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Direct injection liquid chromatography-tandem mass spectrometry as a sensitive and high-throughput method for the quantitative surveillance of antimicrobials in wastewater

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ABSTRACT

Environmental antimicrobial pollution and antimicrobial resistance pose a threat to environmental and human health. Wastewater analysis has been identified as a promising tool for antimicrobial monitoring and the back-estimation of antimicrobial consumption, but current pretreatment methods are tedious and complicated, limiting their scope for high-throughput analysis. A sensitive direct injection method for the quantification of 109 antimicrobials and their metabolites in wastewater samples was developed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method was validated for both wastewater influent and effluent in terms of specificity, calibration range, matrix effect, filtration loss, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Most analytes achieved calibration of $R^2 > 0.99$, and the calibration range was from 0.0002 to 150 μg L^{-1}. Recoveries ranged consistently between ~50 % and ~100 % and losses were attributed to sample filtration. Method LOQs were determined as low as 0.0003 μg L^{-1}, and acceptable accuracy (75 %–125 %) and precision (within 25 %) were achieved for >90 % of the analytes. The method was subsequently further assessed using wastewater of raw influent and treated effluent collected from 6 Australian wastewater treatment plants in 2021. In total, 37 analytes were detected in influent and 22 in effluent. Most of them could be quantified at concentrations ranging from 0.0053 to 160 μg L^{-1}, with benzalkonium chloride-C12, amoxicilloic acid, and cephalexin detected at the highest concentrations. The current study provides a straightforward analytical method for antimicrobial monitoring in wastewater with a fast and simple pretreatment procedure.

1. Introduction

The discovery and use of antimicrobials has brought incremental advances to the treatment of infections in modern medical practice. The selective pressures caused by the improper and excessive use of antimicrobials, however, accelerates the evolution and spread of antimicrobial resistance (AMR) among microorganisms, both in animals and the environment (Larsson and Flach, 2022; Palumbi, 2001). Globally, antimicrobial resistant bacteria (ARB) were the cause of 1.27 million deaths in 2019 (United Nations, 2022). If the current pattern of antimicrobial use prevails, then the number of deaths due to AMR will likely reach 10 million per year by 2050, on par with the death toll from cancer (United Nations, 2022). To combat AMR, key international actions are needed, including enhancing environmental governance, targeting AMR relevant pollutants, improving antimicrobials and AMR surveillance, and prioritizing financing and innovation (United Nations, 2022). Among these, antimicrobial surveillance is one of the key steps in this campaign. Traditional methods of antimicrobial surveillance are based on market surveys, sales, or prescription data (Goossens et al., 2005; Van Boeckel et al., 2014; Zhang et al., 2015). However, for a variety of reasons (e.g., inaccurate, or incomplete data), these approaches make surveillance challenging and may not reflect the actual antimicrobials

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discharged into the environment.

Like other pharmaceuticals, antimicrobials are largely discharged as parent drugs or their metabolites through sewage systems to the environment. This renders wastewater treatment plants (WWTPs) hotspots for investigating both drug use and monitoring environmental releases. Over the past decade, wastewater-based epidemiology (WBE) has increasingly demonstrated its strengths in objectively estimating drug consumption, predicting disease outbreaks, and tracking AMR at the community scale (Ahmed et al., 2020; Bade et al., 2019; Prieto Riquelme et al., 2022). Hitherto, WBE has been widely applied to estimate the use patterns of licit and illicit drugs, and to assess human exposure to industrial chemicals such as pesticides and plasticizers (Ahmed et al., 2021; Gonzalez-Marino et al., 2017; Rousis et al., 2017; Senta et al., 2015; Xu et al., 2017). The occurrence of antimicrobials in wastewater has been quantified for several decades (Zhang and Li, 2011), however, their use as WBE biomarkers to estimate antimicrobial consumption is relatively recent (Gao et al., 2022; Han et al., 2022; Holton et al., 2022a; Yuan et al., 2019). Most of these studies relied upon solid phase extraction (SPE) prior to chemical analysis, but considering that antimicrobials as a group encompass a wide array of structurally different chemicals and chemical properties (Gothwal and Shashidhar, 2015), conventional SPE methods are unsuitable for all analytes and a sensitive but high-throughput method, such as direct injection, is preferred.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has become a sensitive and promising tool for the multi-residue detection of antimicrobials. Most methods published to date for quantifying antimicrobials in wastewater have used a pretreatment procedure for sample clean-up and target analyte concentration, typically SPE, to decrease the matrix effects and improve the limits of detection (LODs) (Han et al., 2021; Holton and Kasprzyk-Hordern, 2021). SPE methods are however harder to automate, have higher cost, use larger volumes of sample and hence are less suitable as high-throughput methods. In addition, many antimicrobials are unstable in solution (Fawaz et al., 2021; Samara et al., 2017), and SPE requires lengthy extraction of samples, often at room temperature, which may increase the uncertainty of analysis (Lin et al., 2021). At present, over 200 unique chemicals are used as antimicrobials in human and veterinary medicine (WHO, 2021). Owing to the distinctions of physicochemical properties of these antimicrobial groups, extracting a larger number of antimicrobials in a single SPE method remains an analytical challenge. By contrast, direct injection only needs straightforward pretreatment procedures, such as filtration, centrifugation, or dilution, which would be fast, economic, and effective. Consequently, if suitable detection limits can be achieved, it would be a preferred high-throughput method.

There are few publications on the analysis of antimicrobials in wastewater by direct injection. Denadai and Cass (2015) determined 6 fluoroquinolones in superficial and wastewater samples by direct injection with a large injection volume (500 μL). Vosough et al. (2015) reported a direct injection method for 6 antibiotics by using filtered influent and effluent wastewater samples. Campos-Manas et al. (2017) and Ng et al. (2020)'s quantitative methods enabled simultaneous determination of 87 and 135 organic contaminants in wastewater, which included 18 and 14 antibiotics, respectively, involving a lower injection volume (10 μL). Nevertheless, the limited number of antimicrobials does not meet the requirements of determining a broad range of antimicrobials. Voigt et al. (2020) reported a multi-residue method for the determination of 47 different antibiotics in filtered and diluted aqueous matrices. However, the indispensable raw wastewater (influent) in WBE was not considered in this method, and most antimicrobial metabolites, as crucial biomarkers widely used in WBE (Han et al., 2022; Holton et al., 2022b), were not involved. Therefore, a more effective and comprehensive analytical method for wastewater is required to meet increasing antimicrobial surveillance needs.

For the improved application of wastewater surveillance tools to assess antimicrobial use and monitor environmental releases, the present study aims to 1) establish a fast and high-throughput direct injection LC-MS/MS method for the determination of a broad spectrum of antimicrobials, including traditional antibiotics, last-resort antibiotics, human and veterinary antibiotics, antifungals, disinfectants, and their metabolites using straightforward sample pretreatment procedures, and 2) test its applicability by applying the method to both influent and effluent wastewater samples collected from 6 WWTPs in Australia.

2. Materials and methods

2.1. Chemicals and reagents

A wide range of antimicrobials and their metabolites belonging to 21 classes for both human and veterinary use were selected. Overall, they consisted of a total of 109 analytes and 26 stable isotope-labeled internal standards (IS) including traditional antibiotics, last-resort antibiotics, antifungals, and disinfectants. The detailed information is collated in Table S1. Stock solutions of all analytes and IS were prepared at 1000 mg L^{-1} in methanol (MeOH, HPLC-grade, Merck, Darmstadt, Germany) or dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany) and stored at −20 °C. Considering the stability in solutions, amoxicillin, penicillin V, meropenem, gentamicin, neomycin, and colistin were freshly prepared from standard powders within 48 h before the experiment was carried out. Formic acid (FA) was purchased from VWR Chemicals (Tingalpa, QLD, Australia), while hydrochloric acid (HCl) was purchased from Merck (Kilsyth, VIC, Australia). Ultra-pure water of 18.2 MΩ cm^{-1} purity was obtained from a Milli-Q system (Merck Millipore, Bedford, MA, USA). Regenerated cellulose syringe filters (0.2 μm RC filters, 4 mm in diameter) were purchased from Agilent (Mulgrave, VIC, Australia).

2.2. Sample collection and pretreatment

Twenty-four-hour composite samples were collected from both influent and effluent wastewaters from 54 sites across Australia in polyethylene terephthalate (PET) bottles using flow or time proportional autosamplers in August 2021. All samples were acidified to pH 2 on-site by adding 2 M HCl and then immediately frozen at −20 °C until the samples were analyzed. Samples were shipped frozen to The University of Queensland, where they were archived at −20 °C in the dark. For method validation, representative wastewater influent and effluent samples were prepared separately by pooling influent and effluent samples, respectively, collected from all sites mentioned above. To test the applicability of the method, 6 sites (3 for influent, 3 for effluent) serving populations ranging from ~10,000 to ~2,000,000 were selected from the 54 WWTPs for analyte concentration determination. Samples were analyzed using the developed direct injection method. One milliliter of thawed wastewater sample was transferred to an amber glass vial and then spiked with 10 μL of IS mix (0.5 mg L^{-1} for each IS). Then, samples were vortexed and filtered through 0.2 μm RC filters into new vials before instrumental analysis.

2.3. Instrumentation

An ultra-high performance liquid chromatography system (Nexera series-LC 40, Shimadzu, Kyoto, Japan) coupled to a tandem mass spectrometer (SCIEX Triple Quad 7500 System, AB SCIEX, Framingham, MA, USA) were used for sample analysis. Compound optimization was achieved through direct infusion of each analyte to determine the ionization mode and two transitions for multiple reaction monitoring (MRM), and to optimize the collision energy (CE) and collision exit cell potential (CXP) for each transition. Entrance potential was set to +10 V for all positive mode transitions and −10 V for all negative mode transitions. Instrument details and parameters of each transition are shown in Table S2. Then, a range of ion source temperatures and electrospray ionization voltages (ISV) were evaluated for optimization of ionization

efficiency. The optimized ion source temperature was 550 °C (considering an LC flow rate of 0.4 mL min^{-1}) and ISV was optimized for each analyte, while the ion source gas 1 and 2 were set at 60 psi and curtain gas at 40 psi. Q0 dissociation (Q0D) was optimized for each transition. The mass spectrometer was run in scheduled multiple reaction monitoring (sMRM) mode and in switching positive and negative ion mode. Dwell times were used as default values of the SCIEX OS 2.1.6 software (AB SCIEX, Framingham, MA, USA).

Chromatographic separation was achieved using a Hypersil Gold C18 selectivity column (100 × 2.1 mm, 1.9 μm, 175 Å, Thermo Fisher Scientific, Waltham, MA, USA) with a Gemini NX-C18 guard column (4 × 2 mm, Phenomenex, Torrance, CA, USA). A Kinetex EVO C18 column (30 × 2.1 mm, 5 μm, 100 Å, Phenomenex, Torrance, CA, USA) was used as a pre-injection column. Mobile phases consisted of 95:5 (v/v) Milli-Q water: methanol with 0.2 % formic acid (mobile phase A) and 95:5 (v/v) methanol: Milli-Q water with 0.2 % formic acid (mobile phase B). The flow rate was set at 0.4 mL min^{-1}. Mobile phase B was initially set at 5 %, followed by linear increase to 40 % over 4 min, linear increase to 100 % over 3 min, held for 3 min, finally returned to 5 % over 0.1 min and kept steady for 2 min to equilibrate the system. The total run time was 12 min. Data were acquired and processed using SCIEX OS 2.1.6.

2.4. Method validation

The method was validated based on The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (ICH Harmonised Tripartite Guideline, 2005). Methodology was evaluated for specificity, calibration range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Matrix effects and filtration loss were also evaluated.

2.4.1. Specificity

Both transitions needed to be present for the identification of one compound (signal-to-noise ratio > 10 for quantifier and >3 for qualifier), otherwise the compound was deemed as <LOD. Meanwhile, the ion ratio variation for all analytes should be within 30 % and the relative standard deviation (RSD) of retention times should be within 2 %.

2.4.2. Calibration standards and range determination

Three sets of calibration standard curves were prepared by using 5 % methanol in Milli-Q (pH = 2), filtered influent sample (pH = 2), and filtered effluent sample (pH = 2). Each set of calibration standard curves was run twice, once at the beginning and once at the end of a batch. Linear, quadratic, or Hill regression models were chosen for each analyte depending on which had the highest goodness of fit (R^2), with a weighting of 1/x. Calibration standard curves were prepared by spiking native standards at 10 concentrations ranging from 0.0002 μg L^{-1} to 150 μg L^{-1} and IS at a concentration of 5 μg L^{-1}. To calculate the calibration range, only the concentrations of calibration curves that showed $R^2 \geq 0.99$ and $N \geq 5$ were included. We were unable to obtain wastewater or even Milli-Q water absent of some interfering signal. Therefore, wastewater samples and Milli-Q water without spiking native standards were included for blanks and the background concentrations were subtracted from all calculated concentrations.

2.4.3. Matrix effects

Relative matrix effects were calculated as the percentage differences of slopes of calibration curves between influent/effluent and solvent based on peak area of native standards within the linear range. A value over 0 indicates percentage signal enhancement, while a value below 0 indicates percentage signal suppression.

2.4.4. Filtration loss

In the present direct injection method, the analyte loss is mostly due to the filtration. Therefore, the recovery was calculated as the percentage change of the instrument response of each analyte spiked before filtration compared to that spiked after filtration ($n = 7$). Three spiking levels (0.1, 1, and 10 μg L^{-1}) were selected and the instrument responses in background were subtracted.

2.4.5. Accuracy and precision

Accuracy and precision were calculated at three spiking levels (low (at LOQs), medium, and high). The specific concentrations are shown in Table S3. Accuracy was calculated as the mean of the observed concentration divided by the theoretical concentration multiplied by 100 % ($n = 7$). The precision was calculated as the relative standard deviation of these repeat injections multiplied by 100 % ($n = 7$).

2.4.6. Limit of detection (LOD) and limit of quantification (LOQ)

Seven vials of 1 mL wastewater were spiked with standards at concentrations of each point of the calibration curves. Generally, it is impossible to obtain wastewater samples free of all the targeted chemicals, so the actual LODs are difficult to determine. Therefore, calculated method LODs were based on the standard deviation of the measured response at low concentration ($n = 7$) multiplied by 3.3 based on the ICH guidelines. Method LOQs were determined for each compound as the lowest concentrations where accuracy (75 %–125 %) and precision (within 25 %) met the acceptable requirement.

3. Results and discussion

3.1. Analyte selection

In the present study, a method to analyze antimicrobials and their metabolites in wastewater samples was developed. Analytes were selected based on common usage and chemicals with known high-risk AMR selection potential (Table S1). Firstly, considering that traditional antibiotics are a main cause of AMR in the environment (Darby et al., 2023), this method covered all major classes of antibiotics: penicillin, cephalosporin, quinolone, sulfonamide, macrolide, tetracycline, lincosamide, rifamycin, amphenicol, aminoglycoside, etc. Apart from some conventional therapeutical antibiotics, such as amoxicillin, cephalexin, ciprofloxacin, sulfamethoxazole, and roxithromycin, several last-resort antibiotics: linezolid, meropenem, colistin, and vancomycin were also included in this method since last-resort antibiotics demonstrated an increasing trend of use in the past few years, indicating a potential threat of causing AMR (Bode et al., 2015; Van Boeckel et al., 2014). In addition, it has been suggested that animals consume much higher amounts of antibiotics compared to humans (Aarestrup, 2012). Common veterinary antibiotics, such as enrofloxacin, sarafloxacin, tilmicosin, tylosin, florfenicol, and salinomycin were also included in the method. Furthermore, there is emerging concern that some non-antibiotic antimicrobials have the ability to cause AMR and/or accelerate AMR spread. These chemicals, such as triclosan, triclocarban, chlorhexidine, and quaternary ammonium compounds (QACs), are widely used as disinfectants or antiseptics and are found in many personal care or disinfection products (Lu et al., 2018; Tandukar et al., 2013; Wand et al., 2017). Therefore, they were also considered in the method. While sulfasalazine is an anti-inflammatory drug, it was included alongside other sulfonamides as well because one of its metabolites, sulfapyridine, is also an antibiotic (Ji et al., 2018). Fluconazole, as a fungicide, was included since it has the same considerations of inducing resistance (Berkow and Lockhart, 2017). Lastly, 31 antimicrobial metabolites were also considered for better application of this method in WBE (Holton et al., 2022b). In brief, a total of 109 antimicrobials and metabolites were selected in this study.

3.2. Method validation

3.2.1. Specificity

Simultaneous detection of the two most intense transitions with an ion ratio tolerance of ≤30 % in wastewater matrix was determined to

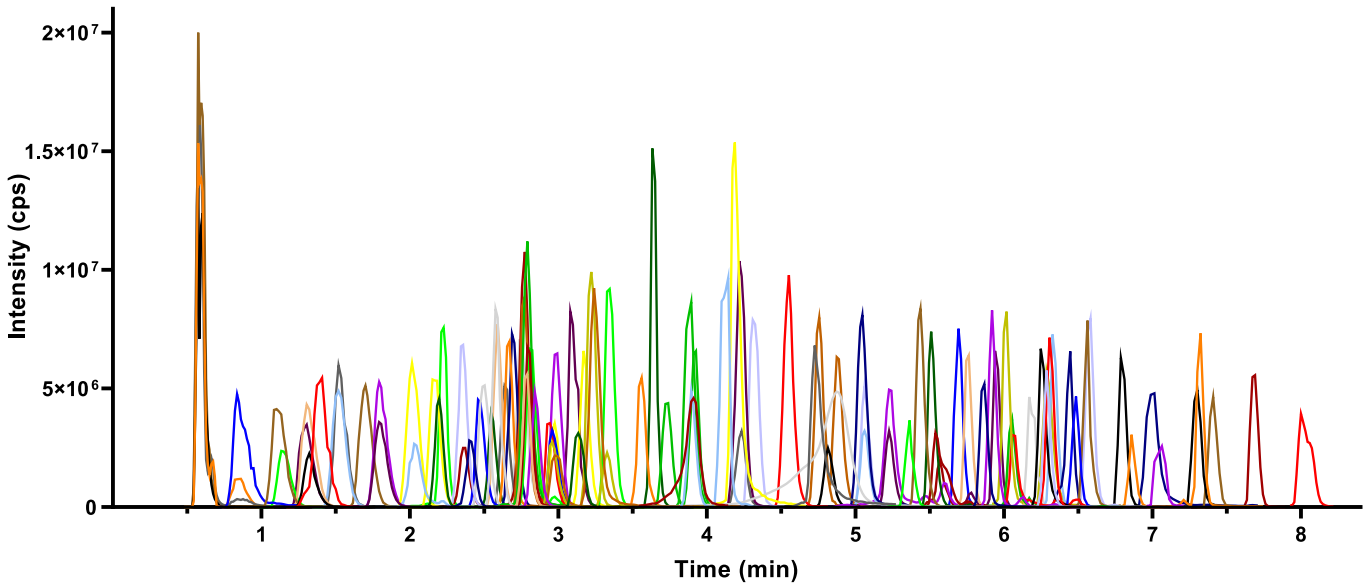

Fig. 1. Extracted Ion Chromatograms (XIC) of all 109 compounds in the present method in solvent. The concentration of each compound was adjusted individually to ensure all analytes were at similar intensity to be seen. The XICs have not been smoothed.

identify an analyte. Mass spectrometry parameters (ISV, CE, CXP, Q0D) were optimized to obtain the best ionization efficiency and MRM signal intensity (Table S2). Total ion chromatograms are shown in Fig. 1. MRM data points across peaks for each analyte were >16. The last analyte was eluted by 8 min. Analytes eluting before 1 min (FFA, GEN1, GEN1a, GEN2, NEO, TAZ-M) were regarded as not retained by the column. However, as the liquid chromatography performed well, the relative standard deviation (%RSD) of retention times ($n = 7$) of all compounds, including the non-retained ones, were still within the acceptable criteria (2 %, Table 1). Due to the accessibility of isotope labeled compounds, only 20 labeled antimicrobials and 6 additional labeled chemicals were used as internal standards (IS). IS were selected based on a comprehensive consideration of retention time, matrix effects, filtration loss, and physicochemical properties of each analyte and were spiked at 5 μg L^{-1} (see Table 1). Approximately 84 % of analytes had relative retention times (RT_{rel}) within the range of 0.60–1.50.

3.2.2. Calibration curves and range

Three models (linear, quadratic, and Hill) were tested for each analyte for the best fit to calibration curves. Regression models were determined by considering both R^2 values and accuracies at low concentrations (Table 1). Compared to solvent, matrix-matched calibration curves fitted regression models better. Most analytes demonstrated satisfactory fittings ($R^2 \geq 0.99$) except for ANP, CFP, dmERY, FA, GEN1, GEN1a, GEN2, NEO, PNU, SAL, TUL in solvent and BAC-12, GEN2 in influent. Nevertheless, ANP, dmERY, GEN1, GEN1a, GEN2, NEO, SAL, and BAC-12 still passed the validation for accuracy and precision, so these compounds could also be considered for quantitative purposes. The range was determined from the concentration level where the signal-to-noise ratio was >10 to the level where the instrumental response approached saturation, meanwhile fulfilling the requirement of $R^2 > 0.99$. Due to the instrumental sensitivity of distinct chemicals, the range could vary a lot, from 0.0002 to 150 μg L^{-1}.

3.2.3. Matrix effects

Relative matrix effects are shown in Table 2. Sixty-three and sixty-five analytes out of 109 had negligible matrix effects within ±10 % for wastewater influent and effluent, respectively, while other chemicals suffered either ion suppression (values < −10 %) or enhancement (values > 10 %). BAC-12, BAC-14, CHL, daRFX, FA, GEN1a, GEN2, MIN, NEO, PNU, RFX, SAL, TAZ-M, TUL in wastewater influent, and ANP, BAC-12, BAC-14, CHL, daRFX, FA, FFA, MIN, NEO, PNU, RFX, SAL, TAZ-M in wastewater effluent suffered matrix effects exceeding ±50 %, indicating major concerns in quantitative mass spectrometry. The greatest ion suppression was observed for TAZ-M (−73 % for influent and −87 % for effluent) and the greatest ion enhancement was observed for NEO (290 %) in influent and FA (190 %) in effluent, respectively.

3.2.4. Filtration losses

Filtration loss is another factor influencing direct injection analysis. In this study, 3 spiking levels (0.1, 1, and 10 μg L^{-1}) were used to test for filtration losses (Table 2). Most compounds were well recovered following filtration (<10 % loss). Certain hydrophobic compounds with high $\log_{Kow}$ values were lost >30 % through filtration, including BAC, FA, SAL, TCC, and TCS, indicating that careful selection of internal standards is required to correct the recoveries for quantification. The largest loss due to filtration was observed on TCC in influent (−48 %) and FA in effluent (−57 %), both at the lowest spiking level. For ANP, CLA, DMC, daRFX, ENR, FF, MIN, RFX, and TET, higher spiking levels reduced filtration losses. An increase of >10 % of instrument response after filtration was also found for aminoglycosides—gentamicin and neomycin.

3.2.5. Accuracy and precision

Three concentrations (low, medium, and high; see Table S3 for the specific concentrations) were spiked to evaluate accuracy and precision ($n = 7$), which are shown in Tables 3, 4, and S4. Accuracy and precision for influent were calculated by using calibration curves prepared both in solvent and in matrix, while for effluent, only solvent was considered. For influent using solvent-based calibration curves, most compounds (89 %) had acceptable accuracies of 75 %–125 % and relative standard deviations of <25 % across all three spiking concentrations. Exceptions were CFP, FA, PNU, TAZ-M, and TMP at all three concentrations, and BAC-10, BAC-12, COL-A, COL-B, dmERY, FF, and NEO at low spiking levels, which was likely due to the matrix effects, stability of the compounds, or the instrumental sensitivities and variation especially at low concentrations. In contrast, using matrix-matched calibration curves provided more satisfactory accuracies. Apart from TAZ-M at all three concentrations, and BAC-10 and BAC-12 at low spiking levels, all analytes met the requirements of validation. Effluents were only validated by using solvent-based calibration curves. Apart from ANP, DLX, FFA, FLX, PNU, TAZ-M, and TUL, all compounds showed acceptable accuracy

Table 1
Calibration curve results and chromatographic performance in wastewater influent and effluent.

Class	Name	Abbr.	RT_{abs} (min; n = 7)	Influent						Effluent			
				IS	RT_{rel} (n = 7)	Calibration curves in solvent		Calibration curves in matrix		IS	RT_{rel} (n = 7)	Calibration curves in solvent	
						R^2	Range (μg L^{-1})	R^2	Range (μg L^{-1})			R^2	Range (μg L^{-1})
Penicillin	Amoxicillin	AMX	1.67 ± 0.01	AMX-d4	1.01 ± 0.00	0.999[a]	0.010–30	0.996[a]	0.050–30	AMX-d4	1.01 ± 0.00	0.998[a]	0.050–30
	Amoxicilloic acid	AMXa	1.46 ± 0.01	AMX-d4	0.88 ± 0.00	0.992[a]	0.050–30	0.994[a]	0.050–30	AMX-d4	0.88 ± 0.01	0.996	0.050–30
	Ampicillin	AMP	3.59 ± 0.01	CFX-d5	1.11 ± 0.00	0.999	0.0050–30	0.999	0.0050–30	AMX-d4	1.97 ± 0.01	0.995[a]	0.050–30
	Cloxacillin	CLX	6.58 ± 0.01	FLX-^{13}C4,^{15}N	1.00 ± 0.00	0.997[b]	0.050–30	0.990[b]	0.10–30	ROX-d7	1.00 ± 0.00	0.990	0.50–30
	Dicloxacillin	DLX	6.76 ± 0.01	FLX-^{13}C4,^{15}N	1.03 ± 0.00	0.994[a]	0.10–30	0.991[a]	0.10–20	ATV-d5	0.95 ± 0.00	0.998	0.10–30
	Flucloxacillin	FLX	6.59 ± 0.01	FLX-^{13}C4,^{15}N	1.00 ± 0.00	0.998	0.10–30	0.990	0.50–20	FLX-^{13}C4,^{15}N	1.00 ± 0.00	0.992	0.10–30
	Penicillin V	PenV	5.70 ± 0.01	CFX-d5	0.95 ± 0.00	0.999	0.50–30	0.997	0.50–30	PIP-d5	0.95 ± 0.00	0.997[a]	0.50–30
	Penicilin V acid	PenVa	5.84 ± 0.03	PIP-d5	0.97 ± 0.01	0.999[a]	0.0050–20	0.994[a]	0.0050–30	PIP-d5	0.97 ± 0.00	0.998[a]	0.0050–30
	Piperacillin	PIP	6.01 ± 0.01	PIP-d5	1.00 ± 0.00	0.996[a]	0.010–30	0.998[a]	0.010–30	PIP-d5	1.00 ± 0.00	0.997[a]	0.010–30
Cephalosporin	Cefaclor	CFC	2.90 ± 0.01	CFX-d5	0.90 ± 0.00	0.999[a]	0.010–20	0.999[a]	0.0050–20	CFX-d5	0.90 ± 0.01	0.998[a]	0.010–30
	Cephalexin	CFX	3.25 ± 0.01	CFX-d5	1.01 ± 0.00	0.999	0.025–150	0.999	0.25–150	PIP-d5	0.54 ± 0.01	0.997[a]	0.050–150
	Cephalothin	CFL	5.24 ± 0.01	PIP-d5	0.87 ± 0.00	0.997	0.050–30	0.996	0.050–5.0	PIP-d5	0.87 ± 0.00	0.998[a]	0.050–30
	Cefazolin	CFZ	3.61 ± 0.01	CTR-d3	1.14 ± 0.00	0.999	0.010–30	0.999	0.010–20	CTR-d3	1.14 ± 0.00	0.995[a]	0.010–20
	Cefepime	CFP	1.51 ± 0.02	AMX-d4	0.92 ± 0.00	0.994	0.10–30	0.995	0.050–30	AMX-d4	0.92 ± 0.01	0.983	0.10–20
	Ceftiofur	CTF	5.32 ± 0.01	LZD-d3	1.14 ± 0.00	0.992	0.0050–30	0.995	0.0050–20	LZD-d3	1.14 ± 0.00	0.999[a]	0.0050–30
	Ceftriaxone	CTR	3.18 ± 0.01	CTR-d3	1.00 ± 0.00	>0.9995	0.050–30	>0.9995	0.050–30	CTR-d3	1.00 ± 0.00	0.996[a]	0.010–30
	Cefuroxime	CRX	3.53 ± 0.01	245-T-^{13}C6	0.51 ± 0.00	0.993[a]	0.50–30	0.993[a]	0.50–30	245-T-^{13}C6	0.51 ± 0.00	0.993[a]	0.50–30
Quinolone	Ciprofloxacin	CIP	3.44 ± 0.01	CIP-d8	1.01 ± 0.00	0.998	0.050–20	0.998	0.0050–20	CIP-d8	1.01 ± 0.00	0.999	0.050–30
	Desethylene ciprofloxacin	deCIP	3.10 ± 0.01	CIP-d8	0.91 ± 0.00	0.997[a]	0.050–10	0.994[a]	0.010–10	CIP-d8	0.91 ± 0.00	0.999	0.050–20
	Enrofloxacin	ENR	3.63 ± 0.01	CIP-d8	1.06 ± 0.00	0.999	0.050–30	0.999	0.050–30	CIP-d8	1.06 ± 0.00	0.997	0.050–30
	Moxifloxacin	MOX	4.61 ± 0.01	ATV-d5	0.65 ± 0.00	0.997[a]	0.010–30	0.990[a]	0.010–30	ATV-d5	0.65 ± 0.00	0.999	0.050–30
	Moxifloxacin sulfate	MOX-SO_4	6.80 ± 0.01	ATV-d5	0.95 ± 0.00	0.999[a]	0.050–30	0.999[a]	0.10–30	ATV-d5	0.95 ± 0.00	0.994[a]	0.10–30
	Norfloxacin	NOR	3.30 ± 0.01	NOR-d5	1.00 ± 0.00	0.999[b]	0.010–10	0.998[b]	0.0050–10	NOR-d5	1.00 ± 0.00	>0.9995	0.050–10
	Desethylene norfloxacin	deNOR	2.88 ± 0.01	NOR-d5	0.88 ± 0.00	0.999	0.050–10	0.992	0.0050–5.0	NOR-d5	0.88 ± 0.00	0.999	0.050–10

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Table 1 (*continued*)

Class	Name	Abbr.	RT_{abs} (min; n = 7)	Influent						Effluent			
				IS	RT_{rel} (n = 7)	Calibration curves in solvent		Calibration curves in matrix		IS	RT_{rel} (n = 7)	Calibration curves in solvent	
						R^2	Range (μg L^{-1})	R^2	Range (μg L^{-1})			R^2	Range (μg L^{-1})
	Ofloxacin	OFL	3.27 ± 0.01	MTZ-d3	2.10 ± 0.01	0.999	0.0050–5	0.999	0.0050–5.0	MTZ-d3	2.10 ± 0.01	0.997	0.010–5.0
	Desmethyl ofloxacin	dmOFL	3.28 ± 0.01	MTZ-d3	2.10 ± 0.01	0.999	0.010–10	0.992	0.010–10	MTZ-d3	2.10 ± 0.01	0.998	0.050–10
	Sarafloxacin	SAR	3.86 ± 0.01	TMP-d9	1.39 ± 0.00	0.999	0.050–30	0.994	0.050–30	TMP-d9	1.39 ± 0.00	0.998[a]	0.050–30
	Oxolinic acid	OXO	4.99 ± 0.01	NOR-d5	1.52 ± 0.00	0.998[a]	0.050–20	0.998[a]	0.050–20	NOR-d5	1.52 ± 0.00	0.999	0.050–30
Sulfonamide	Sulfachloropyridazine	SCP	3.25 ± 0.01	SMX-d4	0.97 ± 0.00	0.999	0.0050–20	0.999	0.0050–10	SMX-d4	0.97 ± 0.00	>0.9995	0.0050–20
	Sulfadiazine	SDZ	1.81 ± 0.01	SMX-d4	0.62 ± 0.00	0.997	0.0050–10	>0.9995	0.0050–10	SMZ-d4	0.62 ± 0.00	>0.9995	0.0050–20
	Acetyl sulfadiazine	aSDZ	2.73 ± 0.01	aSMX-d4	0.63 ± 0.00	0.999	0.0050–30	0.998	0.0050–20	SMZ-d4	0.93 ± 0.00	0.999	0.0050–30
	Sulfadimethozine	SDM	4.68 ± 0.01	SMX-d4	1.39 ± 0.00	0.999	0.0050–10	>0.9995	0.0050–10	SMX-d4	1.39 ± 0.00	>0.9995	0.0050–10
	Sulfamerazine	SMR	2.42 ± 0.01	FCZ-d4	0.58 ± 0.00	0.998	0.0050–20	0.999	0.0050–20	FCZ-d4	0.58 ± 0.00	0.999	0.0050–30
	Acetyl sulfamerazine	aSMR	3.17 ± 0.01	LZD-d3	0.68 ± 0.00	0.997	0.0050–30	0.999	0.0050–20	SMZ-d4	1.08 ± 0.00	0.993	0.0050–30
	Sulfamethazine	SMZ	2.96 ± 0.01	SMZ-d4	1.01 ± 0.00	>0.9995	0.0050–20	>0.9995	0.0050–10	SMZ-d4	1.01 ± 0.00	>0.9995	0.0050–20
	Acetyl sulfamethazine	aSMZ	3.65 ± 0.01	aSMX-d4	1.07 ± 0.00	0.998	0.0050–30	0.998	0.0050–20	aSMX-d4	1.07 ± 0.00	0.999	0.0050–30
	Sulfamethizole	SMT	2.90 ± 0.01	SMX-d4	0.86 ± 0.00	0.999	0.0050–10	0.999	0.0050–5.0	SMX-d4	0.86 ± 0.00	>0.9995	0.0050–10
	Sulfamethoxazole	SMX	3.39 ± 0.01	SMX-d4	1.01 ± 0.00	>0.9995	0.0050–20	0.999	0.0050–30	SMX-d4	1.01 ± 0.00	>0.9995	0.0050–30
	Acetyl sulfamethoxazole	aSMX	4.37 ± 0.01	aSMX-d4	1.00 ± 0.00	0.999[a]	0.010–30	>0.9995[a]	0.0050–30	aSMX-d4	1.00 ± 0.00	0.998[a]	0.010–30
	Sulfapyridine	SPY	2.22 ± 0.01	ATV-d5	0.31 ± 0.00	0.998	0.0050–10	0.993	0.0050–10	aSMX-d4	0.51 ± 0.00	0.999	0.0050–10
	Acetyl sulfapyridine	aSPY	3.07 ± 0.01	aSMX-d4	0.70 ± 0.00	0.999	0.010–20	0.999	0.0050–20	SMZ-d4	1.04 ± 0.00	0.999	0.0050–30
	Sulfasalazine	SLZ	6.54 ± 0.01	ATV-d5	0.92 ± 0.00	>0.9995	0.050–30	0.999	0.0050–20	ATV-d5	0.92 ± 0.00	0.999[a]	0.010–30
	Sulfathiazole	STZ	2.08 ± 0.01	SMX-d4	0.61 ± 0.00	0.999	0.0050–10	>0.9995	0.0050–10	SMZ-d4	0.61 ± 0.00	>0.9995	0.0050–20
	Acetyl sulfathiazole	aSTZ	3.10 ± 0.01	aSMX-d4	0.71 ± 0.00	0.999	0.0050–20	0.999	0.0050–10	aSMX-d4	0.71 ± 0.00	0.997	0.0050–20
Macrolide	Azithromycin	AZI	5.25 ± 0.01	LZD-d3	1.13 ± 0.00	0.992	0.0050–10	0.991	0.0050–20	LZD-d3	1.13 ± 0.00	0.994	0.0050–10
	Desmethyl azithromycin	dmAZI	5.26 ± 0.01	LZD-d3	1.13 ± 0.00	0.999[b]	0.0050–30	>0.9995[b]	0.0050–20	LZD-d3	1.13 ± 0.00	0.996	0.0050–30
	Clarithromycin	CLA	6.57 ± 0.01	ATV-d5	0.92 ± 0.00	0.995	0.0050–10	0.995	0.0050–20	ATV-d5	0.92 ± 0.00	0.996	0.0050–10
	Erythromycin-H_2O	ERY-18	6.39 ± 0.01	ATV-d5	0.89 ± 0.00	0.996[b]	0.0050–20	0.997[b]	0.0050–20	ATV-d5	0.89 ± 0.00	0.991	0.0050–30
	Erythromycin	ERY	6.28 ± 0.01	ATV-d5	0.88 ± 0.00	0.995	0.10–30	0.997	0.10–20	ATV-d5	0.88 ± 0.00	0.996[a]	0.10–30

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Table 1 (*continued*)

Class	Name	Abbr.	RT_{abs} (min; n = 7)	Influent						Effluent			
				IS	RT_{rel} (n = 7)	Calibration curves in solvent		Calibration curves in matrix		IS	RT_{rel} (n = 7)	Calibration curves in solvent	
						R^2	Range (μg L^{-1})	R^2	Range (μg L^{-1})			R^2	Range (μg L^{-1})
	Desmethyl erythromycin	dmERY	6.27 ± 0.02	ROX-d7	0.95 ± 0.00	0.980	0.50–30	0.990	1.0–30	ROX-d7	0.95 ± 0.00	0.990	0.50–30
	Roxithromycin	ROX	6.64 ± 0.01	ROX-d7	1.00 ± 0.00	0.999[a]	0.0050–20	0.998[a]	0.0050–30	ROX-d7	1.00 ± 0.00	0.995	0.0050–30
	Descladinose roxithromycin	dcROX	6.16 ± 0.01	ROX-d7	0.93 ± 0.00	0.997	0.0050–20	0.994	0.0050–30	ROX-d7	0.93 ± 0.00	0.993	0.0050–20
	Spiramycin I	SP-I	5.02 ± 0.01	LZD-d3	1.08 ± 0.00	0.999	0.0080–4.7	0.999	0.0080–4.7	LZD-d3	1.08 ± 0.00	0.998[a]	0.0080–4.7
	Spiramycin III	SP-III	5.63 ± 0.01	LZD-d3	1.21 ± 0.00	0.997	0.010–30	0.999	0.0050–20	LZD-d3	1.21 ± 0.00	0.994[a]	0.050–30
	Monoacetyl spiramycin II	maSP-II	5.42 ± 0.01	CFX-d5	1.68 ± 0.00	0.998	0.0010–7.4	0.999	0.0010–2.5	LZD-d3	1.16 ± 0.00	0.997	0.0020–5.0
	Diacetyl spiramycin II	daSP-II	5.70 ± 0.01	CFX-d5	1.77 ± 0.00	0.999	0.0010–5.2	0.999	0.0010–2.6	LZD-d3	1.22 ± 0.00	0.999[a]	0.0030–2.6
	Monoacetyl spiramycin III	maSP-III	5.75 ± 0.01	CFX-d5	1.78 ± 0.00	0.999	0.0010–6.5	>0.9995	0.0010–2.2	LZD-d3	1.23 ± 0.00	0.996[a]	0.0020–4.4
	Diacetyl spiramycin III	daSP-III	5.93 ± 0.01	CFX-d5	1.84 ± 0.00	0.997	0.0010–4.4	0.999	0.0010–2.2	LZD-d3	1.27 ± 0.00	0.999	0.0020–2.2
	Spiramycin II	SP-II	5.24 ± 0.01	LZD-d3	1.12 ± 0.00	0.998	0.00020–1.0	0.999	0.00030–0.67	LZD-d3	1.12 ± 0.00	0.995[a]	0.00020–1.0
	Tilmicosin	TIL	5.74 ± 0.01	LZD-d3	1.23 ± 0.00	0.998	0.010–10	0.999	0.010–20	LZD-d3	1.23 ± 0.00	0.995[a]	0.010–30
	Tulathromycin	TUL	3.82 ± 0.01	CHX-d8	0.63 ± 0.00	0.980	0.050–20	0.992	0.010–20	ROX-d7	0.58 ± 0.00	0.997	0.050–20
	Tylosin	TYL	6.25 ± 0.01	ROX-d7	0.94 ± 0.00	0.996	0.0050–30	0.992	0.0050–10	ROX-d7	0.94 ± 0.00	0.990	0.0050–30
	Virginiamycin M1	VIR-M	6.61 ± 0.01	PIP-d5	1.10 ± 0.00	0.998	0.0050–30	0.995	0.0050–20	PIP-d5	1.10 ± 0.00	0.999	0.0050–30
	Virginiamycin S1	VIR-S	6.86 ± 0.01	ROX-d7	1.04 ± 0.00	0.995	0.0050–30	0.991	0.0050–30	ROX-d7	1.04 ± 0.00	0.999	0.0050–10
Tetracycline	Chlortetracycline	CTC	4.28 ± 0.01	LZD-d3	0.92 ± 0.00	0.997[a]	0.0050–30	0.999[a]	0.010–30	LZD-d3	0.92 ± 0.00	0.999[a]	0.010–30
	Demeclocycline	DMC	3.62 ± 0.01	SMX-d4	1.08 ± 0.00	0.999	0.050–30	>0.9995	0.050–10	SMX-d4	1.08 ± 0.00	>0.9995[a]	0.050–20
	Doxycycline	DOX	5.22 ± 0.01	LZD-d3	1.12 ± 0.00	0.998	0.050–30	0.999	0.010–30	LZD-d3	1.12 ± 0.00	0.998[a]	0.050–30
	Minocycline	MIN	2.54 ± 0.00	MIN-d7	1.08 ± 0.00	0.998[a]	0.050–20	0.999[a]	0.0050–10	MIN-d7	1.08 ± 0.00	0.994	0.050–20
	Oxytetracycline	OTC	3.16 ± 0.01	CTR-d3	1.00 ± 0.00	0.998	0.0050–20	0.998	0.0050–20	CTR-d3	0.99 ± 0.00	0.999	0.050–20
	Tetracycline	TET	3.13 ± 0.01	CTR-d3	0.99 ± 0.00	0.999	0.0050–30	0.999	0.0050–20	CTR-d3	0.99 ± 0.00	0.999	0.010–30
Azole	Fluconazole	FCZ	4.19 ± 0.01	FCZ-d4	1.00 ± 0.00	>0.9995	0.0050–10	0.999	0.0050–10	FCZ-d4	1.00 ± 0.00	0.999	0.0050–10
	Fluconazole N-oxide	FNO	3.26 ± 0.01	FCZ-d4	0.78 ± 0.00	0.999	0.0050–10	0.998	0.0050–5.0	FCZ-d4	0.78 ± 0.00	0.999	0.0050–10
	Metronidazole	MTZ	1.57 ± 0.01	MTZ-d3	1.01 ± 0.00	0.999	0.0050–10	0.999	0.0050–10	MTZ-d3	1.01 ± 0.00	>0.9995	0.0050–10
	Hydroxy metronidazole	hMTZ	1.28 ± 0.01	2-PY-d3	1.13 ± 0.00	0.997	0.0050–20	0.999	0.0050–20	2-PY-d3	1.13 ± 0.00	0.992	0.0050–30

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Table 1 (*continued*)

Class	Name	Abbr.	RT_{abs} (min; n = 7)	Influent						Effluent			
				IS	RT_{rel} (n = 7)	Calibration curves in solvent		Calibration curves in matrix		IS	RT_{rel} (n = 7)	Calibration curves in solvent	
						R^2	Range (μg L^{-1})	R^2	Range (μg L^{-1})			R^2	Range (μg L^{-1})
Lincosamide	Clindamycin	CLI	5.61 ± 0.01	LZD-d3	1.20 ± 0.00	0.999	0.0050–10	0.999	0.0050–10	LZD-d3	1.20 ± 0.00	0.999	0.0050–10
	Clindamycin sulfoxide	CSO	4.40 ± 0.01	aSMX-d4	1.01 ± 0.00	>0.9995	0.0050–10	>0.9995	0.0050–10	aSMX-d4	1.01 ± 0.00	0.999	0.0050–20
	Lincomycin	LIN	2.68 ± 0.01	SMZ-d4	0.91 ± 0.00	0.999	0.0050–5	>0.9995	0.0050–5.0	TMP-d9	0.96 ± 0.00	0.999	0.0050–5.0
Rifamycin	Rifampicin	RFP	7.01 ± 0.01	CIP-d8	2.05 ± 0.00	0.991	0.050–30	0.995	0.010–30	CIP-d8	2.05 ± 0.00	0.994	0.050–30
	Rifaximin	RFX	7.06 ± 0.01	ATV-d5	0.99 ± 0.00	>0.9995	0.0050–10	0.998	0.0050–10	ATV-d5	0.99 ± 0.00	0.991	0.0050–10
	Desacetyl rifaximin	daRFX	6.82 ± 0.01	ATV-d5	0.96 ± 0.00	0.991	0.0050–10	0.998	0.0050–20	ATV-d5	0.96 ± 0.00	0.994	0.0050–30
Amphenicol	Chloramphenicol	CHL	1.17 ± 0.01	245-T-^{13}C6	1.48 ± 0.00	0.997[a]	0.050–30	0.996[a]	0.050–20	FPN-^{13}C4,^{15}N2	1.48 ± 0.02	0.995[a]	0.050–30
	2-Amino-1-(4-nitrophenyl)-1,3-propanediol	ANP	6.03 ± 0.01	MFM-d6	1.00 ± 0.00	0.984	0.010–10	0.998	0.10–30	MFM-d6	1.00 ± 0.00	0.988	0.050–20
	Florfenicol	FF	3.43 ± 0.01	245-T-^{13}C6	0.48 ± 0.00	0.998	0.050–30	0.993	0.050–30	FPN-^{13}C4,^{15}N2	0.48 ± 0.00	0.994	0.050–20
	Florfenicol amine	FFA	0.92 ± 0.00	MFM-d6	1.16 ± 0.01	0.991	2.5–150	0.992	25–150				
β-lactamase inhibitor	Tazobactam	TAZ	1.51 ± 0.01	MIN-d7	0.64 ± 0.00	0.997	0.010–30	0.998	0.050–20	CFX-d5	0.47 ± 0.00	0.997[a]	0.050–30
	Tazobactam metabolite 1	TAZ-M	0.86 ± 0.01	TCS-^{13}C12	0.11 ± 0.00	0.978[a]	0.50–30	0.997[a]	0.10–30	TCS-^{13}C12	0.12 ± 0.00	0.998	0.10–30
Diaminopyrimidine	Trimethoprim	TMP	2.83 ± 0.01	TMP-d9	1.02 ± 0.00	0.994	0.010–10	0.996	0.0050–10	TMP-d9	1.02 ± 0.00	0.998	0.0050–5.0
	Hydroxy trimethoprim	hTMP	2.97 ± 0.01	NOR-d5	0.91 ± 0.00	0.996	0.0050–5	0.998	0.0050–5.0	NOR-d5	0.91 ± 0.00	0.998	0.0050–10
Oxazolidinone	Linezolid	LZD	4.67 ± 0.01	LZD-d3	1.00 ± 0.00	0.998[a]	0.0050–10	0.992[a]	0.0050–10	LZD-d3	1.00 ± 0.00	>0.9995	0.0050–10
	PNU 142586	PNU	3.22 ± 0.01	MIN-d7	1.37 ± 0.00	0.993	0.010–5	0.999	0.010–10	MIN-d7	1.37 ± 0.00	0.924	0.050–20
Aminoglycoside	Gentamicin C1	GEN1	0.62 ± 0.01	MTZ-d3	0.40 ± 0.00	0.994	1.5–88	0.994	0.10–88	MTZ-d3	0.39 ± 0.01	0.988	0.10–88
	Gentamicin C1a	GEN1a	0.62 ± 0.00	NOR-d5	0.19 ± 0.00	0.985	2.1–64	0.991	0.043–64	MTZ-d3	0.39 ± 0.00	0.972	1.1–64
	Gentamicin C2	GEN2	0.62 ± 0.00	NOR-d5	0.19 ± 0.00	0.983	5.0–150	0.987	0.50–100	MTZ-d3	0.39 ± 0.01	0.979	2.5–150
	Neomycin	NEO	0.62 ± 0.01	CAF-d3	0.20 ± 0.00	0.970[a]	5.0–30	0.991	0.50–30	CAF-d3	0.20 ± 0.00	0.981	5.0–30
Cyclic polypeptide	Colistin A	COL-A	4.85 ± 0.01	LZD-d3	1.04 ± 0.00	0.991[a]	25–150	0.989[a]	2.0–150	LZD-d3	1.05 ± 0.00	0.997[a]	25–10
	Colistin B	COL-B	4.28 ± 0.01	FCZ-d4	1.03 ± 0.00	0.999[a]	25–150	0.999[a]	2.5–150	FCZ-d4	1.03 ± 0.00	0.999[a]	25–10
Fusidane	Fusidic acid	FA	7.89 ± 0.01	245-T-^{13}C6	1.14 ± 0.00	0.998	0.050–30	0.997	0.050–20	245-T-^{13}C6	1.14 ± 0.00	0.982	0.10–30
Carbapenem	Meropenem	MER	2.47 ± 0.01	FCZ-d4	0.59 ± 0.00	0.997[a]	0.050–20	0.998[a]	0.050–30	TMP-d9	0.89 ± 0.00	0.999	0.050–30
Nitrofuran	Nitrofurantoin	NIT	2.55 ± 0.01	TMP-d9	0.92 ± 0.00	0.997	0.050–30	0.999	0.050–30	TMP-d9	0.92 ± 0.00	0.999	0.050–30

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Table 1 (*continued*)

Class	Name	Abbr.	RT_{abs} (min; n = 7)	Influent						Effluent			
				IS	RT_{rel} (n = 7)	Calibration curves in solvent		Calibration curves in matrix		IS	RT_{rel} (n = 7)	Calibration curves in solvent	
						R^2	Range (μg L^{-1})	R^2	Range (μg L^{-1})			R^2	Range (μg L^{-1})
Ionophore	Salinomycin	SAL	8.03 ± 0.01	ATV-d5	1.34 ± 0.00	0.980	0.010–20	0.999	0.0050–30	BAC-12-d5	1.12 ± 0.00	0.985	0.050–20
Glycopeptide	Vancomycin	VAN	2.52 ± 0.01	AMX-d4	1.52 ± 0.01	0.995[a]	0.050–20	0.998[a]	0.0050–30	AMX-d4	1.53 ± 0.01	0.990	0.050–30
Disinfectant	Benzalkonium chloride - C10	BAC-10	6.84 ± 0.01	BAC-12-d5	0.95 ± 0.00	0.990	0.0010–0.30	0.990	0.0050–0.30	BAC-12-d5	0.95 ± 0.00	0.992	0.0050–0.30
	Benzalkonium chloride - C12	BAC-12	7.21 ± 0.01	BAC-12-d5	1.00 ± 0.00	0.992[b]	0.25–150	0.945	0.50–150	BAC-12-d5	1.00 ± 0.00	0.998	0.25–10
	Benzalkonium chloride - C14	BAC-14	7.49 ± 0.01	BAC-12-d5	1.04 ± 0.00	0.990	0.10–50	0.997	0.10–48	BAC-12-d5	1.04 ± 0.00	0.996	0.10–48
	Chlorhexidine	CHX	4.36 ± 0.01	CHX-d8	0.63 ± 0.00	0.996	0.10–30	0.998	0.050–30	CHX-d8	0.63 ± 0.00	0.999[a]	0.050–30
	p-Chloroaniline	p-CA	2.15 ± 0.01	MTZ-d3	1.38 ± 0.01	0.998	0.025–25	0.999	0.025–25	MTZ-d3	1.38 ± 0.00	0.998	0.025–25
	Triclocarban	TCC	7.52 ± 0.01	TCC-^{13}C6	1.00 ± 0.00	0.995[a]	0.0050–20	0.999[a]	0.0050–30	TCC-^{13}C6	1.00 ± 0.00	0.995[a]	0.0050–30
	Triclosan	TCS	7.62 ± 0.01	TCS-^{13}C12	1.00 ± 0.00	>0.9995[a]	0.0050–30	0.999[a]	0.0050–30	TCS-^{13}C12	1.00 ± 0.00	0.999[a]	0.050–30
	Triclosan sulfate	TCS-SO_4	7.58 ± 0.01	FPN-^{13}C4,^{15}N2	1.05 ± 0.00	0.998	0.050–30	0.998	0.010–20	TCS-^{13}C12	1.05 ± 0.00	0.999	0.010–30

IS, internal standard. RT_{abs}, absolute retention time. RT_{rel}, relative retention time.
Direct injection method is infeasible for FFA in effluent.
For isomeric compounds resulting in multiple peaks, only the retention time for the largest peak is reported.
R^2 values without table footnotes indicate calibration curves were fitted with quadratic model.
[a] Calibration curves fitted with linear model.
[b] Calibration curves fitted with Hill model.

Table 2
Matrix effects and filtration loss of antimicrobials and their metabolites in wastewater influent and effluent.

Class	Name	Influent				Effluent			
		Matrix effects (%)	Filtration loss (%, $n = 7$)			Matrix effects (%)	Filtration loss (%, $n = 7$)		
			0.1 μg L^{-1}	1 μg L^{-1}	10 μg L^{-1}		0.1 μg L^{-1}	1 μg L^{-1}	10 μg L^{-1}
Penicillin	AMX	−1	1	10	4	−5	−10	−6	−3
	AMXa	−1	−2	1	−5	−1	5	1	−8
	AMP	−3	−4	2	1	−7	5	−1	2
	CLX	1	−6	1	−3	1	−6	−8	−4
	DLX	2	−9	−5	−5	2	−5	−9	−5
	FLX	2	−2	−3	−2	4	−10	−6	−5
	PenV	6	−4	0	0	6	−2	−5	−3
	PenVa	−1	−8	2	0	−3	4	−6	0
	PIP	10	−5	−5	1	4	−3	−6	−4
Cephalosporin	CFC	−5	−1	10	1	1	4	−3	−1
	CFX	−4	−5	3	3	0	−4	−2	−1
	CFL	−8	9	9	5	−8	−13	−2	−3
	CFZ	−4	−1	4	0	1	−8	−3	−3
	CFP	42	−3	5	4	0	−5	4	11
	CTF	−1	3	1	0	9	5	−3	−5
	CTR	9	−5	4	−4	6	−5	5	−3
	CRX	−13	−6	0	−3	−22	−5	−9	−2
Quinolone	CIP	−26	−9	−7	−1	0	−2	−5	−2
	deCIP	−19	−7	−5	−1	3	0	−2	1
	ENR	−12	−15	−12	−1	3	6	1	1
	MOX	−17	−12	−5	−2	6	1	−4	−1
	MOX-SO_4	13	5	−6	−10	18	−8	−6	−7
	NOR	−28	−9	−10	−2	−4	−2	0	0
	deNOR	−2	−5	−8	−1	−5	1	−1	−1
	OFL	−27	−11	−6	1	−7	4	−4	1
	dmOFL	−25	−2	−7	1	−14	1	−3	2
	SAR	−1	−4	−6	−2	3	−3	−3	−2
	OXO	−9	−4	1	0	12	−1	1	5
Sulfonamide	SCP	−9	3	6	2	1	1	−3	−1
	SDZ	7	0	2	0	40	−1	−2	4
	aSDZ	−10	3	8	1	1	0	−2	2
	SDM	−3	0	1	−1	5	−2	−1	−1
	SMR	21	2	9	2	27	−2	1	2
	aSMR	6	3	8	1	8	−3	−3	−2
	SMZ	6	4	5	3	10	−2	−1	0
	aSMZ	0	2	0	0	−2	0	−2	1
	SMT	−7	1	8	2	4	5	2	−1
	SMX	1	0	5	1	−3	0	−1	0
	aSMX	−4	6	7	2	−4	−1	−2	1
	SPY	−1	−1	6	2	35	−1	−1	2
	aSPY	5	3	6	0	12	1	4	−1
	SLZ	10	−10	−6	−10	2	−10	−8	−11
	STZ	20	−1	9	4	38	2	−3	4
	aSTZ	1	3	6	0	0	−3	1	4
Macrolide	AZI	23	−5	−7	1	17	−4	−3	−2
	dmAZI	2	−3	1	1	−9	−1	−4	0
	CLA	−4	−11	−3	−3	−7	−3	−7	−2
	ERY-18	−27	−2	0	−4	−17	−2	−5	−3
	ERY	−3	−3	4	−1	−7	−7	−6	0
	dmERY	−11	−8	0	−3	8	−2	−10	−2
	ROX	12	−8	−1	−3	9	−8	−8	−1
	dcROX	0	−4	5	−2	3	−5	−3	0
	SP-I	4	1	6	−1	7	−4	−1	0
	SP-III	2	−1	0	0	1	−6	−6	0
	maSP-II	17	−3	5	−4	14	−5	−3	0
	daSP-II	14	−5	2	−2	25	−8	−7	−1
	maSP-III	11	−6	−6	−5	16	−5	−5	−7
	daSP-III	14	−7	2	−3	18	−10	−9	−3
	SP-II	1	−2	4	−1	2	−3	−4	2
	TIL	16	−6	0	−4	15	−6	−2	−4
	TUL	59	−6	2	−3	23	−1	−3	−7
	TYL	7	−6	3	−3	−4	−8	−6	−3
	VIR-M	6	−4	−4	−5	6	−10	−7	3
	VIR-S	3	−11	−7	−7	3	−10	−11	−6
Tetracycline	CTC	−11	−9	−5	−3	−1	6	−5	−2
	DMC	3	−15	−13	−4	21	2	−6	−1
	DOX	17	−7	−3	−6	15	2	−7	−6
	MIN	77	−35	−22	−4	135	5	−14	−5
	OTC	−3	−6	−2	1	8	3	−7	−2
	TET	2	−13	−13	0	19	4	−5	−1
Azole	FCZ	−9	−2	8	1	3	2	−1	−1

(*continued on next page*)

Table 2 (*continued*)

Class	Name	Influent				Effluent			
		Matrix effects (%)	Filtration loss (%, $n = 7$)			Matrix effects (%)	Filtration loss (%, $n = 7$)		
			0.1 μg L^{-1}	1 μg L^{-1}	10 μg L^{-1}		0.1 μg L^{-1}	1 μg L^{-1}	10 μg L^{-1}
	FNO	−3	−1	−2	0	5	1	−4	−2
	MTZ	10	−2	1	0	21	1	0	3
	hMTZ	−2	0	8	0	−20	−2	1	2
Lincosamide	CLI	0	0	2	−3	4	−2	−4	−3
	CSO	0	3	1	1	3	−5	−4	−6
	LIN	32	2	4	−1	38	1	−1	1
Rifamycin	RFP	3	−24	−22	−13	6	−5	−17	−17
	RFX	84	−21	−24	−4	69	−20	−22	−6
	daRFX	57	−26	−18	−6	56	−24	−20	−4
Amphenicol	CHL	−52	−1	5	1	−62	3	2	−2
	ANP	3	−15	−13	−10	52	−15	−16	−1
	FF	−20	−11	11	−1	−16	1	−2	3
	FFA	18			4				
β-lactamase inhibitor	TAZ	9	−1	8	3	38	0	−1	1
	TAZ-M	−73	2	0	9	−87	11	−4	−5
Diaminopyrimidine	TMP	−8	−1	4	0	3	1	−1	0
	hTMP	−3	−1	2	0	6	−2	1	−1
Oxazolidinone	LZD	2	2	4	−1	1	−6	−1	0
	PNU	150	−20	−27	−15	−83	−15	−21	−50
Aminoglycoside	GEN1	12		12	10	32		−9	−6
	GEN1a	65		38	21	30		−3	−11
	GEN2	56		27	6	−2		−8	−5
	NEO	293		27	9	75		5	1
Cyclic polypeptide	COL-A	−10			−2	49			−8
	COL-B	31			−5	41			−8
Fusidane	FA	251	−38	−38	−32	192	−57	−51	−46
Carbapenem	MER	26	2	12	6	8	9	0	4
Nitrofuran	NIT	−6	2	8	0	−6	−9	−1	1
Ionophore	SAL	102	−38	−35	−17	148	−53	−44	−27
Glycopeptide	VAN	0	5	−4	−4	−8	−4	−3	3
Disinfectant	BAC-10	5	−12	−16	−9	1	−9	−9	−7
	BAC-12	−56	−30	−32	−16	51	−30	−33	−15
	BAC-14	126	−23	−40	−41	113	−15	−34	−30
	CHX	4	−7	5	−2	4	2	−4	8
	p-CA	−21	−3	10	3	1	3	−2	0
	TCC	−5	−48	−43	−39	0	−45	−47	−34
	TCS	11	−30	−28	−28	10	−23	−30	−33
	TCS-SO_4	−2	−17	−12	−22	0	−11	−18	−16

Analytes with no values in the columns were below their respective LOQs.

(75 %–125 %) and precision (within 25 %). In addition, those compounds that did not pass method validation at all concentrations were regarded as semi-quantitative analysis.

3.2.6. Limit of detection (LOD) and limit of quantification (LOQ)

It is always challenging to determine the true method LODs in wastewater analysis because of the unavailability of analyte-free wastewater samples, and thus calculated LODs were used (Tables 3, 4, and S4). Most antimicrobial groups, including quinolones, sulfonamides, macrolides, tetracyclines, azoles, and lincosamides, had predicted LODs as low as 0.001–0.01 μg L^{-1}, while for most penicillins and cephalosporins the LODs were below 0.05 μg L^{-1}. The lowest LOD was for SPI-II at 0.00003 μg L^{-1} in effluent. Tables 3, 4, and S4 present the LOQ of each analyte using different calibration curves in influent and effluent. Overall, LOQs ranged from 0.0003 to 50 μg L^{-1}. Some antimicrobial groups could reach remarkably low LOQs at 0.005 to 0.01 μg L^{-1}, such as sulfonamides, macrolides, lincosamides, and azoles. Meanwhile, quinolones and tetracyclines also reached comparatively low LOQs at 0.01 to 0.05 μg L^{-1}. In contrast, LOQs of BAC-12, COL-A, COL-B, dmERY, FFA, GEN1a, GEN2, and NEO were relatively high (>1 μg L^{-1}), possibly because of the low sensitivity of the compounds, the interference of high background concentrations, or the background which varied among the 7 replicates of the spike samples.

3.3. Application to wastewater samples

The present method was applied to 3 influent and 3 effluent samples collected from 6 different municipal wastewater treatment plants in Australia. Thirty-seven of the 109 analytes were detected in the analyzed influents and 22 were detected in the effluents (Table 5). All detected analytes could be quantified in the influent with the exception of CFP, deCIP, and ERY-18 that were at concentrations below their corresponding LOQs for all three sampling sites. The top 3 highest concentrations were observed for AMXa, BAC-12, and CFX at all three sites of influent, at concentrations of between 1.9 and 160 μg L^{-1}. The detection of 9 antibiotic metabolites, previously used as WBE biomarkers (Han et al., 2022; Holton et al., 2022b), demonstrates broader significance of this method for the estimation of antimicrobial consumption in WBE. In addition, two last-resort antibiotics, LZD and VAN, and a veterinary specific antibiotic, SMZ, were also detected. Eleven analytes were quantified in treated effluent. The 3 highest concentrations were observed for SPY, TMP, and SMX ranging from 0.053 to 1.1 μg L^{-1}. There were still many chemicals that were below our LODs in Australian wastewater. The main reasons could be that: 1) some antibiotics are not registered for use in Australia, such as ENR, SAR, DMC, SDM, SMT; 2) some chemicals are veterinary specific antibiotics, like CTF, FF, SMR, TUL, VIR-S, which were not expected to be in domestic wastewater; 3) chemicals were unstable in wastewater since some degraded products could be seen at relatively high concentrations but

Table 3
Accuracy and precision of antimicrobials and their metabolites in wastewater influent using matrix-matched calibration curves.

Class	Name	Accuracy (%, $n = 7$)			Precision (%, $n = 7$)			LOD (μg L^{-1})	LOQ (μg L^{-1})
		Low	Medium	High	Low	Medium	High		
Penicillin	AMX	108	94	95	5	5	2	0.0083	0.050
	AMXa	96	93	110	12	4	6	0.19	0.50
	AMP	84	93	98	5	4	2	0.0079	0.050
	CLX	120	89	112	14	11	19	0.023	0.050
	DLX	113	93	102	19	10	6	0.061	0.10
	FLX	115	107	102	7	13	7	0.12	0.50
	PenV	98	95	93	11	7	3	0.18	0.50
	PenVa	96	105	106	17	11	8	0.0056	0.010
	PIP	98	112	106	8	5	8	0.014	0.050
Cephalosporin	CFC	89	98	100	6	5	3	0.011	0.050
	CFX	81	84	98	17	4	5	0.28	0.50
	CFL	100	105	107	11	7	9	0.018	0.050
	CFZ	80	91	103	4	5	7	0.0068	0.050
	CFP	77	121	109	15	11	13	0.048	0.10
	CTF	103	94	102	9	3	4	0.0015	0.0050
	CTR	100	104	106	5	5	4	0.0089	0.050
	CRX	115	98	98	15	7	7	0.24	0.50
Quinolone	CIP	83	76	95	15	5	3	0.025	0.050
	deCIP	76	116	107	4	10	4	0.013	0.10
	ENR	121	93	99	4	3	3	0.0065	0.050
	MOX	95	103	87	17	6	5	0.0056	0.010
	MOX-SO_4	117	94	98	12	4	3	0.039	0.10
	NOR	115	98	95	9	3	4	0.0031	0.010
	deNOR	104	100	97	4	6	5	0.0012	0.010
	OFL	92	96	102	3	3	3	0.0011	0.010
	dmOFL	84	101	105	3	3	1	0.0048	0.050
	SAR	88	100	104	8	6	4	0.014	0.050
	OXO	105	96	97	6	5	3	0.021	0.10
Sulfonamide	SCP	84	97	100	5	4	2	0.00077	0.0050
	SDZ	96	97	101	7	2	1	0.0012	0.0050
	aSDZ	115	96	99	8	3	2	0.0013	0.0050
	SDM	98	98	101	3	2	2	0.00048	0.0050
	SMR	107	97	97	5	2	3	0.00077	0.0050
	aSMR	117	85	99	10	4	3	0.0016	0.0050
	SMZ	75	95	102	7	1	1	0.0024	0.010
	aSMZ	112	88	104	6	3	4	0.0021	0.010
	SMT	75	95	100	8	3	3	0.0014	0.0050
	SMX	102	90	103	20	2	2	0.0067	0.010
	aSMX	103	95	101	11	1	2	0.0036	0.010
	SPY	92	107	118	16	5	4	0.0051	0.010
	aSPY	95	97	100	23	3	3	0.0076	0.010
	SLZ	79	95	97	11	2	4	0.0037	0.010
	STZ	95	97	98	7	3	1	0.0011	0.0050
	aSTZ	116	89	102	17	4	6	0.0028	0.0050
Macrolide	AZI	108	91	103	4	5	6	0.00069	0.0050
	dmAZI	106	100	100	6	6	5	0.00097	0.0050
	CLA	77	117	109	11	5	10	0.0018	0.0050
	ERY-18	97	109	119	9	10	10	0.014	0.050
	ERY	98	108	100	12	6	6	0.19	0.50
	dmERY	98	89	92	8	8	12	0.28	1.0
	ROX	88	80	94	4	8	7	0.00067	0.0050
	dcROX	75	79	91	25	5	8	0.0041	0.0050
	SP-I	100	90	94	6	4	3	0.0014	0.0080
	SP-III	101	87	97	10	1	3	0.0033	0.010
	maSP-II	93	98	101	5	3	1	0.00050	0.0020
	daSP-II	104	96	98	9	4	2	0.00030	0.0010
	maSP-III	80	98	100	11	2	2	0.00082	0.0022
	daSP-III	108	96	95	12	3	2	0.00043	0.0011
	SP-II	88	100	104	8	6	4	0.00050	0.0020
	TIL	86	91	96	4	4	3	0.0068	0.050
	TUL	95	113	111	13	15	11	0.021	0.050
	TYL	81	104	97	18	13	8	0.0060	0.010
	VIR-M	100	99	105	9	8	6	0.015	0.050
	VIR-S	81	100	99	8	14	7	0.0027	0.010
Tetracycline	CTC	122	87	95	7	2	3	0.0022	0.010
	DMC	109	98	100	6	6	2	0.010	0.050
	DOX	100	92	98	14	4	4	0.0047	0.010
	MIN	117	85	96	8	3	4	0.0013	0.0050
	OTC	120	93	101	7	4	4	0.0011	0.0050
	TET	85	96	104	6	5	3	0.0020	0.010
Azole	FCZ	92	79	97	15	3	2	0.0024	0.0050
	FNO	106	96	99	8	4	6	0.0012	0.0050
	MTZ	110	88	99	8	2	2	0.0013	0.0050

(*continued on next page*)

Table 3 (*continued*)

Class	Name	Accuracy (%, $n = 7$)			Precision (%, $n = 7$)			LOD (μg L^{-1})	LOQ (μg L^{-1})
		Low	Medium	High	Low	Medium	High		
	hMTZ	108	86	96	24	5	4	0.0039	0.0050
Lincosamide	CLI	81	94	95	9	3	3	0.0014	0.0050
	CSO	113	99	101	13	4	2	0.0043	0.010
	LIN	100	92	101	12	1	4	0.0020	0.0050
Rifamycin	RFP	110	105	91	13	10	7	0.021	0.050
	RFX	96	92	98	11	5	2	0.0017	0.0050
	daRFX	87	112	107	7	9	12	0.0024	0.010
Amphenicol	CHL	87	107	110	23	10	6	0.038	0.050
	ANP	86	107	102	14	6	3	0.045	0.10
	FF	87	107	107	12	7	5	0.019	0.050
	FFA	116	119	115	4	8	9	3.5	25
β-lactamase inhibitor	TAZ	109	102	97	5	4	3	0.0081	0.050
	TAZ-M	148	139	131	16	11	16	0.27	0.50[a]
Diaminopyrimidine	TMP	117	82	93	10	9	9	0.0050	0.010
	hTMP	115	96	102	7	3	5	0.0012	0.0050
Oxazolidinone	LZD	99	99	115	5	4	4	0.00090	0.0050
	PNU	85	100	102	4	4	4	0.0072	0.050
Aminoglycoside	GEN1	98	78	88	6	4	6	0.060	0.30
	GEN1a	81	79	99	6	8	8	0.020	0.11
	GEN2	115	110	90	9	13	10	0.14	0.50
	NEO	86	81	94	5	4	3	0.081	0.50
Cyclic polypeptide	COL-A	117	83	94	4	4	6	0.62	5.0
	COL-B	89	93	99	4	6	3	3.0	25
Fusidane	FA	100	85	81	13	7	3	0.050	0.050
Carbapenem	MER	99	108	102	11	5	4	0.019	0.050
Nitrofuran	NIT	99	90	99	7	6	4	0.022	0.10
Ionophore	SAL	102	89	96	7	5	10	0.0012	0.0050
Glycopeptide	VAN	78	95	96	14	7	7	0.024	0.050
Disinfectant	BAC-10	150	111	91	21	13	5	0.035	0.099
	BAC-12	147	118	88	20	7	5	16	50
	BAC-14	91	86	86	11	11	12	0.30	0.80
	CHX	99	94	104	11	7	5	0.018	0.050
	p-CA	82	96	105	3	2	6	0.0050	0.050
	TCC	110	102	104	14	3	2	0.0045	0.010
	TCS	107	101	97	5	4	2	0.0088	0.050
	TCS-SO_4	91	99	99	5	6	4	0.0086	0.050

[a] Chemicals for the purpose of semi-quantification.

their corresponding parent chemicals were all below LODs, such as AMX/AMXa and PenV/PenVa.

3.4. Comparison with previous studies

Table S5 displays the LOQs of selected chemicals from the present study and recently published methods using direct injection or SPE. Compared with the existing direct injection method, our study exhibited lower LOQs for almost all listed chemicals, some of which had over one or close to two orders of magnitude differences, such as SDM, SDZ, SMR, SMX, SPY, AZI, CLA, indicating higher sensitivity of the present method. It should be noted that many analytes, particularly in effluent, achieved LOQs at 0.005 μg L^{-1}, which was based on the lowest concentration for most analytes that were prepared in the calibration curves and passed the validation. Nevertheless, this method still could not achieve LOQs that were subjected to SPE, which allowed LOQs of most substances to be below 0.001 μg L^{-1}. Surprisingly, there were some analytes in the current method that reached similar or even lower LOQs than those in SPE, including AMP (0.05 μg L^{-1} in influent and effluent in our study, 0.185 μg L^{-1} in influent and 0.524 μg L^{-1} in effluent using SPE), CLI (0.005 μg L^{-1} in effluent in our study, 0.00405 μg L^{-1} using SPE), MOX (0.01 μg L^{-1} in influent in our study, 0.0101 μg L^{-1} using SPE), DOX (0.01 μg L^{-1} in influent in our study, 0.0422 μg L^{-1} using SPE), OTC (0.005 μg L^{-1} in influent in our study, 0.0212 μg L^{-1} using SPE), and TMP (0.005 μg L^{-1} in effluent in our study, 0.0031 μg L^{-1} using SPE), which could be attributed to the increased instrumental sensitivity. Additionally, the SPE method is more suitable for environmental matrices with low chemical concentrations, like river water or groundwater. In this study, the majority of the detected chemicals in actual wastewater samples were above our LOQs (Table 5). In this instance, further extraction procedures were deemed unnecessary, and the faster sample pretreatment (filtration) was chosen as the tradeoff with our goal of high-throughput analysis in mind.

4. Conclusions

A comprehensive direct injection method using LC-MS/MS was developed for the wastewater surveillance of 109 antimicrobials and their metabolites covering a broad range of antimicrobial classes. Most analytes achieved acceptable validation results. The LOQs for over half of the analytes were <0.01 μg L^{-1} with the lowest LOQ of 0.0003 μg L^{-1}. The application of this method to wastewater samples collected from WWTPs serving different population sizes in Australia detected 37 antimicrobial residues in influent and 22 in effluent, indicating good applicability of the method to wastewater analysis. The straightforward and fast sample pretreatment process (filtration) made the method easier to automate, more cost-effective, require smaller volumes, reduce degradation, and more suitable for high-throughput workloads.

CRediT authorship contribution statement

Jinglong Li: Conceptualization, Investigation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Katja M. Shimko:** Methodology, Writing - review & editing. **Chang He:** Investigation, Methodology. **Brad Patterson:** Methodology. **Richard Bade:** Writing - original draft, Writing - review & editing. **Ryan Shiels:** Formal analysis. **Jochen F. Mueller:** Resources, Writing - review & editing. **Kevin V. Thomas:** Conceptualization, Funding acquisition,

Table 4
Accuracy and precision of antimicrobials and their metabolites in wastewater effluent using solvent-based calibration curves.

Class	Name	Accuracy (%, $n = 7$)			Precision (%, $n = 7$)			LOD (μg L^{-1})	LOQ (μg L^{-1})
		Low	Medium	High	Low	Medium	High		
Penicillin	AMX	103	93	94	10	3	4	0.017	0.050
	AMXa	97	75	84	6	8	7	0.0094	0.050
	AMP	110	101	105	22	9	7	0.037	0.050
	CLX	86	79	81	4	3	4	0.069	0.50
	DLX	61	71	70	13	4	2	0.043	0.10[a]
	FLX	27	72	71	27	5	4	0.089	0.10[a]
	PenV	103	96	96	15	6	8	0.24	0.50
	PenVa	92	104	106	12	10	5	0.0039	0.010
	PIP	90	103	99	12	7	7	0.020	0.050
Cephalosporin	CFC	84	94	98	17	4	6	0.0055	0.010
	CFX	82	104	102	18	11	6	0.030	0.050
	CFL	85	102	108	11	8	11	0.018	0.050
	CFZ	108	94	98	9	4	4	0.015	0.050
	CFP	92	126	110	5	5	2	0.016	0.10
	CTF	117	79	81	4	4	3	0.00072	0.0050
	CTR	86	108	100	10	7	3	0.032	0.10
	CRX	107	84	83	10	9	5	0.16	0.50
Quinolone	CIP	97	80	80	2	3	4	0.0038	0.050
	deCIP	95	78	79	2	2	4	0.0039	0.050
	ENR	84	97	78	7	6	6	0.023	0.10
	MOX	107	120	109	6	3	2	0.0099	0.050
	MOX-SO_4	107	95	101	6	4	5	0.092	0.50
	NOR	85	83	86	3	3	2	0.0055	0.050
	deNOR	97	84	83	2	4	2	0.0032	0.050
	OFL	105	87	91	2	2	2	0.00074	0.010
	dmOFL	98	77	86	2	2	3	0.0034	0.050
	SAR	95	95	93	8	5	5	0.013	0.050
	OXO	82	82	84	15	4	2	0.049	0.10
Sulfonamide	SCP	111	114	118	11	3	3	0.0018	0.0050
	SDZ	112	104	112	4	4	3	0.00071	0.0050
	aSDZ	119	105	103	5	6	5	0.00083	0.0050
	SDM	118	100	104	3	3	2	0.00056	0.0050
	SMR	122	115	121	3	3	4	0.00046	0.0050
	aSMR	117	115	110	4	4	3	0.0014	0.010
	SMZ	121	98	101	6	3	2	0.00099	0.0050
	aSMZ	117	110	115	6	4	5	0.0011	0.0050
	SMT	116	116	117	7	5	4	0.0011	0.0050
	SMX	97	90	93	15	1	2	0.0025	0.0050
	aSMX	94	95	98	10	2	2	0.0033	0.010
	SPY	109	102	105	18	4	3	0.0029	0.0050
	aSPY	124	107	101	5	2	4	0.0015	0.010
	SLZ	99	107	99	18	3	3	0.0059	0.010
	STZ	119	116	122	10	2	4	0.0017	0.0050
	aSTZ	109	119	119	13	5	7	0.0022	0.0050
Macrolide	AZI	108	91	94	9	5	5	0.0014	0.0050
	dmAZI	80	80	86	5	4	3	0.00076	0.0050
	CLA	125	102	98	12	3	3	0.0019	0.0050
	ERY-18	83	122	110	14	7	17	0.0023	0.0050
	ERY	100	102	101	15	7	5	0.049	0.10
	dmERY	117	117	112	18	6	5	0.30	0.50
	ROX	112	101	103	9	7	9	0.0014	0.0050
	dcROX	110	102	112	10	3	7	0.0017	0.0050
	SP-I	117	75	79	4	1	2	0.0010	0.0080
	SP-III	95	80	90	4	2	3	0.0061	0.050
	maSP-II	83	75	84	2	2	3	0.00070	0.012
	daSP-II	102	80	89	8	2	3	0.00070	0.0030
	maSP-III	122	74	83	6	2	2	0.00044	0.0022
	daSP-III	98	76	87	6	3	3	0.00041	0.0022
	SP-II	92	82	91	3	2	3	0.000030	0.00030
	TIL	80	89	91	7	4	5	0.0024	0.010
	TUL	70	61	77	6	7	7	0.0092	0.050[a]
	TYL	90	101	105	20	7	6	0.0068	0.010
	VIR-M	102	95	98	10	10	5	0.0033	0.010
	VIR-S	89	96	102	14	11	11	0.0022	0.0050
Tetracycline	CTC	109	102	106	7	3	3	0.0022	0.010
	DMC	111	108	109	3	3	2	0.0054	0.050
	DOX	123	92	91	2	3	2	0.0037	0.050
	MIN	118	117	125	5	5	4	0.0082	0.050
	OTC	118	110	115	2	4	3	0.0041	0.050
	TET	93	112	114	4	4	3	0.0013	0.010
Azole	FCZ	79	89	94	19	2	2	0.0032	0.0050
	FNO	102	92	96	7	4	3	0.0011	0.0050
	MTZ	95	93	98	6	2	2	0.00097	0.0050

(*continued on next page*)

Table 4 (*continued*)

Class	Name	Accuracy (%, $n = 7$)			Precision (%, $n = 7$)			LOD (μg L^{-1})	LOQ (μg L^{-1})
		Low	Medium	High	Low	Medium	High		
	hMTZ	92	100	84	16	3	4	0.0027	0.0050
Lincosamide	CLI	107	86	88	3	1	3	0.00046	0.0050
	CSO	114	107	106	8	2	1	0.0013	0.0050
	LIN	119	87	91	4	4	4	0.00067	0.0050
Rifamycin	RFP	86	87	87	7	9	8	0.012	0.050
	RFX	124	113	119	10	5	5	0.0016	0.0050
	daRFX	101	110	113	18	14	16	0.0030	0.0050
Amphenicol	CHL	78	107	108	10	4	4	0.032	0.10
	ANP	40	43	34	3	2	1	0.049	0.50[a]
	FF	79	87	88	14	8	4	0.024	0.050
β-lactamase inhibitor	TAZ	108	125	98	8	3	3	0.013	0.050
	TAZ-M	68	65	53	7	5	3	0.11	0.50[a]
Diaminopyrimidine	TMP	84	91	99	6	4	3	0.0010	0.0050
	hTMP	81	94	101	6	6	4	0.0011	0.0050
Oxazolidinone	LZD	114	91	95	2	2	2	0.00035	0.0050
	PNU	96	295	453	51	45	26	0.050	0.050[a]
Aminoglycoside	GEN1	123	90	91	6	6	3	0.30	1.5
	GEN1a	120	105	102	5	6	4	0.33	2.1
	GEN2	101	98	96	5	10	2	0.83	5.0
	NEO	96	92	87	6	8	8	1.0	5.0
Cyclic polypeptide	COL-A	99	83	90	4	3	4	3.2	25
	COL-B	97	89	96	4	2	3	3.7	25
Fusidane	FA	82	91	81	6	3	6	0.050	0.50
Carbapenem	MER	106	82	76	6	2	3	0.0091	0.050
Nitrofuran	NIT	92	75	76	8	2	2	0.013	0.050
Ionophore	SAL	80	114	102	7	10	7	0.011	0.050
Glycopeptide	VAN	99	80	85	16	6	5	0.026	0.050
Disinfectant	BAC-10	101	87	78	11	6	3	0.0040	0.010
	BAC-12	124	122	97	18	5	4	0.15	0.25
	BAC-14	115	87	77	16	18	14	0.085	0.16
	CHX	105	80	88	11	3	3	0.019	0.050
	p-CA	115	79	84	3	2	2	0.0050	0.025
	TCC	106	98	94	6	3	3	0.0018	0.010
	TCS	93	96	98	10	4	3	0.017	0.050
	TCS-SO_4	101	122	117	13	6	9	0.0042	0.010

[a] Chemicals for the purpose of semi-quantification.

Table 5
Quantification of analytes in wastewater samples.

Class	Compound name	Abbr.	Influent (μg L^{-1})			Effluent (μg L^{-1})		
			Site_1	Site_2	Site_3	Site_4	Site_5	Site_6
Penicillin	Amoxicilloic acid	AMXa	15	20	16			
	Ampicillin	AMP	0.81	0.81	0.47			
	Penicillin V acid	PenVa	0.97	1.9	1.3			
Cephalosporin	Cefepime	CFP	<LOQ	<LOQ	<LOQ			
	Cefaclor	CFC	0.058	0.062		<LOQ	<LOQ	<LOQ
	Cefazolin	CFZ		0.18				
	Cephalexin	CFX	3.2	3.4	1.9		0.052	<LOQ
Quinolone	Ciprofloxacin	CIP	0.59	0.58	0.36	<LOQ	<LOQ	<LOQ
	Desethylene ciprofloxacin	deCIP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	Moxifloxacin	MOX	0.078		<LOQ			
	Norfloxacin	NOR	0.12	0.091	0.069	<LOQ	<LOQ	
	Ofloxacin	OFL	0.061	<LOQ	0.067		<LOQ	
Sulfonamide	Sulfadiazine	SDZ		0.012	0.0072			
	Sulfamethazine	SMZ	0.016	0.011	0.034			
	Sulfamethoxazole	SMX	0.2	1.3	0.35	0.19	0.053	0.25
	Acetyl sulfamethoxazole	aSMX	0.12	0.40	0.23	<LOQ		
	Sulfapyridine	SPY	0.4	2.6	0.92	1.1	0.25	0.79
	Acetyl sulfapyridine	aSPY	0.067	0.15	0.099	<LOQ	<LOQ	<LOQ
	Sulfasalazine	SLZ	0.12			0.032		0.079
Macrolide	Descladinose roxithromycin	dcROX	0.12	0.062	0.07	0.014	0.033	<LOQ
	Erythromycin-18	ERY-18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tetracycline	Doxycycline	DOX	0.49	0.45	0.17	<LOQ	<LOQ	<LOQ
	Minocycline	MIN	0.16	0.084	0.084			
Azole	Fluconazole	FCZ	0.17	0.27	0.11	0.15	0.17	0.25
	Metronidazole	MTZ	0.085	0.028	0.12	<LOQ	0.0089	0.0079
	Hydroxy metronidazole	hMTZ	0.11	0.040	0.13	0.021	0.021	0.045
Lincosamide	Clindamycin	CLI	0.021	0.0096	0.011	0.047	0.033	0.032
	Clindamycin sulfoxide	CSO	0.11	0.11	0.044	0.17	0.048	0.097
	Lincomycin	LIN		0.030	0.0053			<LOQ
Other	Rifaximin	RFX	0.041	0.031	0.027			
	Trimethoprim	TMP	0.43	0.73	0.35	0.11	0.12	0.36
	Linezolid	LZD		0.012	<LOQ			
	Vancomycin	VAN	0.10	<LOQ	0.079			
Disinfectant	Benzalkonium chloride - C12	BAC-12	100	120	160			
	Benzalkonium chloride - C14	BAC-14	1.1	0.98	1.4			
	Chlorhexidine	CHX	2.1	2.9	1.5			
	p-Chloroaniline	p-CA	0.13	0.11	0.050	<LOQ		<LOQ

LOQ, limit of quantification. Influent and effluent site numbers are not related.
Analytes with no values in the columns were below their respective limits of detection.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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