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RESEARCH ARTICLE

Fully-automated SPE coupled to UHPLC-MS/MS method for multiresidue analysis of 26 trace antibiotics in environmental waters: SPE optimization and method validation

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Abstract

Achieving simultaneous determination of antibiotic multiresidues in environmental waters by solid phase extraction (SPE) coupled with ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) with detection limits ≤ ng L^{-1} is still a huge challenge. Moreover, the offline SPE procedure was performed manually, costly, and time-consuming, while the online SPE required precision pretreatment instruments that require highly-skilled personnel. In this paper, a fully automated SPE coupled with UHPLC–MS/MS method was developed for analysis of antibiotics (sulfonamides, quinolones, and macrolides) in water matrices. Sample preparation optimization included SPE materials and configuration (HLB disks), sample volume (500-1000 mL), and pH (pH = 3) with a flow rate at 2~5 mL min^{-1}, and an elution procedure with 2 × 6 mL methanol, and 2 × 6 mL acetone. Meanwhile, the parameters for UHPLC-MS/S detection of analytes were optimized, including LC retention time, and MS parameters. The instrumental limits of detection (LOD) and quantification (LOQ) of analytes ranged from 0.01-0.72 μg L^{-1} and 0.05-2.39 μg L^{-1}, respectively, with satisfactory linear calibration ($R^2 > 0.995$) and precision (< 9.9%). Recoveries in spike samples ranged between 77.5-104.9% in pure water, 59.4-97.8% in surface water (SW), and 58.2-108.6% in wastewater effluent (WWE) with relative standard deviations ≤ 12.8%. The matrix effects observed for most analytes were suppression (0-28.1%) except for five analytes having presented enhancement (0-14.6 %) in SW or WWE. This method can basically meet the needs of trace antibiotic residues detection in waters, with examples of concentrations of detected antibiotics being lower than LOQ (LLQ) -94.47 ng L^{-1} in WWEs and LLQ-15.47 ng L^{-1} in SW in the lower reaches of the Yangtze River Basin.

Keywords SPE optimization · UHPLC–MS/MS · Environmental water samples · Sulfonamide, quinolone, and macrolide antibiotics · Validation

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Introduction

The widespread occurrence of antibiotics in the aquatic environment triggered a great concern because it led to an alarming increase in antimicrobial resistance, thereby posing potential ecological and health risks (Danner et al. 2019, Roberts &Zembower 2021, Zhang et al. 2015). Antibiotics including macrolides, quinolones, sulfonamides, β-lactams, tetracyclines, chloramphenicol, etc. have been widely used to prevent or treat bacterial infections in humans and animals, as well as promoting the animal growth (Kovalakova et al. 2020; Luo et al. 2011; Pedrouzo et al. 2008). The continuous discharge of antibiotics in the environment has resulted in surface water (SW) concentrations ranging from ng L^{-1} to μg L^{-1}, leading to the emergence of antimicrobial resistance (Kovalakova et al. 2020; Roberts and Zembower 2021). The World Health Organization (WHO) has reported that the increase in antimicrobial resistance was one of the top ten threats to global health in 2019 (Roberts and Zembower 2021). Furthermore, exposure to antibiotics in waters may induce systemic effects to the intestinal flora of humans, and cause many diseases related to immunity and metabolism (Zhang et al. 2015). Therefore, a reliable analytical method for the detection of antibiotics in waters is necessary to understand the fate of antibiotics in water matrices.

Recently, a new trend in antibiotics analysis is to develop methods for simultaneous determination of multiple compounds in various environmental matrices (Li et al. 2020; Xue et al. 2020). Solid phase extraction (SPE) coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) is now the most commonly used method to simultaneously detect antibiotics, with acceptable recovery and sensitivity, and low solvent consumption (Xue et al. 2020). The analytical technique consists of sample preparation (collection and pretreatment) and detection method, which sample pretreatment largely determines the accuracy and repeatability of residues detection and analysis (Choi et al. 2007). SPE procedure is one of the key techniques for processing water samples and extracts in laboratories due to its simplicity, speed, low solvent consumption, good reproducibility, and acceptable recovery rates and sensitivity (Ramos 2012). Since many antibiotics are non-volatile with high molecular weight, the most common method for antibiotics detection utilizes LC-MS/MS, with multiple reaction selection (MRM) as the preferred mode for increased analytical sensitivity and selectivity in complex matrices (Mokh et al. 2017; Panditi et al. 2013; Zhi et al. 2020).

Researches on SPE-LC-MS/MS methods to simultaneously detect antibiotics in waters have been reported (Li et al. 2020; Mokh et al. 2017; Panditi et al. 2013; Xue et al. 2020). Gusmaroli et al. (2018) successfully used online SPE coupled with ultra-high-performance LC-MS/MS (UHPLC-MS/MS) to detect 17 compounds in SW and wastewater influent and effluent (WWE/WWI) with limits of detection (LOD) ranging from sub- to low-nanogram per liter levels. Nevertheless, national standards have not yet been established for the detection and control of antibiotics in environmental water matrices in China. Firstly, achieving stable routine detection limits at ng L^{-1} concentration or lower in the simultaneous determination of trace antibiotics in environmental waters by SPE-LC-MS/MS is still a huge challenge due to the substantial differences in the physicochemical properties of different antibiotics (such as polarity, solubility, pKa, and stability), as well as the specific complexity of water matrices (Mokh et al. 2017). Secondly, nowadays, the multiresidue methods for antibiotic analysis include and have shifted from offline to online SPE-LC-MS/MS. Table S1 reveals the main comparative assessments of sample pretreatments by different SPE modes. The traditional offline SPE was often usually performed manually with multi-steps, high cost and particularly time-consuming, that may result in high artificial error rate, and unstable recovery rate and method detection limit (MDL), in addition to requiring bulky solvents and loading sample (Gusmaroli et al. 2018; Mokh et al. 2017). By contrast, the online SPE procedure with direct injection automatically performed sample pre-concentration and chromatographic separation (Panditi et al. 2013). These may reduce tedious sample manipulation to increase productivity and sample throughput, as well as sample volume (< 10 mL), and organic solvents (< 10 mL) (Panditi et al. 2013; Rubirola et al. 2017). However, it needed extra precision instruments that require highly skilled operators for sample pretreatment, accompanied by lower concentration multiples with less sample volume and unknown whether the analytes are fully enriched and separated with less eluent. Xue et al. (2020) established an offline SPE-LC-MS/MS (UHPLC-MS/MS) method to simultaneously detect 44 drug residues in aquatic samples with recoveries of 75.7-108% and good detection limits of 0.0111–0.966 ng L^{-1}. Panditi et al. (2013) established an online SPE-LC-MS/MS method to simultaneously determine 31 antibiotics in drinking water, SW, and reclaimed water with recoveries between 50 and 150%, and the MDLs ranging from 1.2–9.7, 2.2–15, and 5.5–63 ng L^{-1}, respectively. Therefore, it is necessary to continue developing automated multiresidue methods for efficient separation and enrichment of antibiotics, and evaluate the effectiveness and influencing factors, simultaneously. In addition, the huge differences in sample substrates in different regions also require to establish new analytical methods of antibiotics suitable for specific region such as the Yangtze River valley in China.

This study develop a simple method for rapid simultaneous analysis of 26 antibiotics (sulfonamides, quinolones, and macrolides) in the sewage treatment plant (STP) effluents and Yangtze River with an automated cartridge-disk universal SPE coupled to a UHPLC-MS/MS. The significant feature of this method is automated

SPE mode as compared to manual offline-SPE, reducing sample manipulation to decrease the possibility of high contamination and loss of analytes (Table S1). Compared to online SPE, this mode avoids the need for sophisticated sample pretreatment instruments that require highly skilled personnel, and the prepared sample in vials is flexible and mobile for subsequent UHPLC-MS/MS detection (Table S1). Since the SPE effect is mostly affected by the selectivity of SPE materials (Zhi et al. 2020), this study mainly considered the optimal selection of SPE cartridge/disk materials, in addition to eluent procedure, water sample pH, MS parameters, and LC separation.

Experimental

Materials and reagents

Methanol (MeOH), dichloromethane (DCM), formic acid, and acetone of analytical grade were purchased from MERCK, Germany. Hydrochloric acid (superior grade) was purchased from Sigma, USA. EDTA-2Na (analytical grade, Chinese medicine, China) at 1.0 g L^{-1} was added to water samples to prevent photo-degradation and chelation with metal ions. Others such as sulfuric acid (H_2SO_4) were purchased from Shanghai Sinopharm Group Chemical Preparation Co., Ltd. Pure water (PW) was produced with a Milli-Q system (Millipore, USA).

Analytical standards of antibiotics (> 97% purity) were purchased from Dr. Ehrenstofer GmbH (Germany). The selection of 26 analytes was based on data acquired from provided literatures about the detection frequency, concentration, toxicological relevance, and availability of reliable analytical methods (Anumol and Snyder 2015; Han et al. 2015, Zhi et al. 2020). The selected antibiotics consists of three categories: sulfonamides, quinolones, and macrolides. Detailed information about all the target analytes is presented in Table 1. Isotopically labeled surrogates (ISs) including sulfamethazine-$^{13}C_6$, sulfamethoxazole-$^{13}C_6$, difloxacin hydrochloride-D_3, ofloxacin hydrochloride-D_3, ofloxacin hydrochloride-D_5, ciprofloxacin hydrochloride-D_8, and erythromycin-^{13}C-D_3 were purchased from Cambridge Isotope Labs (USA). Stock solutions of mixed analytical standards (1 mg L^{-1}) and mixed IS standards (50 μg L^{-1}) were prepared in MeOH.

SPE cartridges/disks included Oasis HLB SPE cartridge (500 mg/6 mL, Waters, USA), C18 SPE cartridge (500 mg/6 mL, Lab Tech, China), C18 SPE cartridge (1 g/6 mL, IST, Sweden), Florisil SPE cartridge (500 mg/6 mL, Chromatography, USA), HLB SPE disk (ϕ 47 mm, Atlantic, USA), and C18 SPE disk (ϕ 47 mm, Atlantic, USA).

Sample collection and treatment

Triplicates of SW and WWE samples (1.0 L) were collected in clean brown glass bottles from the Yangtze River (a depth of ~ 0.5 m below the water surface) and effluents of Bailonggang sewage treatment plant (STP) which adopts multi-mode anaerobic-anoxic-aerobic activated sludge process in Pudong area, Shanghai. Detailed information including the longitude and latitude are provided in Table S2. Samples were transported to the laboratory in iceboxes overnight, immediately filtered by 0.45-μm mixed fiber membrane to remove suspended solids, acidified by adding H_2SO_4 (pH 3.0), followed by Na_2EDTA (1.0 g L^{-1}) addition, and spiked with a mixture of all ISs to achieve a final concentration of 50-100 μg L^{-1} depending on the type of analyte and sample matrix.

The conditions of actual sample preparation were determined based on the results of recoveries from experiments using PW consisting of suitable acidified condition (pH 3.0, 4.0 or 5.0), flow rate (1-5 mL min^{-1}), and volume of loading sample (0.10-1.00 L), whether preserved with 1.0 g L^{-1} of Na_2EDTA.

Fully automatic solid phase extraction and enrichment

A fully automatic cartridge-disk universal SPE system (LabTech, China) coupled with a MultiVap-8 channel parallel concentrator (LabTech, China) (Figure S1) were used for sample extraction and enrichment of target antibiotics. The system automatically executed the activation, sample passing, cleaning, nitrogen drying, soaking, and elution of 12 SPE cartridges/disks, simultaneously.

The recoveries using six SPE cartridges/disks described above were compared. Eluent using either MeOH, water, acetone, MeOH-DCM (V/V = 1/1), MeOH-acetone (V/V = 1/1), or DCM with the corresponding volume of 3 × 6 mL, 4 × 6 mL, or 5 × 6 mL was also evaluated for all analytes by using HLB SPE disk. Preliminary experiments were performed with the PW spiked with 100 μg $L^{-1}$of mixed analytical standards and the absolute recoveries were compared. Unless otherwise specified, these experiments were conducted in quintuplicates.

The optimized SPE procedure can be briefly described as follows: The systems sequence first preconditioned the SPE HLB disks with 15 mL MeOH, 5 mL acetone-MeOH (V/V = 1/1), and 10 mL PW successively. Then, water samples (0.5~1 L) adjusted to pH 3 by adding H_2SO_4 and Na_2EDTA (1.0 g L^{-1}) were passed through SPE with a flow rate of 2~5 mL min^{-1}, followed by 2 × 5 mL acidified PW (pH 3) to rinse the HLB disks. After samples loading, the disk were dried for 10 min with N_2-blowdown, and then eluted into 50-mL nitrogen blowpipe using eluent consisting of 2 × 6 mL of MeOH, and 2 × 6 mL of acetone. Finally, the collected eluent was

Table 1 Categories, names, and molecular weights of target antibiotics and their compound-dependent parameters in MS/MS analysis

Category	Compound	Molecular formula	Molecular weight	Abbreviation	Scope of use	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (V)	Retention time (min)
Sulfonamides	Sulfadiazine	$C_{10}H_{10}N_4O_2S$	250.3	SD	H & A[a]	251.06	156	110	14	3.436
							92	110	26	
	Sulfamerazine	$C_{11}H_{12}N_4O_2S$	264.3	SDMD	H & A	265.08	155.9	126	14	5.123
							92.1	126	34	
	Sulfamethazine	$C_{12}H_{14}N_4O_2S$	278.33	SM2	H & A	279.09	186	138	18	7.068
							92	138	34	
	Sulfamethoxadiazole	$C_9H_{10}N_4O_2S_2$	270.33	SMT	H & A	271.03	156	118	14	7.414
							92	118	34	
	Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28	SMX	H & A	254.06	155.9	86	10	9.624
							92.1	86	26	
	Sulfamethoxine	$C_{12}H_{14}N_4O_4S$	310.36	SDM	H & A	311.08	156	134	20	15.719
							92.1	134	36	
Quinolones	Mabofloxacin	$C_{17}H_{19}FN_4O_4$	362.36	MAR	Animal	363.37	320	122	14	7.461
							72.2	122	20	
	Enrofloxacin	$C_{19}H_{22}FN_3O_3$	359.4	ENR	Animal	360.41	342.1	128	18	10.672
							316.1	128	18	
	Danofloxacin mesylate	$C_{20}H_{24}FN_3O_6S$	453.5	DAM	Animal	358.51	340.1	132	22	10.988
							82.1	132	46	
	Difloxacin	$C_{21}H_{19}F_2N_3O_3{\cdot}HCl$	438.85	DFLX	Animal	400.31	382.1	132	22	11.413
							356.1	132	18	
	Ofloxacin	$C_{18}H_{20}FN_3O_4$	361.37	OFL	H & A	362.38	318.1	138	18	8.78
							261.1	138	26	
	Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.33	NOR	H & A	320.31	302.1	108	22	9.415
							276.1	108	14	
	Lomefloxacin hydrochloride	$C_{17}H_{19}F_2N_3O_3{\cdot}HCl$	387.81	LOM	H & A	352.51	314	129	42	10.817
							231	129	42	
	Nalidixic acid	$C_{14}H_{12}FNO_3$	261.25	NAL	H & A	233.25	215.1	96	13	21.115
							187	96	25	
	Flumequine	$C_{12}H_{12}N_2O_3$	232.24	FLU	Animal	262.26	244.1	98	18	22.458
							202	98	34	
	Ciprofloxacin hydrochloride	$C_{17}H_{18}FN_3O_3{\cdot}HCl$	367.8	CIP	H & A	332.51	314	129	18	10.073
							231	129	42	

Table 1 (continued)

Category	Compound	Molecular formula	Molecular weight	Abbreviation	Scope of use	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (V)	Retention time (min)
Macrolides	(7S)-Lincomycin	$C_{18}H_{34}N_2O_6S$	406.54	LIN	H & A	407.22	359.1	128	18	5.881
							126.1	138	30	
	Spiramycin	$C_{43}H_{74}N_2O_{14}$	842.51	SPM	H & A	843.52	174.1	204	42	16.705
							101	204	54	
	Tilmicaoxin	$C_{46}H_{80}N_2O_{13}$	869.2	TILM	Animal	869.61	174	108	48	20.974
							88.1	108	70	
	Tylosin	$C_{46}H_{77}NO_{17}$	916.1	TYL	Animal	916.53	174	238	44	26.159
							101	238	50	
	Leucomycin A5	$C_{39}H_{65}NO_{14}$	771.93	LM	H & A	772.4	174	146	32	26.162
							109	146	48	
	Anhydroerythromycin A	$C_{37}H_{65}NO_{12}$	715.91	ETM-H_2O	H & A	716.46	558.3	150	14	26.65
							158.1	132	30	
	Clarithromycin	$C_{38}H_{69}NO_{13}$	747.96	CTM	H & A	748.51	158.1	162	29	27.338
							83.1	156	60	
	Azithromycin dihydrate	$C_{38}H_{72}N_2O_{12}{\cdot}2H_2O$	784.98	AZM·$2H_2O$	H & A	749.41	591.3	130	30	27.337
							83.1	138	60	
	Roxithromycin	$C_{41}H_{76}N_2O_{15}$	837.1	ROM	H & A	837.51	679.4	158	18	27.565
							158.1	158	38	
	Clindamycin hydrochloride	$C_{18}H_{33}ClN_2O_5S{\cdot}HCl{\cdot}H_2O$	479.46	CLI	H & A	425.19	377.1	150	18	19.605
							126.1	150	34	

[a] *H&A* Human & animal

dried with N_2-blowdown in nitrogen blowpipe submerged at 45 °C water in the concentrator, and then diluted to 2 mL using MeOH-water (V/V = 1/1) containing 0.01% formic acid, filtered through 0.2-μm nylon syringe filters, and transferred to a 1.0-mL injection vial for subsequent UPLC-MS/MS analysis.

Liquid chromatography

LC separation was performed on an Agilent 1290 UHPLC coupled with an Agilent ZORBAX Eclipse Plus C18 (2.1 mm × 100 mm, 1.8 μm) column. The column was maintained at 40 °C, and the injection volume was 2.0 μL. A dual eluent mobile phase consisted of MeOH with 0.05% formic acid (A) and water with 0.1% acetic acid (B) at 0.300 mL min^{-1}. The suitable gradient procedures were set up as follows: 0–3 min, 90–85 % A; 3–24 min, 85–50 % A; 24–30 min, 50–10% A, and post-time was 5 min. The separation effect of target antibiotics standards is shown in Figure S2.

Mass spectrometry

Mass spectrometry was performed on an Agilent 6460 triple quadrupole mass spectrometer. Samples were analyzed in positive electron spray ionization (ESI+) with MRM mode. The ESI+ operating conditions of the source were as follows: collision nitrogen flow at 7 L min^{-1}, sheath nitrogen flow at 11 L min^{-1} at 350 °C, spray source pressure at 45 psi, desolvation temperature at 300 °C, and capillary voltage at 3500 V. In the scanning range of 150–1000 m/z, the first-order MS spectrum of target antibiotics was presented as precursor ions at $[M+H]^+$ mode. The optimized MS/MS product ions, collision voltage (V), fragmentation voltage (V), retention time (Table 1), and the mass spectrum of each analyte are shown in Figure S3 (a-z).

Method validation

The analytical method was evaluated through the estimation of the linearity, (absolute) recoveries, instrumental limits of detection (LOD) and LOQ, precision expressed as repeatability in terms of the relative standard deviation (RSD), and matrix effects (MEs) of the SW and WWE samples.

Linearity was verified by establishing matrix-matched calibration (MMC) curves (fitted as Eq. (S1)) made using the peak area ratio of seven standards at 2-100 μg L^{-1} in relation to IS at 50 or 100 μg L^{-1}, which can compensate the matrix effects that influence the analytical response (Monteiro et al. 2016). Notably, standards of 5-100 μg L^{-1} were set when the LOQ was higher than 2 ug L^{-1}. The corresponding coefficient of determination (R^2) was > 0.990 and usually exceeded 0.995 for quantification (Ho et al. 2012). Fit parameters, standard deviations, and 95% confidence intervals were obtained using origin (origin 2018).

The instrumental LOD and LOQ were defined as the lowest concentrations with an analyte signal to noise ratio (SNR) equal to or greater than 3 and 10, respectively, using the MMC curves (Mokh et al. 2017; Zhi et al. 2020). The absolute recoveries were calculated by comparing the peak areas of each analyte obtained in the matrix samples followed by SPE and the standard followed by direct chromatographic injection mode in five replicates (Eq. (S2)). For each batch of sample analysis, one relevant blank matrix was spiked with ISs (50 ug L^{-1}) as quality control (QC) sample to check for the possible background of the analytes from the matrix samples.

The precision of the method was verified using the intra-day (in five hours) and inter-day (five days) reproducibility expressed by RSD through analyzing five replicate injections of a 100 μg L^{-1} standard by means of a one-way ANOVA.

The matrix spike recoveries calculated in PW, SW, and WWE represented the isotope corrected recoveries (Eq. (S3)) while absolute recoveries were usually lower (Anumol &Snyder 2015). The concentrations of target analytes and ISs were set at 5 and 50 μg L^{-1}. The ME value indicated the enhancement or reduction of the antibiotics signal in SW and WWE, which was calculated by comparing the difference between the peak areas obtained in the standard and those in matrices with the peak areas obtained in the standard at 50 ng L^{-1}, using Eq. (S4).

Quality control procedures were carried out, using batch samples, blank solvents, blank samples, and new MMC curves to obtain matrix sample concentrations, to evaluate whether there was carryover or background contamination, and to verify the performance of the method. Each analyte retained the same retention time as the corresponding calibration standard within 5%, and the same ion ratio < 20%.

Results and discussion

SPE optimization

Selection of SPE cartridges/disks

The extraction and elution effect of analytes were affected by SPE cartridge/disk type, eluent, elution procedure, sample pH, etc., with the cartridge/disk type being the primary factor. The extraction efficiency of SPE cartridges/disks depends on its ability to retain target analytes from the aqueous phase and the ability to elute the analytes using the organic phase. C18 is the most widely used material for SPE, which can adsorb a variety of organic substances in the range of weak to moderate polarity; Florisil cartridges can extract polar compounds from non-polar solutions; hydrophilic-esterophilic HLB can retain acidic, basic, and neutral compounds in a wide range of polarities

(from polar to non-polar), and has a good enrichment effect on polar compounds in particular (Hennion 1999, Thurman and Mills 1998). In this study, six kinds of cartridges/disks were investigated to identify their enrichment and elution effects on target analytes. The absolute recoveries were calculated (Eq. (S2)) for SPE of 1.0 L PW spiked with analytes at 100 μg L^{-1}. A total of 40 mL of elution solvent (MeOH-acetone (V/V = 1/1)) was used for the elution of these analytes.

Figure 1 (a) shows the range of absolute recoveries obtained for 26 target analytes tested with each cartridge/disk. A recovery between 70 and 130% was considered acceptable and used as the criteria for cartridge selection (Anumol &Snyder 2015). The C18 and HLB disk had 10 analytes in this range and good reproducibility (RSD < 10%, n = 5, Table S3), followed by five for HLB cartridge, three for C18 cartridge (IST), one for C18 cartridge (Lab tech), and even zero for Florisil cartridge. Meanwhile, only HLB disk was able to extract all 26 analytes (recoveries > 0%), followed by the HLB cartridge and C18 disk with 24, the C18 cartridge (IST) with 23, and the C18 cartridge (Lab tech) and Florisil cartridge with 15 (Table S3). The HLB disk showed the best recovery for most analytes since it can enrich and elute all analytes, regardless of its polarity or it being acidic, basic, or neutral. Meanwhile, the C18 disk performed well for the sulfonamide antibiotics. The HLB cartridge and C18 cartridge (IST) eluted most antibiotics, but the elution efficiency was not as good as HLB disk since the numbers of analytes that were not recovered or had a recovery > 130% was ≥ 4 (Figure 1(a)). The poor recoveries of the C18 cartridge (LabTech) and the Florisil cartridge were due to their poor elution effect with a solvent of MeOH-acetone. Thus, HLB disk was the most suitable choice. Notably, the recovery was greater than 130% for FLU using C18 disk, FLU and TYL using HLB cartridge, and FLU, SDM, and TYL using C18 cartridge (IST) (Table S3). This may be attributed to carryover from one injection to the next (Anumol and Snyder 2015).

Significant differences in antibiotic physicochemical properties, usually having acidic or alkaline functionalities or being protonated/deprotonated, will affect the retention and elution capability of antibiotics in the SPE procedure (Mol et al. 2008). For the HLB disk SPE, the recoveries of sulfonamides were 60.2–84.2%, higher than most quinolones (e.g., ENR, DAM DFLX, OFL, LOM, NAL, and CIP), probably because these quinolones with the pKa at 5.45-7.79 are in protonated (cationic) state in acid conditions, and their polar is relatively lower, which is relatively adverse to HLB enrichment, but HLB has a better enrichment effect on polar or neutral compounds. For some macrolides (e.g., LIN, and TILM) with relative lower recoveries, it may be due to their relatively large molecular weight and high non-polarity, or they are basic compounds in cationic state, which were not conducive to enrich in HLB under acidic conditions.

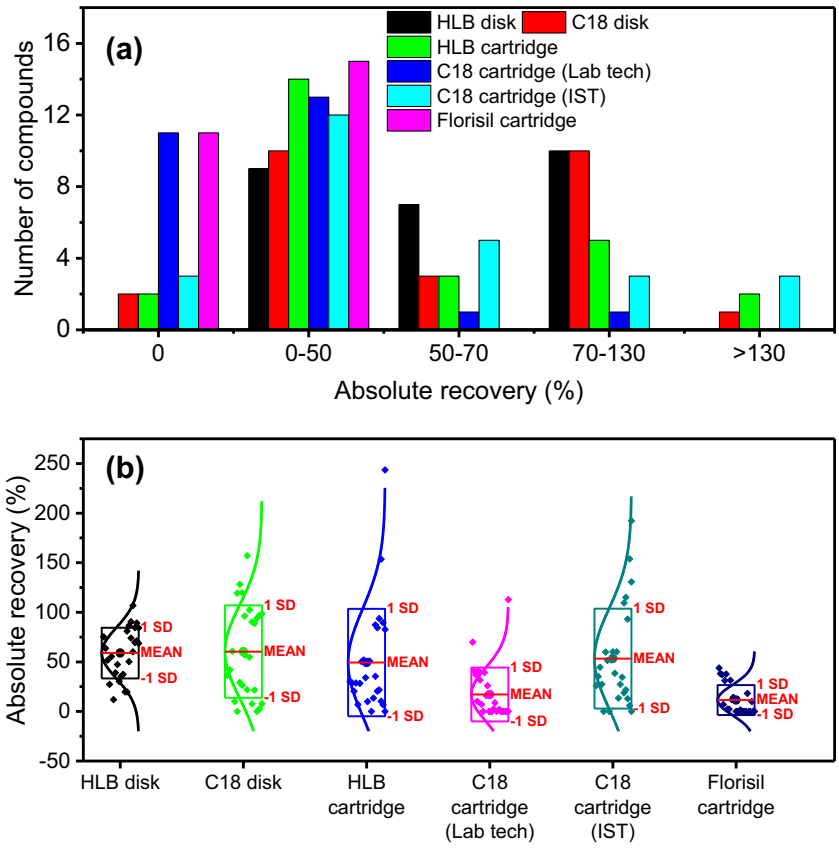

Fig. 1 (a) Range of absolute recoveries and (b) box plot of absolute recoveries for target analytes with 6 different SPE cartridges/disks. (Analytes of 100 μg L^{-1} were set; elution solvent: MeOH-acetone (V/V = 1/1), n = 5)

Furthermore, Figure 1 (b) shows the distribution of absolute recoveries, for which the total mean value and standard deviation (SD) were calculated. The absolute recoveries for the HLB disk had a relatively concentrated distribution with the maximum mean at 58.9%. The C18 disk, HLB cartridge, and C18 had acceptable mean values, while the distribution was relatively dispersed with a SD of 46.6, 54.1, and 50.3%, respectively. The C18 cartridge (Lab tech) and Florisil cartridge had a smaller SD while the mean recoveries were quite low with values at 17.2% and 11.5%, respectively. It is always desirable to obtain the maximum recoveries with the best sensitivity but with respect to a highly diverse analyte list trade-offs are inevitable. Therefore, the HLB disk was selected for subsequent analyses.

Selection of the eluent and the optimum volume using HLB disk

The MeOH, acetone, or DCM are the most widely used elution solvents for solid-phase extraction of antibiotics based on similar physicochemical polarity (from large to small) (Behera et al. 2011; Ghosh et al. 2009). In this paper, MeOH, acetone, MeOH-DCM (V/V = 1/1), MeOH-acetone (V/V = 1/1), and DCM with a 6-mL volume were selected as elution solvents to investigate, and the optimal solvent for each analyte was shown in Table S4 (recoveries not shown). The number of compounds that were eluted with the optimal tested solvent is shown in Figure 2 (a). MeOH and acetone exhibited better elution results for eight analytes, followed by that of DCM (four analytes), MeOH-DCM (three analytes), and MeOH-acetone (three analytes). Thus, both MeOH and acetone were selected as elution solvents for target antibiotics. Accordingly, the effect of the eluent's volume (6 mL × 3, × 4, and × 5) on the elution effect was studied (recoveries not shown), and the results are summarized in Table S5 and Figure 2 (b). The maximum number of analytes with the optimal elution occurred when the volume was set at 4 × 6 mL for both MeOH and acetone, while a smaller elution volume (3 × 6 mL) could not completely elute all of the targeted antibiotics, and an excessive volume (5 × 6 mL) lead to the loss of recovery of some antibiotics, especially for sulfonamides (Table S5). To sum up, 2 × 6 mL of MeOH was selected as the main eluent with the supplement eluent of 2 × 6 mL acetone.

Determination of pH, flow rate, and volume of water samples and EDTA-2Na addition

The various pH values of water samples may result in different recoveries for the analytes. The extraction of antibiotic from the water matrix is usually performed under acidic conditions because antibiotics usually presenting as acidic or alkaline compounds could interact with the matrix, while this could

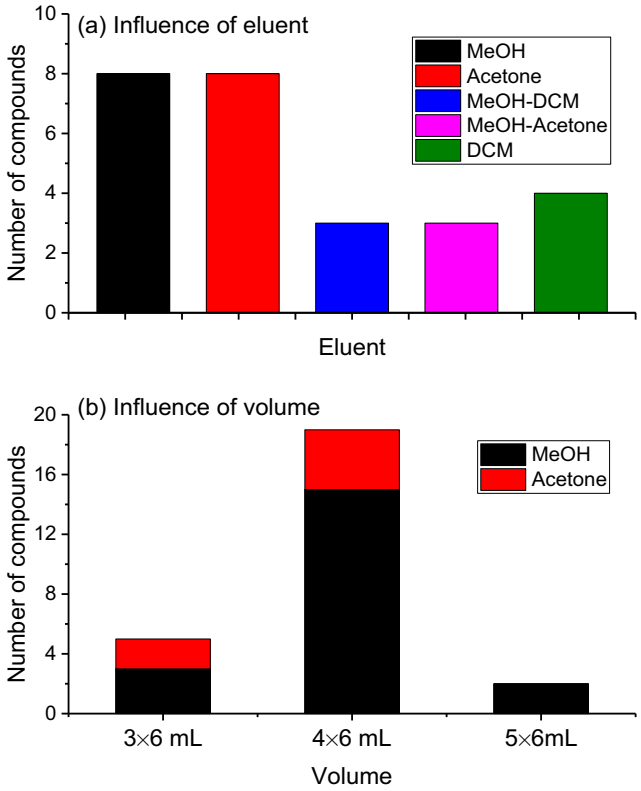

Fig. 2 Numbers of compounds with optimal recovery (closest to 100%) using (a) different eluents (volume was set at 6 mL), and (b) different volumes of MeOH and acetone (each volume was 6 mL). (Analytes of 100 μg L^{-1} were set)

be avoided by extracting at low pH (Englert 2007; Mol et al. 2008). At neutral conditions, acidic compounds are deprotonated and could interact with (protonated) matrix amino functionalities while basic compounds could interact with deprotonated matrix acidic functionalities, thus reducing the extraction efficiency (Mol et al. 2008). At basic conditions, the antibiotics may undergo hydrolytic degradation or the H^+ dissociation degree of the amine moiety of antibiotics will increase, leading to a lower extraction efficiency than that under neutral conditions (Kim et al. 2018; Mol et al. 2008). However, under the condition of low pH, acidic groups are protonated and in their molecular state, while basic functionalities are either neutral or protonated. Thus, the analytes may remain dissolved in the solution without linking to the matrix component (Mol et al. 2008). In this paper, the pH of 1.0 L of PW was adjusted from an initial 6.8 to a final 3.0, 4.0, and 5.0 using H_2SO_4 (Figure S4), (recoveries not shown). The results reveal that acidic sulfonamides and quinolones had higher recoveries under the condition of pH = 3; however, some macrolides had lower but acceptable recoveries, probably because they are weak basic compounds, and were in cationic state, not the most conducive to adsorption on HLB disk, with examples of SPM, CTM, and, AZM·$2H_2O$ (Drugbank 2021, Wishart et al. 2018). Therefore, when trying to achieve

acceptable recovery for all 26 antibiotics, it is recommended the water samples to be acidified to a pH of 3.0 before performing SPE. Pervious study also reported the pH value at 2.5-4 was the most suitable sample pH for simultaneous extraction of multiple antibiotic residues from aqueous matrices by SPE (Kim et al. 2018).

According to the instructions for the HLB disk provided by the manufacturer, the flow rate of the water sample through this sorbent should not exceed 5 mL min^{-1}. However, previous literatures (Monteiro et al. 2016; Zhi et al. 2020) have shown that this can vary depending on the various characteristics of the water samples. For example, water samples with complex matrices such as STP samples should have a lower sample loading speed. Thus, the flow rate through the disk in this experiment was controlled at 2~5 mL min^{-1} in this study.

When analyzing antibiotics in water matrices, the sample must be reasonably concentrated during pretreatment while removing the interfering substances as much as possible. The concentration factor was limited by the breakthrough volume of the SPE cartridge/disk, i.e., the maximum loading volume that can be percolated through the SPE cartridge/disk without losing the analytes. Considering the concentration of analytes and the influence of impurities in actual waters, the ideal loading volume was determined to be 1000 mL of water sample for PW, and surface water, and 500 mL for WWE.

Metal ions that are naturally present in certain water sample may form antibiotic-metal complexes with antibiotics (Aristilde and Sposito 2008; Cuprys et al. 2018, Pulicharla et al. 2017), which results in a reduction in the recoveries of target analytes. Antibiotics with more electron-rich groups containing N and O may lead to a stronger complexation with metal ions. Macrolides owned the strongest complexing ability, followed by quinolones and sulfonamides. This is because macrolides are more complex in structure and usually have 12–16 carbon lactone rings, while quinolones contain -COOH and -C=O, as well as piperazine groups, and sulfonamides containing N-functional groups are involved in the coordination. Considering the water sample matrix and published literatures (Mokh et al. 2017; Zhi et al. 2020), EDTA-2Na at 1.0 g L^{-1} was added to water samples to reduce metal ion interference in water, while the amount of EDTA-2Na will be increased for metal polluted sample to ensure the removal of metal ion interference.

Figures of merit

In this study, none of target analytes was detected in the blanks through detection of blanks (PW) with and without isotopically labeled standards. Good linearity was found for 26 antibiotics by analyzing the concentrations calibration curves of standards (2-100 μgL^{-1}) except for SM2 (5-100 μgL^{-1}) with all analytes having an $R^2 > 0.995$. The calculated instrumental LODs (SNR >3) and LOQs (SNR >10) for the 26 analytes ranged from 0.01-0.72 μg L^{-1} and 0.05-2.39 μg L^{-1}, respectively, which was comparable to those reported in a previous study using the same calculation method (Mokh et al. 2017). Detailed linearity, LODs, and LOQs are summarized in Table 2. The precision of the analytical method was verified using the intra-day and inter-day reproducibility, calculated as RSD (%) which were determined by analyzing five replicates of a 100 μg L^{-1} standard. The intra-day RSD ranged from 1.0 to 9.9% and the inter-day RSD ranged from 1.5 to 8.7%, indicating a good repeatability and reliability of the method.

Matrix spike recoveries and matrix effect assessment

The viability of the analytical method was verified by the recovery of analytes spiked in water samples from three different matrices (PW, SW, and WWE). Water samples were spiked with a mix of analytical standards (5 or 50 μg L^{-1}) and ISs (50 μg L^{-1}), followed by SPE pretreatment. The matrix spike recoveries (Eq. (S3)) and ME (Eq. (S4)) were calculated and shown in Table S6 and Figure 3. The matrix spike recoveries of analytes in PW were acceptable (70-130%) (Anumol and Snyder 2015)), with percentage at 77.5-101.8% for analytes of 5 μg L^{-1} and 78.2-104.9% for analytes of 50 μg L^{-1} (Figure 3(A)). The reproducibility (RSD) was ≤ 10% except for CIP with RSDs of 10.4% at 10 μg L^{-1} and 10.7% at 50 μg L^{-1} (Table S6). In SW, the recoveries ranged from 59.4 to 89.5 % for analytes of 5 μg L^{-1} and 77.0-97.8% for analytes of 50 μg L^{-1}, with five compounds < 70% being only at 5 μg L^{-1} (Figure 3(A)). The reproducibility for all compounds at both 5 and 50 μg L^{-1} was good with respect to RSDs < 10.7% (Table S6). For the WWE samples, the matrix spike recoveries were within 58.2-108.6% and 67.5-104.5% for low and high concentrations of analytes with seven and four compounds outside the acceptable range, respectively (Figure 3(A)), and meanwhile, the corresponding RSDs were ≤ 12.8% and ≤ 10.8% (Table S6). The recoveries for all analytes obtained in this paper were within EPA 1694 (Englert 2007) range of 5–200% for water matrices. This wide range is due to the special structure of antibiotics and their physicochemical properties. A previous study reported that the recoveries for tetracyclines and quinolones were 88–112% in river water samples, 41–87% in well water samples, and 23–103% in wastewater influent and effluent using SPE-LC-MS/MS (Reverté et al. 2003). The recoveries for 63 antibiotics in PW were in the range of 70.7-133.9% (Mokh et al. 2017). Furthermore, the ME had an insignificant influence on the RSD as most compounds had a similar RSD < 10% (Table S6), which was also previously reported (Mokh et al. 2017).

The ME caused by interferences of co-eluting constituents in the matrix can lead to a loss of sensitivity and reproducibility for trace quantification of analytes using ESI-LC–MS/MS (Anumol and Snyder 2015). The MEs for target analytes in

Table 2 Linear equation and the corresponding stand errors, 95% confidence intervals, and correlation coefficients, instrumental LODs (SNR > 3) and LOQs (SNR > 10), and precision of the analytical method

Category	Compound	Linear equation	Standard error of linear fit		95% confidence interval		Correlation coefficients (R^2)	LOD (μg L^{-1})	LOQ (μg L^{-1})	Intra-day variability (n = 5) RSD (%)	Inter-day variability (n = 5) RSD (%)
			Slope	Intercept	Slope	Intercept					
Sulfonamides	SD	y = 0.0078x+0.0042	0.00014	0.00778	0.00076	0.04321	0.9988	0.04	0.14	5.6	7.4
	SDMD	y = 0.0074x+0.0022	0.00022	0.01242	0.00122	0.06898	0.9965	0.32	1.08	3.9	4.0
	SM2	y = 0.01x-0.0038	0.00019	0.01091	0.00106	0.06059	0.9985	0.72	2.39	5.3	5.0
	SMT	y = 0.0068x-0.0057	0.00014	0.00788	0.00077	0.04375	0.9983	0.38	1.26	2.0	3.3
	SMX	y = 0.0238x-0.0104	0.00035	0.02012	0.00197	0.11172	0.9991	0.15	0.49	1.9	5.0
	SDM	y = 0.0758x+0.0338	0.00157	0.08934	0.00872	0.49608	0.9983	0.03	0.09	5.2	2.5
Quinolones	MAR	y = 0.0259x-0.0103	0.00028	0.01593	0.00155	0.08846	0.9995	0.04	0.12	2.4	2.2
	ENR	y = 0.0215x+0.0055	0.00027	0.01520	0.00149	0.08438	0.9994	0.09	0.30	3.3	3.1
	DAM	y = 0.0043x-0.0117	0.00016	0.00886	0.00087	0.04919	0.9958	0.10	0.33	2.1	1.5
	DFLX	y = 0.0136x-0.0068	0.00009	0.00496	0.00049	0.02754	0.9998	0.05	0.18	5.9	8.7
	OFL	y = 0.0234x-0.0022	0.00034	0.01931	0.00189	0.10723	0.9992	0.05	0.15	1.9	2.7
	NOR	y = 0.0033x-0.0044	0.00013	0.00754	0.00074	0.04185	0.9955	0.59	1.98	2.4	3.6
	LOM	y = 0.008x-0.0041	0.00009	0.00513	0.0005	0.02848	0.9995	0.09	0.31	7.6	6.3
	NAL	y = 0.0229x+0.8748	0.00052	0.02961	0.00289	0.16442	0.9979	0.08	0.27	2.1	2.0
	FLU	y = 0.0123x-0.0043	0.00013	0.00749	0.00073	0.04159	0.9995	0.16	0.54	3.3	4.1
	CIP	y = 0.014x-0.0224	0.00036	0.02036	0.00199	0.11305	0.9974	0.69	2.29	5.6	6.5
Macrolides	LIN	y = 0.035x+0.0092	0.00044	0.02481	0.00242	0.13776	0.9994	0.01	0.04	3.4	3.8
	SPM	y = 0.0008x-0.0001	0.00001	0.00076	0.00007	0.00422	0.9990	0.13	0.43	1.0	1.7
	TILM	y = 0.0043x+0.0002	0.00007	0.00425	0.00042	0.02361	0.9988	0.09	0.29	3.5	1.8
	TYL	y = 0.0036x-0.0017	0.00005	0.00264	0.00026	0.01466	0.9994	0.07	0.23	3.3	4.5
	LM	y = 0.0035x-0.0038	0.00012	0.00706	0.00069	0.03919	0.9950	0.15	0.50	2.8	4.5
	ETM-H_2O	y = 0.01881x-0.001	0.00017	0.00948	0.00093	0.05262	0.9998	0.03	0.10	3.3	1.7
	CTM	y = 0.0460x+0.004	0.00025	0.01443	0.00141	0.08014	0.9998	0.01	0.04	3.3	3.0
	AZM·$2H_2O$	y = 0.0071x+0.0004	0.00034	0.01941	0.00190	0.10778	0.9995	0.07	0.22	9.9	3.7
	ROM	y = 0.0161x+0.0026	0.00017	0.00962	0.00094	0.05340	0.9994	0.02	0.05	1.9	3.5
	CLI	y = 0.1006x+0.0226	0.00093	0.05288	0.00516	0.29361	0.9997	0.02	0.05	1.6	1.7

LOD, limit of detection; *LOQ*, limit of quantitation; *RSD*, relative standard deviations. Standards of 2-100 μg L^{-1} were set for linearity evaluated, except for SM2 using standards of 5-100 μg L^{-1}. Standards of 100 μg L^{-1} were set for intra-day and inter-day reproducibility evaluated

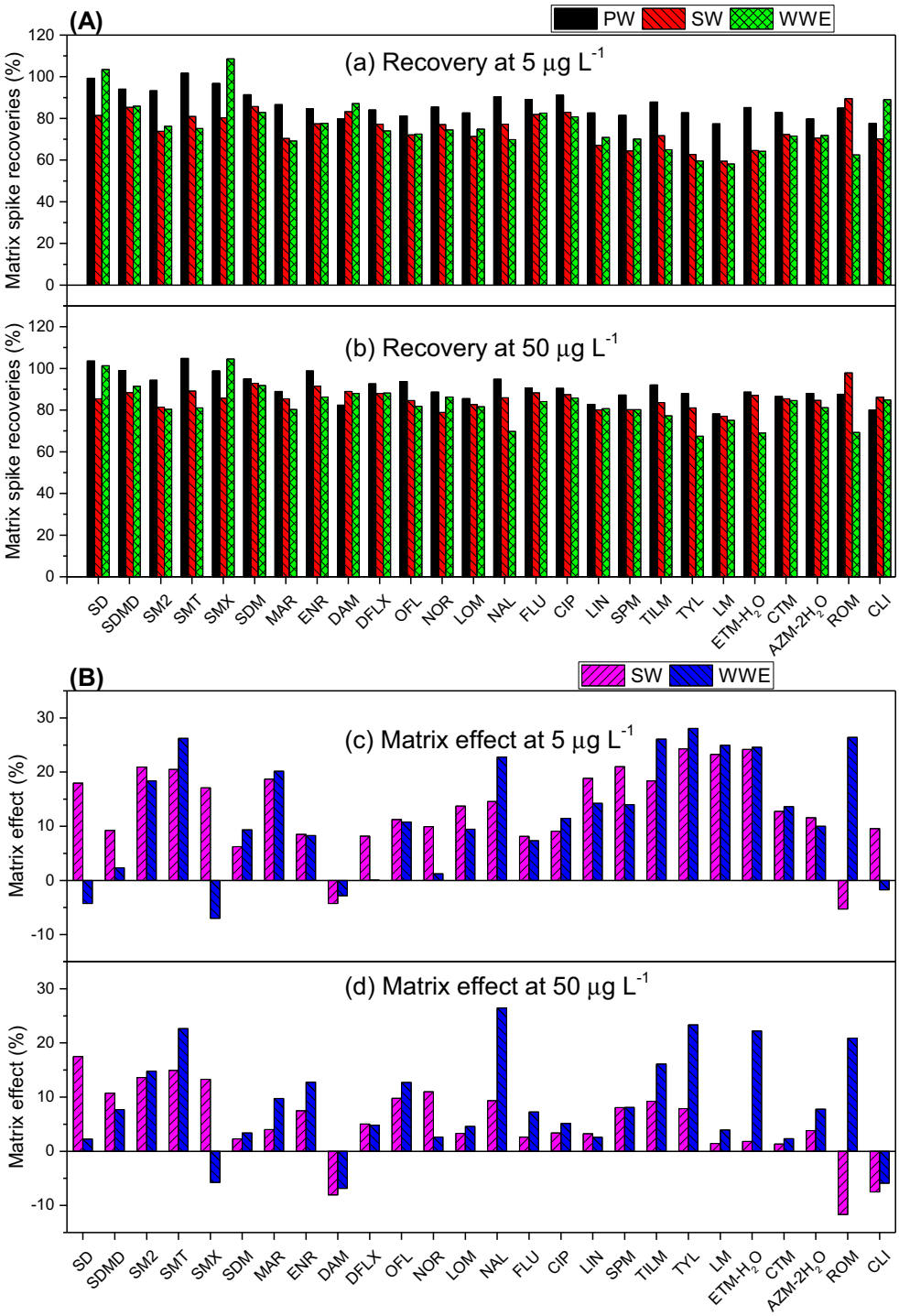

Fig. 3 (A) Matrix spike recoveries, and (B) matrix effect (%) in pure water (PW), surface water (SW), and wastewater effluent (WWE) with target analytes setting at 5 and 50 μg L^{-1}. (The isotopically labeled surrogates (ISs) were set at 50 μg L^{-1})

SW and WWE at both 5 and 50 μg L^{-1}(Figure 3(B) and Table S6) indicate that all analytes were affected by suppression or enhancement. The suppression of 6.2-24.3% and 1.3-17.5% for 5 and 50 μg L^{-1} analytes was separately observed for most analytes in SW, with the exception of DAM, ROM, and CLI, which showed slight enhancement of 4.3-8.1%, 5.3-11.7%, and 7.5% (only 50 μg L^{-1}), respectively (Figure 3(B)). The MEs in WWE were comparable to those in SW, where most compounds had a suppression of 7.3-28.1% and 2.2-26.5%, whereas slight enhancements were observed for 5 μg L^{-1} of SD, SMX, DAM, and CLI at 4.3-14.6%, and 50 μg L^{-1} of SMX, DAM, and CLI at 5.8-6.9% (Figure 3(B)). Moreover, the magnitude of MEs was vastly different; for instance, SDM had < 9.3% ME in the two different water qualities at the two spiking concentrations whereas SMT experienced much stronger suppression in all water qualities (14.9-26..2% in

Table 3 Occurrence and concentration levels of antibiotics in different water matrices (ng L^{-1}) (n = 5)

Category	Compound	WWEs		SWs		
		WWE 1	WWE 2	SW1	SW2	SW3
Sulfonamides	SD	4.54 ± 0.09	6.15 ± 0.00	3.79 ± 0.99	7.10 ± 1.44	5.41 ± 0.14
	SDMD	8.29 ± 1.25	4.20 ± 0.68	1.59 ± 0.72	2.45 ± 1.19	1.63 ± 0.59
	SM2	LLQ	LLQ	2.30 ± 1.05	6.81 ± 0.62	6.02 ± 0.86
	SMT	8.86 ± 0.11	8.82 ± 0.09	4.33 ± 0.02	6.82 ± 2.95	4.36 ± 0.37
	SMX	4.00 ± 0.32	29.67 ± 1.96	8.20 ± 1.60	11.30 ± 0.46	11.68 ± 0.51
	SDM	LLQ	LLQ	LLQ	LLQ	LLQ
Quinolones	MAR	4.97 ± 0.96	2.73 ± 1.09	1.43 ± 0.25	1.60 ± 0.02	1.40 ± 0.35
	ENR	3.97 ± 0.61	0.46 ± 0.66	LLQ	LLQ	LLQ
	DAM	LLQ	18.57 ± 7.30	2.09 ± 0.26	13.81 ± 0.86	1.40 ± 0.73
	DFLX	6.49 ± 1.20	3.74 ± 0.89	2.36 ± 0.33	2.99 ± 0.41	2.15 ± 0.45
	OFL	60.52 ± 4.13	94.47 ± 7.82	9.08 ± 0.94	3.97 ± 0.72	3.20 ± 0.14
	NOR	31.37 ± 9.81	37.62 ± 7.52	9.06 ± 1.04	13.95 ± 0.78	15.47 ± 0.87
	LOM	LLQ	8.81 ± 2.46	6.05 ± 0.64	2.78 ± 0.94	6.55 ± 0.71
	NAL	4.00 ± 1.25	5.51 ± 1.18	2.54 ± 0.31	2.53 ± 0.66	2.77 ± 0.85
	FLU	4.79 ± 0.59	4.52 ± 0.59	2.49 ± 0.57	3.21 ± 1.02	2.83 ± 0.78
	CIP	9.85 ± 0.64	5.89 ± 0.05	2.19 ± 0.76	4.27 ± 0.12	4.26 ± 0.73
Macrolides	LIN	14.20 ± 1.08	19.31 ± 5.25	0.94 ± 0.42	1.56 ± 0.56	1.18 ± 0.58
	SPM	4.86 ± 0.87	LLQ	LLQ	LLQ	LLQ
	TILM	0.12 ± 0.05	0.08 ± 0.12	LLQ	LLQ	0.03 ± 0.04
	TYL	2.98 ± 0.22	LLQ	LLQ	LLQ	0.99 ± 0.71
	LM	4.63 ± 0.02	4.52 ± 0.04	LLQ	LLQ	2.28 ± 0.67
	ETM-H_2O	52.35 ± 6.22	41.32 ± 1.30	5.58 ± 0.56	6.37 ± 0.33	6.26 ± 0.44
	CTM	23.08 ± 1.30	27.02 ± 1.89	3.75 ± 0.93	3.89 ± 0.64	3.48 ± 0.30
	AZM·$2H_2O$	24.12 ± 1.36	28.07 ± 1.49	4.02 ± 1.01	4.39 ± 0.57	3.86 ± 0.43
	ROM	17.08 ± 2.39	18.26 ± 0.04	3.41 ± 0.97	3.49 ± 0.62	3.13 ± 0.36
	CLI	LLQ	29.94 ± 0.29	LLQ	LLQ	LLQ

LLQ below limit of quantitation (LOQ). Error bars represent ± one standard deviation

SW and WWE) (Table S6). Generally, the MEs were greater in more complex matrices with the average ME in the WWE (10.1 %), being slightly higher than SW (7.4%) at such as 5 μg L^{-1} (Table S6). The occurse suppression or enhancement for both low and high concentrations of analytes did little change, except for SD being slightly enhanced at low concentration but suppressed at high concentration. This phenomena of various MEs may be due to the matrix complexity of the SW and WWE (Zhi et al. 2020) and the dependence of ESI methods on matrix effects (Anumol and Snyder 2015).

Notably, both suppression and enhancement could be minimized using the analytical standards and internal standards addition method (Mokh et al. 2017). The matrix spike recoveries shown in Table S6 for the isotope corrected recoveries were usually fluctuated less than the absolute recoveries, e.g., 50 μg L^{-1} of analytes in Figure S5, which was also reported previously (Anumol and Snyder 2015).

Implementation of multi-residue analysis of environmental water samples

This validated method was applied for the multiresidue analysis of 26 antibiotics in WWE and SW samples from the scaled Yangtze River and Bailonggang STP, respectively, during the summer. Two types of WWE samples were collected from two different outlets of the Bailonggang STP (WWE1 and WWE2), and three types of SW samples were collected from the Yangtze River near STP outlets (SW1), 5 km upstream of SW1 (SW2), and 4 km downstream of SW1(SW3). Further details can be found in Table S2. As shown in Table 3, the concentrations of the target antibiotics detected in the WWE samples ranged from lower than LOQ (LLQ)-60.52 ng L^{-1} for WWE1 and LLQ-94.47 ng L^{-1} for WWE2, with 21 and 22 of the antibiotics quantitatively detected, respectively. The quinolones (LLQ-94.47 ng L^{-1}) were the most frequently detected group of antibiotics, where OFL

and NOR was detected at the highest concentration of 60.52-94.47 ng L^{-1} and 31.37-37.62 ng L^{-1} in the two WWE samples. A previous study also proved that the quinolones were the most frequently detected compounds in six STPs around the Pudong New Area of Shanghai, China. The concentrations of OFL and NOR were in the range of 8.84-246.76 ng L^{-1} and 17.16-66.53 ng L^{-1}, respectively (Pan et al. 2020). OFL and NOR were hardly removed by STP using traditional activated sludge method. Gao et al. (Gao et al. 2012) reported 36-130 ng L^{-1} of NOR and 58-75 ng L^{-1} of OFL in the WWEs using anaerobic/anoxic/oxic (A^2/O) activated sludge process, 45 ng L^{-1} of NOR and 72 ng L^{-1} of OFL using oxidation ditch (OD), 9.4 and 150 ng L^{-1} of OFL using anoxic/oxic (A/O) activated sludge process, and 56 ng L^{-1} of NOR and 120 ng L^{-1} of OFL using A^2/O combined cilium nutrient removal (CNR) technology. As to macrolides, over eight compounds were detected in the WWE samples, and ETM-H_2O was the highest concentration antibiotic detected (41.32-52.35 ng L^{-1}). ETM-H_2O was also widely detected in the USA (mean concentration, 76-110 ng L^{-1}) (Nelson et al. 2011), the UK (mean concentration, 696-1385 ng L^{-1}) (Kasprzyk-Hordern et al. 2009), Singapore (maximum concentration, 267.5-381 ng L^{-1}) (Tran et al. 2016), and other areas of China (mean concentration, 358-2980 ng L^{-1}) (Leung et al. 2012). Table 3 displays four of the six sulfonamides detected. SMX was detected at the highest concentration at 29.67 ng L^{-1} in WWE2. SMX has been detected in WWEs in many countries, e.g., with mean concentrations at 18-910 ng L^{-1} in the USA (Kostich et al. 2014; Nelson et al. 2011), 519 ng L^{-1} in Canada (Basiuk et al. 2017), and 153-3375 ng L^{-1} in France (Dinh et al. 2017).

There were 19 antibiotics detected in SW1 near the STP, 19 antibiotics detected 5 km upstream of Yangtze (SW2), and 22 detected 4 km downstream of Yangtze (SW3). Their concentrations ranged between LLQ-15.47 ng L^{-1}. The detected antibiotics with a higher concentration in SWs were SMX (8.20-11.68 ng L^{-1}) for sulfonamides, NOR (9.06-15.47 ng L^{-1}) for quinolones, and ETM-H_2O (5.58-6.37 ng L^{-1}) for macrolides, which is similar to those in the WWEs. However, the total concentrations of antibiotics in SWs (9.08-15.47 ng L^{-1}) were significantly lower than those in WWEs (60.52-94.47 ng L^{-1}). This is likely due to the unremoved antibiotics from the STPs being diluted upon entering the river. Moreover, the concentrations of antibiotics in river near the STP were not significantly higher than those upstream of the STP, as well as the downstream sampling location, which is probably due to the rapid dilution by large amounts of water from the river and the presence of background antibiotics from other sources. The frequently detected SMX (a sulfonamide), NOR and OFL (quinolones), and ETM-H_2O (a macrolide) have been reported in rivers in different countries (Danner et al. 2019; Kovalakova et al. 2020), with mean concentrations of four compounds up to such as 1209 ng L^{-1} in France (Dinh et al. 2017), 443 ng L^{-1} in the USA (Massey et al. 2010), and 172 ng L^{-1} in Lebanon (Mokh et al. 2017).

Conclusions

A method using automated SPE coupled to UHPLC–MS/MS under MRM mode was developed and validated for the analysis of 26 antibiotics in water matrices. The optimized sample preparation for the best extraction and effective elution included applying a HLB SPE disk, 500-1000 mL water sample (pH = 3) with a flow rate at 2~5 mL min^{-1}, and elution procedure (6 mL × 2 MeOH, and 6 mL × 2 acetone). The recoveries for all analytes at 10 and 50 μg L^{-1} were between 77.5-104.9% in PW, 59.4-97.8% in SW, and 58.2-108.6% in WWE with the corresponding reproducibility within 13%. The ME observed for most analytes was suppression (0-28.1%) with slight enhancement (0-14.6 %) in SW and WWE. Furthermore, the instrumental LODs and LOQs of analytes were < 1 μg L^{-1} and 2.39 μg L^{-1} respectively, with satisfactory linear calibration ($R^2 > 0.995$) and precision (RSD < 9.9 %). Water samples from WWEs and SWs were analyzed to evaluate the effectiveness of the proposed method. The concentrations of sulfonamides, quinolones, and macrolides ranged between LLQ-94.47 ng L^{-1} in WWEs and LLQ-15.47 ng L^{-1} in SWs. SMX, OFL, and ETM-H_2O were detected at the highest concentration for the three types of antibiotics analyzed in the WWEs, and SMX, NOR, and ETM-H_2O were present in the highest residual concentration among the tested antibiotics in the Yangtze River. The establishment and verification of this method enables the determination of antibiotic residues in actual water matrices for further research.

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Data availability All data generated or analyzed during this study are included in this article (and its supplementary material).

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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