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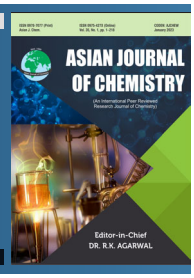

Development and Validation of UPLC-MS/MS Method for Simultaneous Determination of Multi-Class Antibiotics, Antivirals and Antifungals in Water

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According to a WHO report, antimicrobial resistance is one of the top 10 health threats. Environmental sources are extremely contaminated with high levels of antibiotics, antivirals and antifungals due to their excessive and unsafe usage. Hence, there is a need to detect and quantitate the antimicrobial agents in water. Consequently, a simultaneous analytical method is developed and validated, employing a solid-phase extraction methodology with an HLB cartridge. This method was utilized to quantify a minimum of twenty different chemicals derived from antibiotics, antivirals and antifungal drugs. Antibiotics include quinolones, isonicotinic acid, macrolides, oxazolidinones, penicillin, sulphonamides, antivirals that contain nucleotide class compounds and antifungals that comprising of triazoles and imidazoles. Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) was used to quantify the analytes. Detection was achieved using multiple reaction monitoring with positive electron spray ionization. All the compounds were well separated with the column, Xterra MS C18 4.6 × 50 mm 2.5 µm, using water and acetonitrile with 0.1% formic acid as a mobile phase. The calibration curve range was set at 50 to 2000 ng mL^{-1}. Validation parameters like precision and accuracy, matrix effect, sensitivity, autosampler stability, the limit of detection and recovery were performed.

Keywords: Antimicrobials, UPLC-MS/MS, Solid-phase extraction, Antimicrobial resistance, Validation.

INTRODUCTION

The consumption of antimicrobials is directly linked to antimicrobial resistance (AMR) in public health. In the year 2015, WHO established Global Antimicrobial Resistance and Use Surveillance System (GLASS) to monitor AMR in common bacteria and invasive fungi and antimicrobial consumption in humans. The recent GLASS report, 2020, stated that more than a 15% increase in AMR for meropenem and third-generation cephalosporin resistance in bloodstream *E. coli*, ciprofloxacin resistance in *Salmonella* spp. and azithromycin resistance in gonorrhea compared to 2017 [1]. Water sources play a crucial role in the dissemination of antibiotics and that leads to resistance [2]. Release of antibiotics from the hospital, pharma industries, agriculture and animal husbandry waste into various environmental sources has considerably increased. This triggers the development of antimicrobial resistances (AMRs) and antimicrobial resistance genes (ARGs) in the ecosystem. The wastewater treatment plants are meant to control pollution and pathogens but are not designed to inactivate antimicrobial agents [3]. Continuous exposure to sublethal doses of antibiotics leads to the development of drug resistance and the transfer of the resistance genes to other bacteria. Antimicrobial resistance (AMR) has a direct impact on the management and efficacy of illness treatments [4]. Antibiotics are not only found in water sources like wastewater, surface water, household water, *etc.*.

The other sources of antibiotics that have direct or indirect contact with the environment are dairy products, animal feed, food products like honey, meat including chicken, pork, beef, *etc.* and animal feces [5-13].

The other two major elements that contaminate water are antivirals and antifungals. The presence of azole antifungals in the environment is a growing ecotoxicological threat that contributes to the development of drug-resistant fungi in the ecosystem [14]. In particular, the azole fungicide concentration that impacts crops and the inefficient removal of those from water creates significant resistance [15]. Certain fungal species like *Aspergillus*, *Candida*, *Fusarium*, *etc*, are developing resistance against some commonly consumed azole derivative compounds [16]. A tool known as SELECT (SELection Endpoints in Communities of baCTeria), which determines the lowest concentration required for antimicrobial resistance, was followed and a report was generated to re-discover the opportunities to adopt a similar type of approach for the determination of antifungal resistance in the environment [17].

Many studies have also shown widespread accumulation of antiviral drugs in wastewater during the COVID-19 pandemic period due to excessive use of these drugs. Since these drugs are cost-effective and easily available in low and middle income countries, they were used indiscriminately and the development of antiviral resistance is inevitable [18]. The levels of these antiviral agents range from ng L^{-1} to μg L^{-1} in surface waters [19]. Hence, it raises serious concerns about the ecotoxicological effects on living beings that depend on surface water. The risk for ecotoxicology from antivirals like favipiravir, lopinavir and ritonavir was estimated to be high [20] with a risk quotient > 1.

Several LC-MS/MS analytical methods are available for the simultaneous estimation of multiple classes of antimicrobials, including β-lactams, cephalosporins, macrolides, sulfonamides, *etc.* in various water sources such as wastewater from hospitals, water treatment plants, surface and groundwater and environmental samples like soil [4,21-28]. However, this work aims to develop a robust analytical method and validate the same to simultaneously detect antibiotics, antivirals and antifungals in water using tandem mass spectrometry. The validation parameters include precision and accuracy, matrix effect, recovery, autosampler stability, sensitivity and carryover. The method was validated for at least 20 commonly used commercially available compounds including antibiotics from macrolides, quinolones, penicillin, tetracyclines, oxazolidinone, antibacterial and sulfonamides, antivirals from nucleotide analogs and antifungals from triazole and imidazole groups. All these selective compounds have the potential to develop antimicrobial, antiviral and antifungal resistance. The method has a shorter run time of 5 min following an isocratic LC flow for quicker analysis.

EXPERIMENTAL

Acetonitrile, methanol and water were purchased from Biosolve (Biosolve Chimie, France). Formic acid of analytical grade was purchased from Sigma Aldrich. Acquity UPLC and TQD mass spectrometer systems were obtained from Waters Inc. (Milford, USA). Positive pressure SPE manifold was purchased from Athena Technologies, Mumbai, India. Zymark TurboVap LV nitrogen evaporator was purchased from Biotage, India. Hi-Purit Dura HLB SPE cartridges (1 mL tubes with 30 mg sorbent) were procured from National Chromatography. Xterra MS C18, (4.6 mm × 50 mm, 2.5 μ) was procured from Waters Inc., All other reagents used in the study were of analytical grade or higher and procured from standard chemical suppliers. Waters Acquity UPLC from Waters Corporation was used as HPLC System. The reference standards are amoxicillin (AMX), azithromycin (AZI), clarithromycin (CLA), erythromycin (ERY), roxithromycin (ROX), clofazimine (CLO), doxycycline (DOX), ethambutol (ETH), isoniazid (ISO), rifampicin (RIF), linezolid (LIN), moxifloxacin (MOX), ofloxacin (OFL), sulfamethoxazole (SUL), trimethoprim (TRIM), remdesivir (REM), favipiravir (FAV), oseltamivir (OSEL), fluconazole (FLU), ketoconazole (KET), imipramine (internal standard) and were procured from Sigma Aldrich, USA and TCI Chemicals, India.

Preparation of stocks, standards and quality control samples: All the reference standards were weighed and transferred to a suitable container. Dissolved the contents by adding DMSO to achieve 1 mg/mL as a final stock solution. The intermediate or working stock solutions, *i.e.* standards and quality control (QCs), were prepared in water. Standard and QCs were prepared by adding 50 μL of the working solution into 450 μL of blank water. The final concentration of standards (STD 1 to STD 8) ranges from 50, 100, 200, 500, 750, 1000, 1500 and 2000 ng/mL and three QCs *i.e.*, LQC, MQC and HQC with the final concentration of 175, 950 and 1600 ng/mL, respectively.

Similarly, an internal standard stock solution was prepared by weighing and dissolving in DMSO to achieve 1 mg/mL. The internal standard (ISTD) working solution was prepared by adding 50 μL of stock solution into a 100 mL volumetric flask. Made up the volume with acetonitrile that contains 0.1% formic acid. All the stock and working solutions were stored at 4 ºC until use.

LC-MS/MS conditions: Waters UPLC system was equipped with a binary pump, autosampler, column oven and degasser coupled with a mass spectrometric detector (TQD) system. Mass spectrometry was equipped with an ion source with triple quadrupole primarily using the electrospray ionization (ESI) technique. All the compounds were detected using ESI+, whereas favipiravir was detected using ESI-mode. The TQD system detects compounds in multiple reaction monitoring (MRM) modes at unit resolution. Xterra MS C_{18}, (4.6 mm × 50 mm, 2.5 μm) column was used for chromatographic separations. 0.1% formic acid in water was used as mobile phase A (MP-A) and 0.1% formic acid in acetonitrile was used as mobile phase B (MP-B) in the proportion of 25:75 (MP-A:MP-B) at a flow rate of 0.3 mL/min. The injection volume was set at 7 μL whereas the column and autosampler temperature was maintained at 40 and 15 ºC, respectively. Separation of analytes was achieved at 5 min run time using isocratic elution. Mass spectrometry parameters such as collision energy, desolvation gas, desolvation temperature and source temperature were optimized using the auto-tuning mode. Mass spectrometry parameters for all the compounds (except favipiravir) including ISTD were capillary: 4.0 (kV), cone: 30 (V), extractor: 3.0 (V), RF

lens: 0.1, source temperature: 150 ºC, desolvation temperature: 450 ºC, desolvation gas: 900 L/h and cone gas flow: 50 L/h. Whereas for favipiravir, ESI negative, the capillary is fixed at 2.5 kV, the cone is 26 V and other parameters remain the same. For Data acquisition and regression were performed using MassLynx software (Version 4.1) from the Waters UPLC-MS/MS system. The mass spectrometry details are given in Table-1.

Sample preparation and extraction procedure: Standards and QC samples were prepared by adding 50 µL from the working solution to 450 µL of water. Vortexed to ensure proper mixing. To this, 500 µL of ISTD working solution was added and vortexed. Centrifuged at 4500 rpm for 10 min at 4 ºC. Transferred the supernatant to a labeled tube. The solid-phase extraction procedure, using HLB cartridges (1 cc with 30 mg sorbent) was followed to extract the compounds. The extraction procedure is as follows, condition the HLB cartridges with 1 mL of methanol followed by 1mL of ultra-pure water. Load 1 mL of prepared samples into the cartridges. Elute the content by applying nitrogen pressure and then washed the cartridges with 1 mL of 10% methanol in water. Elute the compound by adding 1 mL of 100% methanol that contains 0.1% formic acid in a separate tube. Using a nitrogen evaporator, evaporate the eluted samples under nitrogen pressure for 20 min at 45 ºC. Upon evaporation, reconstitute the dried sample using 100 µL of reconstitution solution that contains 0.1% formic acid in water and 0.1% formic acid in acetonitrile (25:75). Transfer the content into the autosampler vials and load into the autosampler for analysis.

RESULTS AND DISCUSSION

Optimization of LC-MS/MS conditions

Chromatographic conditions: A protein precipitation technique was initially followed for the sample processing method. However, the response of many compounds was not sufficient at lower-level concentrations. Though the precipitation technique is low-cost and easy to perform, the quality of analysis and quantitation cannot be compromised. Hence, a solid-phase extraction technique was used. The analytes were eluted using the HLB sorbents, which are then evaporated and concentrated. The concentrated analytes were reconstituted to achieve to lowest quantitation limit. The 1 cc of 30 mg MCX and MAX SPE cartridges were also used for elution, however, these cartridges failed to elute certain compounds. All the compounds were eluted on HLB cartridges. Different types of chromatography columns such as phenyl, cyano, C_{18} and C_8 columns were checked for the optimization to obtain an acceptable peak shape, column suitability and better response for all the analytes. The above parameters were achieved by using Xterra MS C_{18}, (4.6 mm × 50 mm, 2.5 µm) column. Initially, the mobile phase consists of methanol and water with 0.1% formic acid. However, the peak shape was not acceptable for some antibiotics like doxycycline, amoxicillin and azithromycin. The same was rectified by changing the mobile phase from methanol to acetonitrile. Upon changing the mobile phase, the response was found suppressed for certain other antibiotics. The response was optimized by altering the formic acid strength in the mobile phase. Various formic acid strengths were experimented *i.e.*, from 0.01% to 0.5% in the mobile phase. Finally, 0.1% formic acid in the mobile phase was shown optimum intensity due to its low pH value that helps in enhanced ionization. Since there were no shoulder or merged peaks, the chromatography was achieved on the isocratic flow and no gradient flow was attempted. The separation was well achieved at 40 ºC column temperature compared with 25 and 30 ºC. Since, it is practically difficult to use a deuterated internal standard for every compound, a common analyte that can be well-eluted similar to other analytes

TABLE-1
SUMMARY OF MOLECULAR WEIGHT, CHEMICAL FORMULA AND MASS SPECTROMETRY PARAMETERS FOR ALL THE COMPOUNDS

Antimicrobials	m.w.	m.f.	Parent/daughter ion (*m/z*)	Collision energy (eV)	Cone voltage (V)	RT (min)
Amoxicillin (AMX)	365.40	$C_{16}H_{19}N_3O_5S$	366.2/113.99	22	22	1.2
Azithromycin (AZI)	749.00	$C_{38}H_{72}N_2O_{12}$	749.79/158.17	30	44	1.2
Clarithromycin (CLA)	747.95	$C_{38}H_{69}NO_{13}$	748.76/83.0	45	32	1.1
Erythromycin (ERY)	733.93	$C_{37}H_{67}NO_{13}$	734.74/158.11	22	32	1.2
Roxithromycin (ROX)	837.05	$C_{41}H_{76}N_2O_{15}$	837.94/82.99	50	36	1.2
Clofazimine (CLO)	473.40	$C_{27}H_{22}N_4Cl_2$	473.28/283.41	72	64	1.3
Doxycycline (DOX)	444.40	$C_{22}H_{24}N_2O_8$	445.31/428.30	20	30	1.2
Ethambutol (ETH)	204.31	$C_{10}H_{24}N_2O_2$	205.21/116.07	16	24	1.1
Isoniazid (ISO)	137.14	$C_6H_7N_3O$	137.96/78.85	26	14	1.7
Rifampicin (RIF)	822.94	$C_{43}H_{58}N_4O_{12}$	823.75/791.75	18	32	1.3
Linezolid (LIN)	337.35	$C_{16}H_{20}N_3O_4F$	338.24/296.27	18	36	1.9
Moxifloxacin (MOX)	401.43	$C_{21}H_{24}N_3O_4F$	402.33/110.08	22	44	1.2
Ofloxacin (OFL)	361.37	$C_{18}H_{21}N_3O_4FCl$	361.85/317.77	18	36	1.2
Sulfamethoxazole (SUL)	253.28	$C_{10}H_{11}N_3O_3S$	253.89/97.64	26	16	1.9
Trimethoprim (TRIM)	290.32	$C_{14}H_{18}N_4O_3$	290.91/229.68	24	44	1.2
Remdesivir (REM)	602.59	$C_{27}H_{35}N_6O_8P$	603.51/200.15	42	32	1.9
Favipiravir (FAV)	157.10	$C_5H_4N_3O_2F$	155.90/112.97	14	30	2.0
Oseltamivir (OSEL)	312.40	$C_{16}H_{28}N_2O_4$	312.85/165.50	18	26	1.2
Fluconazole (FLU)	306.27	$C_{13}H_{12}N_6OF_2$	307.20/238.15	16	28	1.8
Ketoconazole (KET)	531.43	$C_{26}H_{28}N_4O_4Cl_2$	531.37/81.96	40	54	1.2
Imipramine (ISTD)	280.41	$C_{19}H_{24}N_2$	281.16/86.03	16	24	1.2

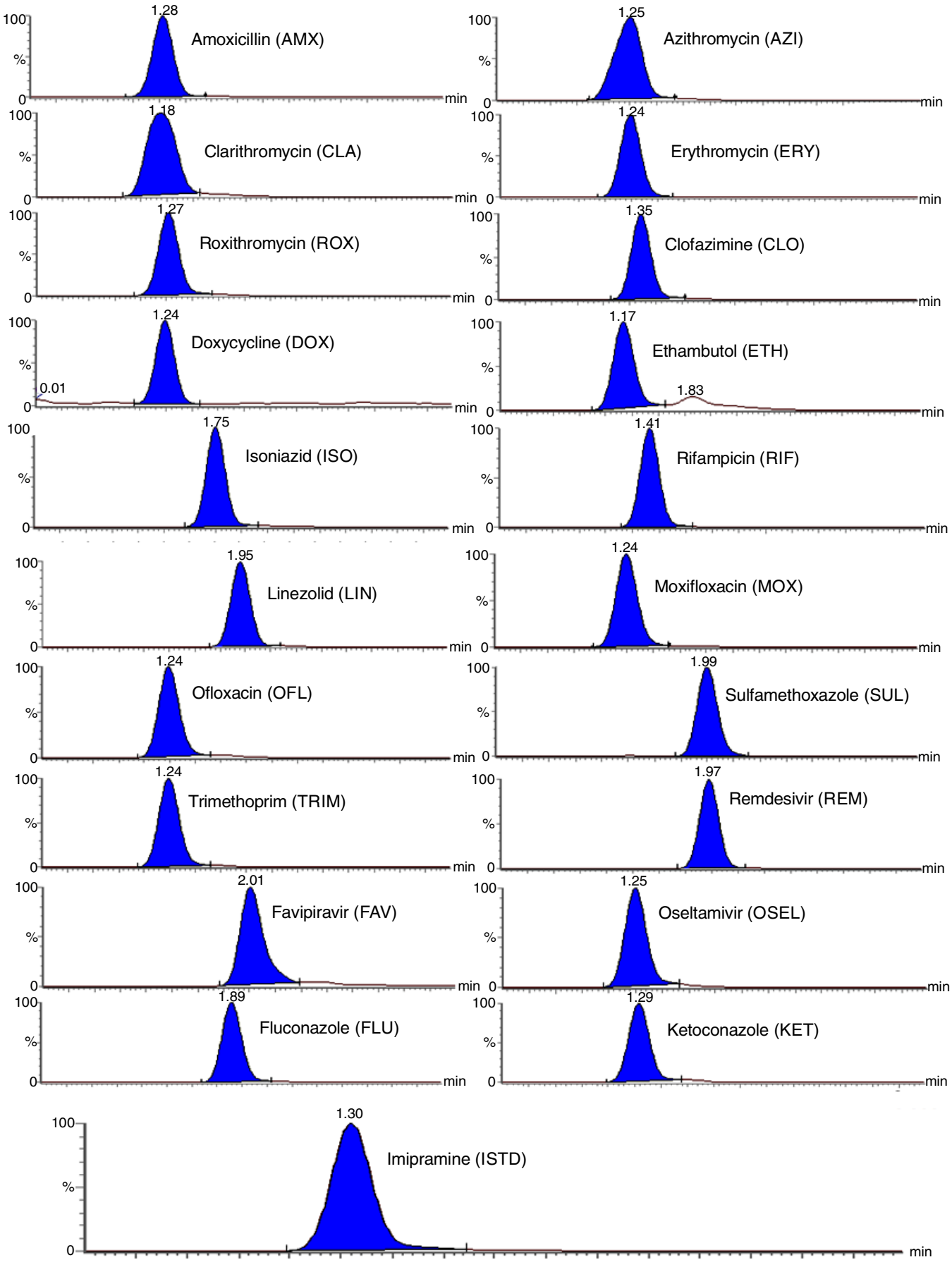

Fig. 1. Representative chromatograms for antibiotics, antivirals and antifungals

was used as an internal standard. Imipramine was used as an internal standard due to its consistent results during the processing and analysis. Other parameters like mobile phase proportions and stock diluent trials were evaluated for their optimum response.

Mass spectrometry: A suitable solvent was used for dissolving the analytes and a tuning solution was prepared at 50 µg/mL concentration to perform mass parameters tuning. All the compounds were optimized using ESI, positive mode except Favipiravir which was optimized with ESI negative mode. Though the source & desolvation temperature and cone & desolvation gas remain the same for all compounds, the collision energy and cone voltage varies according to the sensitivity of the compound. The temperature and gas parameters were optimized based on the observed intensity of the molecules. With the MRM options, the most predominant parent and daughter ions were selected. With the proposed optimized parameters, no suppression in the ionization was found and a maximum intensity was observed. The representative chromatograms of all the analytes were shown in Fig. 1. Also, the functional groups of the antimicrobials are shown in Fig. 2.

TABLE-2
SUMMARY OF THE CORRELATION COEFFICIENT (r^2)

Antimicrobials	CC range (ng/mL)	Average[a] (r^2)
AMX	50-2000	0.9921
AZI	50-2000	0.9934
CLA	50-2000	0.9916
ERY	50-2000	0.9916
ROX	50-2000	0.9949
CLO	50-2000	0.9958
DOX	50-2000	0.9919
ETH	50-2000	0.9930
ISO	50-2000	0.9939
RIF	50-2000	0.9937
LIN	50-2000	0.9924
MOX	50-2000	0.9925
OFL	50-2000	0.9925
SUL	50-2000	0.9923
TRIM	50-2000	0.9957
REM	50-2000	0.9929
FAV	50-2000	0.9930
OSEL	50-2000	0.9953
FLU	50-2000	0.9926
KET	50-2000	0.9980

[a]Average r^2 was calculated from three precision and accuracy batches.

Method validation

Calibration curve: From the three precision and accuracy batches, the average correlation coefficient (r^2) for all the compounds ranged between 0.9916 to 0.9980, respectively. The results are given in Table-2. The calibration curves for 20 antimicrobials are shown in Fig. 3.

Precision, accuracy and carry-over effect: The precision and accuracy for LQC, MQC and HQC ranged between 2.0 to 14.20% and 87.97 to 109.26%, respectively. The obtained results showed that the batches met the acceptance criteria with the limit of ≤ 15% for precision and ± 15% for accuracy. Table-3 summarizes the results of precision and accuracy. No significant peaks were observed in the blank samples that were injected after the upper limit of quantitation samples. This ensured that no carry-over issue with the proposed analytical method.

Recovery, matrix effect and limit of detection: The determination of extraction recovery and matrix effect efficiency was conducted using LQC and HQC level concentrations

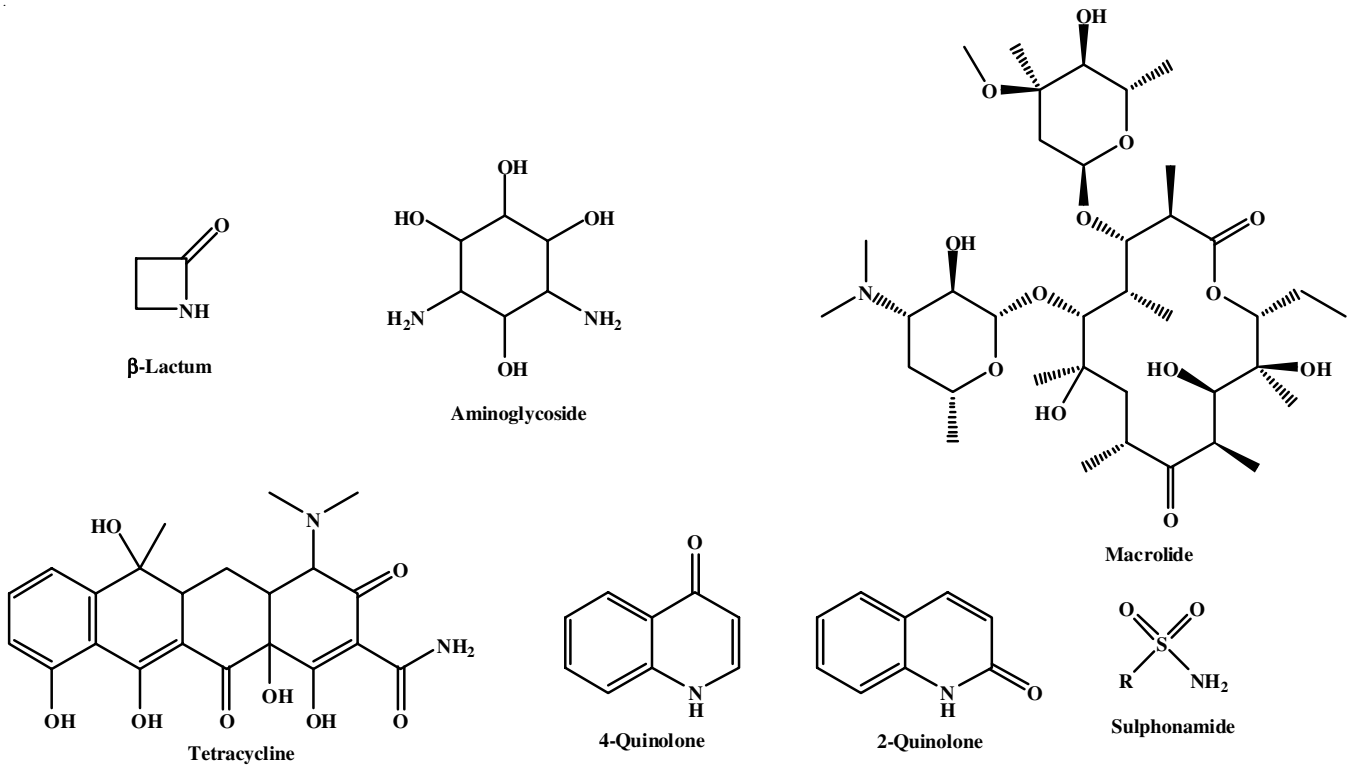

Fig. 2. Functional groups of the major antimicrobials

TABLE-3
SUMMARY OF THE PRECISION AND ACCURACY

Antimicrobials	Precision (% RSD)[a]			Accuracy[b]		
	LQC (%)	MQC (%)	HQC (%)	LQC (%)	MQC (%)	HQC (%)
AMX	13.29	6.83	4.93	92.19	107.68	87.97
AZI	10.15	3.21	3.85	101.07	107.28	99.23
CLA	11.10	5.81	3.73	98.82	101.88	96.81
ERY	6.79	7.98	5.39	89.52	95.96	91.77
ROX	3.99	2.50	2.02	104.38	106.25	94.45
CLO	12.71	6.30	4.82	98.59	107.87	97.73
DOX	12.67	8.87	5.40	98.40	106.86	97.80
ETH	7.29	9.59	8.68	99.58	102.85	103.78
ISO	12.22	4.52	5.65	100.33	102.23	98.98
RIF	11.79	3.42	4.73	97.80	105.48	105.70
LIN	9.18	9.97	12.33	98.86	101.08	97.23
MOX	8.75	5.52	6.27	101.79	107.43	98.73
OFL	5.84	3.66	4.65	100.76	107.30	97.86
SUL	9.70	7.63	10.00	101.64	103.83	98.41
TRIM	11.56	5.24	2.74	100.66	106.54	93.10
REM	13.13	13.20	14.21	95.77	92.87	105.51
FAV	6.63	5.10	3.25	99.02	102.79	94.57
OSEL	6.01	3.56	2.76	105.83	107.70	90.35
FLU	10.85	6.44	7.46	109.26	100.39	96.28
KET	6.15	5.42	4.33	99.92	106.17	99.62

[a]%RSD was calculated from three precision and accuracy batches. [b]Accuracy was calculated against the nominal concentration of the respective QC concentrations.

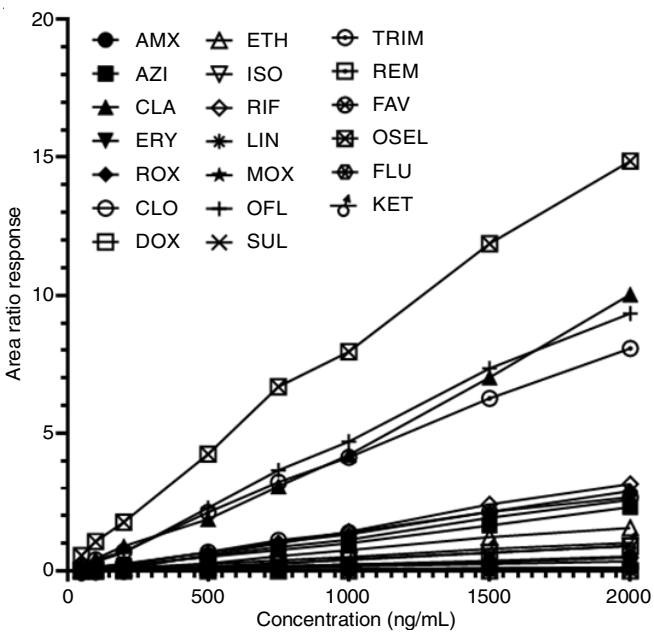

Fig. 3. Representative calibration curve for the antimicrobials from precision and accuracy batch

spiked in water. The matrix effect was performed to ensure that there is no ion suppression and or enhancement in the proposed analytical method. The recovery at both levels for all the compounds ranged between 70.13% to 93.92%. Whereas the matrix effect of the extracted LQC and HQC against the neat solution was found between 70.32% and 105.82%, respectively. The limit of detection was determined using an S/N ratio produced by mass spectrometry. The limit of detection is the lower limit of concentration producing the S/N ratio at the values of three.

The obtained results for recovery, matrix effect and limit of detection are given in Table-4. The average recovery of antimicrobials is presented in Fig. 4.

TABLE-4
SUMMARY OF THE EXTRACTION RECOVERY, MATRIX EFFECT AND THE LIMIT OF DETECTION

Antimicrobials	Recovery[a]		Matrix effect[b]		Limit of detection (ng/mL)
	LQC (%)	HQC (%)	LQC (%)	HQC (%)	
AMX	85.72	89.06	89.04	93.68	7.60
AZI	83.73	84.90	87.43	93.15	17.54
CLA	93.92	83.08	94.51	94.51	10.85
ERY	86.32	84.55	70.32	87.24	20.71
ROX	89.02	87.71	90.86	90.55	18.23
CLO	82.48	90.03	83.87	91.65	15.26
DOX	93.24	96.64	73.51	87.07	12.81
ETH	86.43	74.29	89.93	83.71	15.04
ISO	88.21	85.07	88.02	88.19	12.49
RIF	84.05	79.54	97.36	85.10	26.13
LIN	82.66	84.89	97.00	85.73	11.55
MOX	70.13	86.44	85.36	90.17	3.69
OFL	78.40	85.93	85.68	89.86	16.81
SUL	84.61	80.25	105.82	85.76	4.49
TRIM	85.33	87.85	92.46	89.59	1.68
REM	80.04	73.14	77.90	81.30	11.83
FAV	89.71	89.36	96.53	89.09	12.97
OSEL	88.30	89.07	90.72	92.73	4.04
FLU	86.64	81.49	91.61	90.32	16.56
KET	86.58	88.56	91.08	91.65	7.02

[a,b]Average of five replicates.

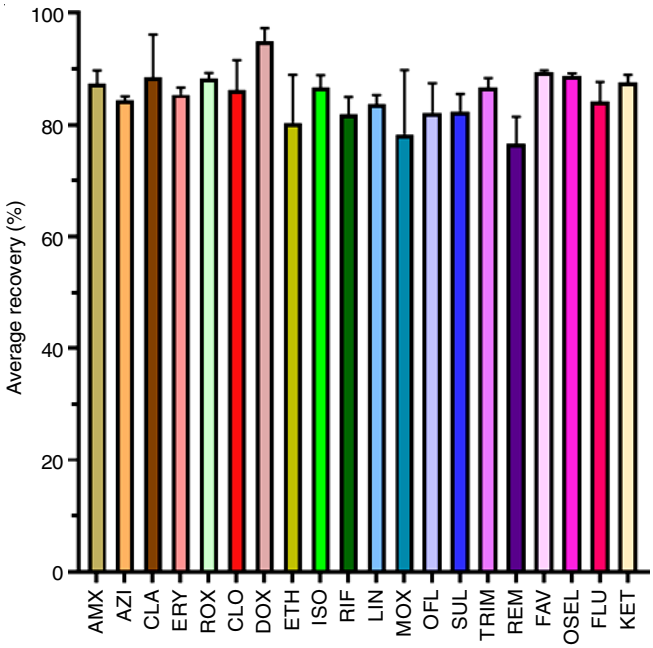

Fig. 4. Average recovery of antimicrobials in water

Sensitivity: Five replicates of the lower limit of quantitation were analyzed against the calibration standards. The average accuracy of LLOQ for all the compounds ranged within ± 20% from the nominal concentration as given in Table-5.

Autosampler stability: The stability of the antimicrobials in the autosampler was determined by storing the processed

TABLE-5
SUMMARY OF SENSITIVITY EXPERIMENT

Antimicrobials	Average LLOQ conc. (ng/mL) of 5 replicates	Average % accuracy of 5 replicates against the nominal concentration (50 ng/mL)
AMX	46.60	93.20
AZI	51.20	102.38
CLA	56.40	112.98
ERY	54.60	108.92
ROX	52.40	104.94
CLO	52.40	104.78
DOX	51.60	103.28
ETH	50.20	100.30
ISO	54.80	110.08
RIF	49.40	99.10
LIN	51.40	102.80
MOX	51.20	102.52
OFL	48.00	96.18
SUL	52.80	105.44
TRIM	54.20	109.10
REM	54.00	107.86
FAV	51.00	101.92
OSEL	52.60	105.44
FLU	55.80	112.12
KET	45.80	91.18

samples within the autosampler for at least duration of 24 h and 32 min. The stability was determined at lower and higher quality control samples against the freshly prepared calibration standards. The autosampler temperature was maintained at 15 ºC. The obtained % bias results are provided in Table-6.

TABLE-6
SUMMARY OF % BIAS OF AUTOSAMPLER STABILITY

Antimicrobials	% Bias [a]LQC (175 ng/mL)	% Bias [b]HQC (1600 ng/mL)
AMX	3.20	-10.14
AZI	2.17	-9.51
CLA	0.11	-9.04
ERY	-8.11	4.60
ROX	10.06	-8.33
CLO	7.62	-9.18
DOX	-2.86	1.04
ETH	-2.17	2.11
ISO	4.46	-8.36
RIF	3.54	-6.64
LIN	-10.51	-9.76
MOX	2.43	-2.43
OFL	-4.91	-7.24
SUL	-3.14	-14.25
TRIM	5.49	-7.34
REM	-4.69	-4.03
FAV	3.54	-11.60
OSEL	3.20	-12.66
FLU	-12.57	-5.53
KET	8.00	-1.04

[a,b]Average of five replicates.

Conclusion

In this study, a novel analytical approach was developed and validated to identify and quantify antimicrobial compounds frequently detected in water. The method demonstrated rapidity, sensitivity, high reliability and reproducibility, employing LC-MS/MS. The solid-phase extraction efficiency was achieved using HLB cartridges. The analytical method was found to be more precise and accurate within ± 20% of standard deviation and accuracy. Since, it is expensive to use isotopically labeled internal standards for all the compounds, imipramine which was compatible with the analysis was chosen as an internal standard. All the compounds were eluted within 5 min of run time with the least matrix effect. The executed validation parameters like precision and accuracy, sensitivity, recovery, matrix effect, the limit of detection and stability show that the validated method is reliable for the detection of commonly used antimicrobials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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