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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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## ARTICLE INFO

## 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

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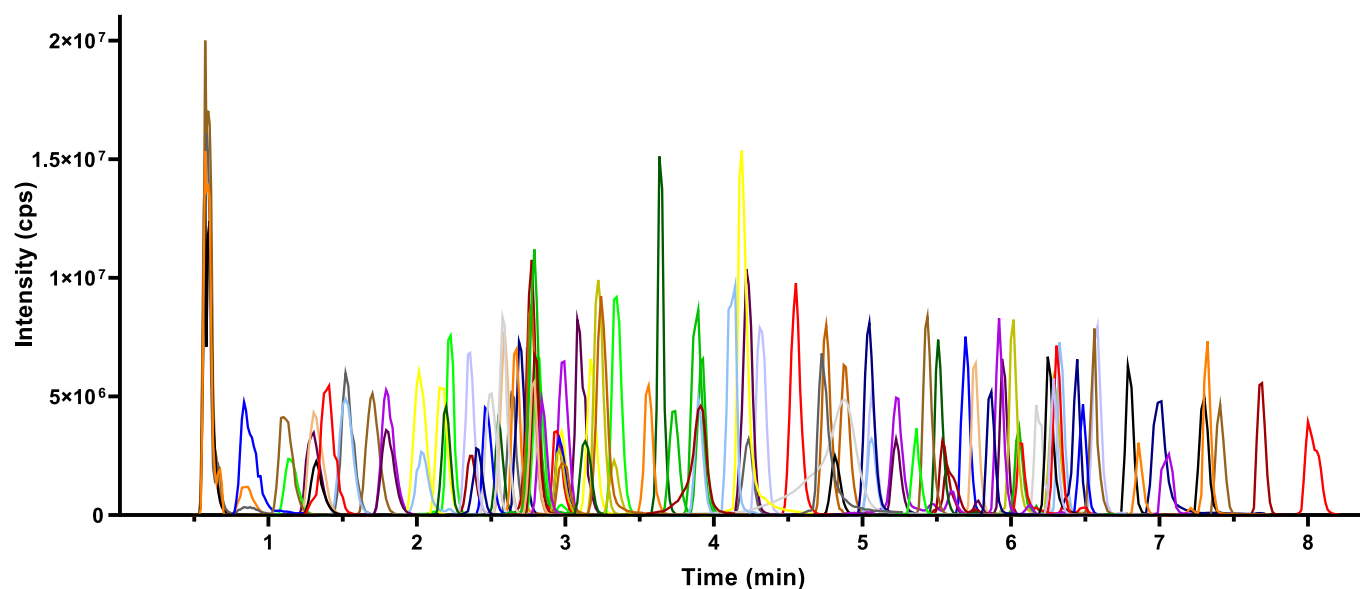
**Fig. 1.** Extracted Ion Chromatograms (XIC) of all 109 compounds in the present method in solvent. The concentration of each compound was adjusted individually to ensure all analytes were at similar intensity to be seen. The XICs have not been smoothed.

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 IS | RT_rel (n = 7) | Calibration curves in solvent R² | Range (µg L⁻¹) | Calibration curves in matrix R² | Range (µg L⁻¹) | Effluent IS | RT_rel (n = 7) | Calibration curves in solvent R² | 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-¹³C4,¹⁵N | 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-¹³C4,¹⁵N | 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-¹³C4,¹⁵N | 1.00 ± 0.00 | 0.998 | 0.10–30 | 0.990 | 0.50–20 | FLX-¹³C4,¹⁵N | 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-¹³C6 | 0.51 ± 0.00 | 0.993[a] | 0.50–30 | 0.993[a] | 0.50–30 | 245-T-¹³C6 | 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-SO₄ | 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 |

**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$_2$O | 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 |

**Table 1** (*continued*)

| Class | Name | Abbr. | RT$_{abs}$ (min; $n = 7$) | Influent IS | RT$_{rel}$ ($n = 7$) | Calibration curves in solvent $R^2$ | Range (µg L$^{-1}$) | Calibration curves in matrix $R^2$ | Range (µg L$^{-1}$) | Effluent IS | RT$_{rel}$ ($n = 7$) | Calibration curves in solvent $R^2$ | 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 |

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 |

**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-SO₄ | 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 |

**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 bio-markers (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 |

**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 |

**Table 4** (*continued*)

| Class | Name | Accuracy (%, $n = 7$) | | | Precision (%, $n = 7$) | | | LOD ($\mu g\ L^{-1}$) | LOQ ($\mu g\ L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| | | 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.

## 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165825.

## References

Aarestrup, F., 2012. Get pigs off antibiotics. Nature 486, 465.

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., et al., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764.

Ahmed, F., Tscharke, B., O'Brien, J.W., Zheng, Q., Thompson, J., Mueller, J.F., et al., 2021. Wastewater-based prevalence trends of gout in an Australian community over a period of 8 years. Sci. Total Environ. 759, 143460.

Bade, R., Tscharke, B.J., White, J.M., Grant, S., Mueller, J.F., O'Brien, J., et al., 2019. LC-HRMS suspect screening to show spatial patterns of new psychoactive substances use in Australia. Sci. Total Environ. 650, 2181–2187.

Berkow, E.L., Lockhart, S.R., 2017. Fluconazole resistance in *Candida* species: a current perspective. Infect. Drug Resist. 10, 237–245.

Bode, C., Muenster, S., Diedrich, B., Jahnert, S., Weisheit, C., Steinhagen, F., et al., 2015. Linezolid, vancomycin and daptomycin modulate cytokine production, Toll-like receptors and phagocytosis in a human in vitro model of sepsis. J. Antibiot. 68, 485–490.

Campos-Manas, M.C., Plaza-Bolanos, P., Sanchez-Perez, J.A., Malato, S., Aguera, A., 2017. Fast determination of pesticides and other contaminants of emerging concern in treated wastewater using direct injection coupled to highly sensitive ultra-high

performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1507, 84–94.

Darby, E.M., Trampari, E., Siasat, P., Gaya, M.S., Alav, I., Webber, M.A., et al., 2023. Molecular mechanisms of antibiotic resistance revisited. Nat. Rev. Microbiol. 21, 280–295.

Denadai, M., Cass, Q.B., 2015. Simultaneous determination of fluoroquinolones in environmental water by liquid chromatography-tandem mass spectrometry with direct injection: a green approach. J. Chromatogr. A 1418, 177–184.

Fawaz, S., Merzouk, M., Barton, S., Nabhani-Gebara, S., 2021. Stability of amoxicillin and clavulanic acid in separate containers for administration via a Y-site. Drug Des. Dev. Ther. 15, 3979–3984.

Gao, J., Li, L., Duan, L., Yang, M., Zhou, X., Zheng, Q., et al., 2022. Exploring antibiotic consumption between urban and sub-urban catchments using both parent drugs and related metabolites in wastewater-based epidemiology. Sci. Total Environ. 827, 154171.

Gonzalez-Marino, I., Rodil, R., Barrio, I., Cela, R., Quintana, J.B., 2017. Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers. Environ. Sci. Technol. 51, 3902–3910.

Goossens, H., Ferech, M., Vander Stichele, R., Elseviers, M., 2005. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 365, 579–587.

Gothwal, R., Shashidhar, T., 2015. Antibiotic pollution in the environment: a review. CLEAN - Soil Air Water 43, 479–489.

Han, S., Li, X.Y., Huang, H.M., Wang, T., Wang, Z.L., Fu, X.F., et al., 2021. Simultaneous determination of seven antibiotics and five of their metabolites in municipal wastewater and evaluation of their stability under laboratory conditions. Int. J. Environ. Res. Public Health 18.

Han, S., Wang, Z., Huang, H., Wang, T., Zhou, Z., Bai, Y., et al., 2022. Estimating antibiotics use in major cities in China through wastewater-based epidemiology. Sci. Total Environ. 826, 154116.

Holton, E., Kasprzyk-Hordern, B., 2021. Multiresidue antibiotic-metabolite quantification method using ultra-performance liquid chromatography coupled with tandem mass spectrometry for environmental and public exposure estimation. Anal. Bioanal. Chem. 413, 5901–5920.

Holton, E., Archer, E., Fidal, J., Kjeldsen, T., Wolfaardt, G., Kasprzyk-Hordern, B., 2022a. Spatiotemporal urban water profiling for the assessment of environmental and public exposure to antimicrobials (antibiotics, antifungals, and antivirals) in the Eerste River Catchment, South Africa. Environ. Int. 164, 107227.

Holton, E., Sims, N., Jagadeesan, K., Standerwick, R., Kasprzyk-Hordern, B., 2022b. Quantifying community-wide antimicrobials usage via wastewater-based epidemiology. J. Hazard. Mater. 436, 129001.

ICH Harmonised Tripartite Guideline, 2005. Validation of Analytical Procedures: Text and Methodology Q2 (R1). International conference on harmonization, Geneva, Switzerland, pp. 1–13.

Ji, Y., Yang, Y., Zhou, L., Wang, L., Lu, J., Ferronato, C., et al., 2018. Photodegradation of sulfasalazine and its human metabolites in water by UV and UV/peroxydisulfate processes. Water Res. 133, 299–309.

Larsson, D.G.J., Flach, C.F., 2022. Antibiotic resistance in the environment. Nat. Rev. Microbiol. 20, 257–269.

Lin, X., Choi, P.M., Thompson, J., Reeks, T., Verhagen, R., Tscharke, B.J., et al., 2021. Systematic evaluation of the in-sample stability of selected pharmaceuticals, illicit drugs, and their metabolites in wastewater. Environ. Sci. Technol. 55, 7418–7429.

Lu, J., Jin, M., Nguyen, S.H., Mao, L., Li, J., Coin, L.J.M., et al., 2018. Non-antibiotic antimicrobial triclosan induces multiple antibiotic resistance through genetic mutation. Environ. Int. 118, 257–265.

Ng, K.T., Rapp-Wright, H., Egli, M., Hartmann, A., Steele, J.C., Sosa-Hernandez, J.E., et al., 2020. High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry. J. Hazard. Mater. 398, 122933.

Palumbi, S.R., 2001. Humans as the world's greatest evolutionary force. Science 293, 1786–1790.

Prieto Riquelme, M.V., Garner, E., Gupta, S., Metch, J., Zhu, N., Blair, M.F., et al., 2022. Demonstrating a comprehensive wastewater-based surveillance approach that differentiates globally sourced resistomes. Environ. Sci. Technol. 56, 14982–14993.

Rousis, N.I., Zuccato, E., Castiglioni, S., 2017. Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. Environ. Int. 99, 213–220.

Samara, E., Moriarty, T.F., Decosterd, L.A., Richards, R.G., Gautier, E., Wahl, P., 2017. Antibiotic stability over six weeks in aqueous solution at body temperature with and without heat treatment that mimics the curing of bone cement. Bone Joint Res. 6, 296–306.

Senta, I., Gracia-Lor, E., Borsotti, A., Zuccato, E., Castiglioni, S., 2015. Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Res. 74, 23–33.

Tandukar, M., Oh, S., Tezel, U., Konstantinidis, K.T., Pavlostathis, S.G., 2013. Long-term exposure to benzalkonium chloride disinfectants results in change of microbial community structure and increased antimicrobial resistance. Environ. Sci. Technol. 47, 9730–9738.

United Nations, 2022. Environmental Dimensions of Antimicrobial Resistance: Summary for Policymakers.

Van Boeckel, T.P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., et al., 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect. Dis. 14, 742–750.

Voigt, A.M., Skutlarek, D., Timm, C., Schreiber, C., Felder, C., Exner, M., et al., 2020. Liquid chromatography-tandem mass spectrometry as a fast and simple method for the determination of several antibiotics in different aqueous matrices. Environ. Chem. 17, 54–74.

Vosough, M., Rashvand, M., Esfahani, H.M., Kargosha, K., Salemi, A., 2015. Direct analysis of six antibiotics in wastewater samples using rapid high-performance liquid chromatography coupled with diode array detector: a chemometric study towards green analytical chemistry. Talanta 135, 7–17.

Wand, M.E., Bock, L.J., Bonney, L.C., Sutton, J.M., 2017. Mechanisms of increased resistance to chlorhexidine and cross-resistance to Colistin following exposure of Klebsiella pneumoniae clinical isolates to chlorhexidine. Antimicrob. Agents Chemother. 61 (e01162-16).

WHO, 2021. WHO Access, Watch, Reserve (AWaRe) Classification of Antibiotics for Evaluation and Monitoring of Use, 2021. World Health Organization, Geneva.

Xu, Z., Du, P., Li, K., Gao, T., Wang, Z., Fu, X., et al., 2017. Tracing methamphetamine and amphetamine sources in wastewater and receiving waters via concentration and enantiomeric profiling. Sci. Total Environ. 601-602, 159–166.

Yuan, S.F., Liu, Z.H., Yin, H., Dang, Z., Wu, P.X., Zhu, N.W., et al., 2019. Trace determination of sulfonamide antibiotics and their acetylated metabolites via SPE-LC-MS/MS in wastewater and insights from their occurrence in a municipal wastewater treatment plant. Sci. Total Environ. 653, 815–821.

Zhang, T., Li, B., 2011. Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. Crit. Rev. Environ. Sci. Technol. 41, 951–998.

Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49, 6772–6782.