteristics of hexanoic acid 2-(diethyl amino) ethylester (DA-6) in water. J Ecol Rural Environ. 2014;23:45–8.
48. Vergeynst L, Haeck A, Wispelaere P. Multiresidue analysis of pharmaceuticals in wastewater by liquid chromatography-magnetic sector mass spectrometry: method quality assessment and application in a Belgian case study. Chemosphere. 2015;119:S2–8.
49. European Commission. Commission Decision (2002/657/EC) of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off J Eur Communities L. 2002;221:8–36.
50. Tang N, Zhang SH, Chen MH, Song NH. Contamination level and risk assessment of 12 sulfonamides in surface water of Nanjing reach of the Yangze River. Environ Chem. 2018;7:505–12.
51. Cahill JD, Furlong ET, Burkhardt MR, Kolpin D. Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography–electrospray ionization mass spectrometry. J Chromatogr A. 2004;1041:171–80.
52. Li PC. The harm of antibiotic abuse to breeding industry and its control countermeasure. China Animal Health Inspection. 2009;26(6):23–4.