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Development and Validation of UFLC-MS/MS Analytical Method for the Simultaneous Quantification of Antibiotic Residues in Surface Water, Groundwater, and Pharmaceutical Waste Water Samples from South India

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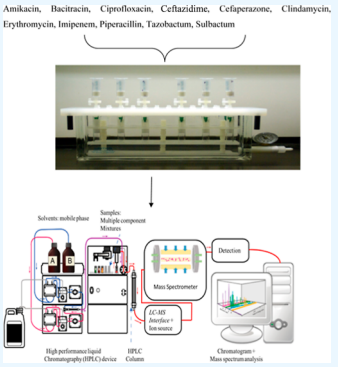
ABSTRACT: Antibiotic residues in pharmaceutical wastewater pose a significant environmental concern due to their potential role in fostering antimicrobial resistance. South Indian pharmaceutical companies produce a wide range of antibiotics. As a result, the industries that discharge water may include antibiotic residues, which could be harmful to the environment. In this study, a novel, quick, accurate, and sensitive approach for the simultaneous detection of 11 antibiotics was established, and triple quadrupole mass spectrometry, ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS), and selective solid-phase extraction (SPE) were used for validation. Utilizing a mixed mode reversed-phase/cation-exchange cartridge (SPE using Strata X, 33 μm), the single-cartridge extraction procedure was performed and validated. Relative standard deviations for most of the antibiotics ranged from 3.5 to 0.56 with recoveries ranging from 57 to 85%. The samples were injected into the UFLC-MS/MS apparatus at a volume of 10 μL for analysis. The auto sampler cooler temperature was kept at 150 °C, while the column temperature was kept at 40 °C. After validation, the technique was determined to be linear in the range of 2.0−1000.0 ng/mL. The retention period for antibiotics was between 1.2 and 1.5 min. Antibiotics transitions for multiple reaction monitoring were between 235.1/105.9 and 711.5/467.9 m/z. The method of analysis took 2.5 min to run completely. Antibiotic residues were efficiently analyzed using the established analytical approach in pharmaceutical wastewater (influent and effluent), surface, and groundwater. Eleven antibiotics were found in the water samples during examination with concentrations ranging between 2.313 and 95.744 ng/L. The procedure was shown to be much more environmentally friendly than other contemporary methods based on the green analytical procedure index's evaluation of greenness. Blue applicability grade index tool indicated the developed method's practicality in comparison with that of other reported method.



INTRODUCTION

Medicines can end up in the environment for several reasons, including the release of leftover medicines into the water system by humans and animals. The incorrect disposal of these pharmaceuticals in water resources has led to the discovery of drug residues in many countries and contaminated water supplies, which has a knock-on effect on the environment and living things both directly and indirectly.[1] Although antibiotics are thought to be harmful to human health, a significant source of contamination is their widespread use in the environment, and if these medications are not properly disposed, it could contribute to the growth of resistant bacteria. However, if the medications are not destroyed or diminished, they will straightly enter drinking water, groundwater, and surface water.[2,3] Unused antibiotics, on the other hand, are frequently disposed of through the sewage system.

High aqueous solubility and low degradability make antibiotics easily pass through filtration processes and end up in drinking water.[4,5] So there is a chance that wastewater treatment in the pharmaceutical industry could contain traces of antibiotics. These have received increased attention and contribute to the growth of antibiotic-resistant bacteria in both natural settings and other environments.[6,7] Antibiotics have drawn great attention as a consequence of the development of resistant bacteria. Fluoroquinolones are broad-spectrum antibiotics that are effective against a variety of pathogenic illnesses, especially β-lactam amoxicillin, ciprofloxacin, and levofloxacin. The growth of multidrug-resistant germs that are resistant to aminoglycosides, macrolide, and beta-lactam have been demonstrated to be inhibited by ciprofloxacin.[8−11]

A surge in the formation of resistant bacteria that harms human health has been connected to low levels of antibiotics. Investigations have demonstrated the existence of micro-

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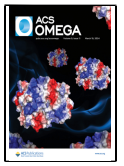

organisms resistant to antibiotics in drinking water systems in the US and Europe.[12,13] Cross-resistance may emerge because veterinary and human drugs share a similar identity.[6,13] Due to antibiotic contamination, antibiotics in the environment and in different aquatic systems, including drinking water, surface water, groundwater, municipal wastewater, hospital wastewater, and industrial wastewater, has drawn considerable attention.[7,14,15]

When it comes to antibiotics, comprehensive preclinical and clinical testing are necessary to ascertain their viability prior to commercialization, as long as they are categorized as environmental pollutants.[16] Antibiotic risk assessments for humans have been studied in the UK, Australia, and the US, where the minimal therapeutic dose, or lowest active drug concentration, of an antibiotic in drinking water is typically more than a thousand times lower (WHO, 2011).[17]

One of the largest challenges to world health is antibiotic resistance, which can afflict people of any age in any nation and raise hospital stays, death rates, and related expenses. Pharmaceutical industry is feeling the rise of superbugs through pollution in its supply chains. According to recent research, certain wastewater effluents from factories that manufacture antibiotics include a significant amount of antibiotics, which contaminate lakes and rivers.

To determine the occurrence of antibiotics in pharmaceutical industrial wastewater, surface water, and groundwater across South India, serving as a baseline for future influent, effluent, and surface water treatment approaches and to ascertain the extent of antibiotic residue levels, research studies were carried out in response to the antibiotics' concentration in aquatic environments.[18−21] Solid-phase separation techniques, high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and capillary electrophoresis have been used to extract antibiotics from a variety of samples for identification and quantification.[9]

There are few works published for the quantification of antibiotics from various medicinal classes in ground and surface wastewaters. Developing a technique for a laboratory may require balancing costs, time commitment, and research objectives. Certain approaches that have been described for quantifying residual antibiotics in influent, effluent, groundwater, and surface water are assumed to use costly liquid−liquid extraction sample extraction techniques. Few liquid chromatography-tandem mass spectrometry (LC−MS/MS) techniques were reported for the analysis of pharmaceutical substances in effluent, influent, surface, groundwater, and wastewater treatment plant using solid phase extraction (SPE),[22−25] electrospray ionization source,[26,27] and MS/MS.[28] ultra-performance LC (UPLC)-MS/MS methods have also been reported.[29,30]

The intention of the present research is to establish an advanced ultra-fast LC (UFLC)-MS/MS analytical technique that would be sensitive enough to find any probable antibiotic residues in pharmaceutical industry wastewater as well as in surrounding surface and groundwater. Target medications were chosen due to their widespread use among humans and aquatic organisms, as described in the scientific literature.

In this paper we, present for the first time, a sensitive, selective, and advanced analytical technique for the simultaneous estimation of 11 antibiotics in surface, ground, and pharmaceutical wastewater samples in south India using SPE-UFLC-MS/MS. Chromatographic conditions have been optimized and validated using this technique in compliance with USFDA regulations. Compared to the other available approaches, this leads to a very sophisticated, easy, time-saving, and sensitive quantification technique.

The GAPI green chemistry tool was used throughout the investigation, from sample collection, extraction, and cleanup to final quantification by the instrument to assess the greenness of the method. A new metric tool called blue applicability grade index (BAGI) could be used to assess an analytical method's feasibility.

MATERIALS AND METHODS

Chemicals and Reagents. Ceftazidime, cefaperazone (Orchid Pharma limited, Chennai), sulbactum, piperacillin, tazobactum, ciprofloxacin, amikacin (MMC Health Care Ltd., Chennai), imipenem (Aurobindo, Visakhapatnam) erythromycin, clindamycin (Intermed 4GK industrial estate, Chennai), and bacitracin (Yancheng youhua pharmaceuticals and chemical technology co., Ltd., China) analytical standards used were of high-purity grade (>99%).

Ultrapure water (Millipore, Billerica, MA), HPLC-grade methanol (Thermo Fisher scientific, India), acetonitrile (Merck Specialties Pvt. Ltd. Germany), HPLC-grade water (Rankem, Delhi), formic acid, 0.1% (Fisher scientific, Waltham, MA, USA), 10 mMammonium formate, and buffer, 500 μL (Thermo Fisher scientific, India) were used in this study.

Instrumentation. The instrument used for the analysis is linear triple quadrupole active pharmaceutical ingredients (API) 4000 from Sciex (USA), combined with an UFLC system from Shimadzu (Shimadzu, Japan). Analysis 1.6.3 software was used for estimation.

Column Details. The cartridges used for SPE were Strata X 33 μm, reverse phase 30 mg/1CC from Inertsil ODS C18 analytical column (50 mm × 4.6 mm, 5 μm particle size, Phenomenex, USA). 0.5 mL/min was the flow rate set for the analytical method.

Sample Collection. The pharmaceutical factories producing antibiotic drugs [API + Drug Profile] located in southern India (Chennai, Tamil Nadu; Nellore, Andhra Pradesh; Hyderabad, Telangana; Kottayam, Kerala; and Bangaluru, Karnataka) contributed a total of 55 water samples, of which 25 samples were influent, 15 samples were effluent, 5 samples were ground, and 10 samples were surface water (Figure 1). The samples were obtained and transported to the lab in 1 L sterile polypropylene bottles using a thermo cold box and analyzed.

The selection of 11 antibiotics is based on the pharmaceutical industries, which are involved in the manufacturing of selected antibiotics in South India. A diverse range of antibacterial and antimicrobial was incorporated in the list of selected molecules (Table S1).

For the simultaneous estimation of antibiotics (ceftazidime, cefaperazone, sulbactum, piperacillin, tazobactum, ciprofloxacin, imipenem, amikacin, erythromycin, clindamycin, and bacitracin) in HPLC-grade water, a reliable extraction method was devised in the current work.

Based on their therapeutic effectiveness and physiochemical characteristics, target chemicals are categorized in Table S2.

Methodology. For analysis, 10 μL of the samples were injected to the UFLC-MS/MS system. The column temperature and auto sampler cooler temperature were maintained at 40 and 15 °C. The method was linear in the range of 2.000−1000.00 ng/mL. Antibiotics retention time was ranges from 1.2

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Figure 1. Water sample sites (south India).

to 1.5 min. The multiple reaction monitoring transitions for antibiotics were between 235.1 and 105.9 m/z and 711.5/467.9 m/z. The total run time is 2.5 min. Utilizing this technique, the amount of antibiotic contamination in effluent, influent, surface water, and groundwater samples from South India could be measured.

Preparation of Buffer. A volume of 1000 mL was achieved by adding 990 mL of Millipore water to 1 mL of formic acid.

Mobile Phase Preparation. Before use, 20 mL of buffer and 80 mL of HPLC-grade methanol were mixed and sonicated in a 20:80 v/v ratio before use.

Preparation of Standard. The appropriate quantity of each antibiotic was dissolved in a methanol/water (50:50% v/v) mixture to obtain a stock solution (1.0 mg/mL). Aliquot of 0.2 μL of antibiotics respective stock dilution, spiked in blank water, and final volume was made up with plasma to obtain 10 mL.

Standard Stock Preparation in Diluents. Using a mixed standard solution containing 20 μL (of each analyte), eight replicate samples were spiked to a concentration of 980 μL of each analyte to give eight concentrations to each analyte ranging from 100 to 120 μL and analyzed. 0.1% formic acid was added in methanol, and the aliquot liquid is vortexed and centrifuged. After that, the aliquot supernatant was collected and passed to the conditioned Strata X 33 cartridge. The samples were collected in auto sampler vials and quantified via UFLC-MS/MS (Figure 2).

Mobile Phase Extraction. Mobile phase used is methanol and water with formic acid (0.1%) in the ratio of 80:20% v/v. 200 μL of the effluent, influent, surface, and groundwater samples were used for sample preparation.

Solid phase extraction. A 200 μL sample of influent, effluent, surface, or groundwater was mixed with 200 μL of formic acid (0.1%) in water and vortexed well. 1 mL of methanol followed by 1 mL of water were used to condition the cartridges. After this, the samples were percolated over the cartridge under vacuum at a pressure of −10 KPa. 1 mL of water and 1 mL of 10% methanol in water were used to wash the cartridges once each. After the cartridges were dried, 1 mL of methanol was used to elute the cartridges. In the low-volume evaporator, a nitrogen stream was used to dry the eluted solution. The dried residue was reconstituted using 80:20% v/v of methanol and water (200 μL) solution, and the sample was transferred into injector vial containing a glass

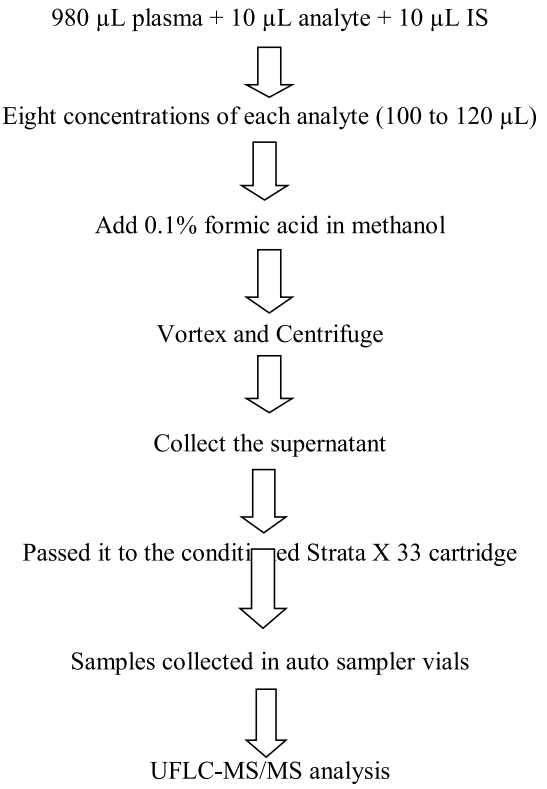

Figure 2. Flowchart of sample preparation.

insert into it. The analysis was performed using UFLC-MS/MS (Figure S1).

Chromatographic Conditions. A constant temperature of 40 °C was maintained for the auto sampler. Ultrasonically, the mobile phase was degassed and then used after passing through a 0.22 m Millipore membrane filter. Maintaining a temperature of 40 °C in the column oven, LC ran for 2.5 min. The rinse solution contained methanol and HPLC-grade water in a ratio of 80:20 v/v. The analysis was performed on the API 4000 UFLC-MS/MS system, which was provided with an autoinjector. The acquisition and processing of the mass data were controlled by the Analyst 1.6.3 software, and the details are given in Table 1. The mass spectrometer's positive mode operating conditions were as mentioned in Table 2.

Method Development and Optimization. In order to attain optimal chromatographic conditions, the mobile phase underwent optimization to yield adequate selectivity and sensitivity within brief separation duration. By using methanol, the peak symmetry was improved, and the analysis time was shortened. The Inertsil ODS C18, 50, 4.6 mm, and 5 μm

Table 1. UFLC-MS/MS Conditions Triple Quadrupole Mass Spectrometer

stationary phase	intersil ODS C18 (5 μm, 50 mm × 4.6 mm)
mobile phase	80:20% v/v of methanol and water with 0.1% formic acid
diluent	methanol/water (70:30)
flow rate	0.5 mL/min
injection volume	10 μL
column oven temperature	40 °C
auto sampler	10 °C
run time	2.5 min

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Table 2. UFLC-MS/MS Chromatographic Conditions for Antibiotics

scan type	MRM
polarity	positive
ion source	turbo spray
source temperature (at set point)	300 °C
mass (Da)	59.000 to 2997.200
channel electron multiplier	2100.0
deflector	100.0
software version	Analyst 1.6.3

analytical column was chosen after many sources of columns were analyzed. This column offered the highest chromatographic performance and appropriate peak characteristics, including the tailing factor. Additionally, a satisfactory resolution of antibiotics was achieved, demonstrating the suggested method's capacity to indicate stability. With the mobile phase flowing at a rate of 0.5 mL/min, a decent separation with acceptable peak symmetry and a stable baseline were obtained.

Method Validation. The whole process was validated in accordance with FDA guidelines.[31] System suitability, selectivity, linearity, sensitivity, [limit of detection (LOD), limit of quantitation (LOQ)], specificity, recovery studies, reproducibility, and repeatability were established in order to assess the SPE-UFLC-MS/MS method's accuracy and precision. Stability testing was not conducted; instead, blank plasma and analyte at a moderate concentration level were spiked and left on the benchtop for 4−6 h at room temperature. Subsequently, extraction procedure was carried out using the benchtop sample.

System Suitability. On every day of the study period, a system suitability test was conducted to verify the detector's response to the analyte before the analytical batch run.

Selectivity. By contrasting the chromatograms produced from the samples containing antibiotic standard to those produced from blank samples, the method's selectivity was assessed.

Linearity. Regression analysis was used to establish the linearity between the peak area and concentration. The linearity of response was determined with standard solutions of concentration ranging from 2 to 1000 ng/mL of 11 antibiotics. The linearity was evaluated at various ranges for different antibiotics with a coefficient of variation of 0.99 or higher for all of the analytes.

LOQ and LOD. The signal-to-noise ratios of 3:1 and 10:1 were used to calculate the LOD and instrument LOQ, respectively. By dividing the average concentration obtained for the blank by two and ten times its standard deviation, respectively, the LOQs and LODs were determined.

Specificity. The method's specificity was verified by analysis of 11 antibiotic samples and a blank.

Recovery Studies. The recovery of 11 antibiotics (n = 3) from plasma [ethylenediaminetetraacetic acid (EDTA)] was performed by contrasting peak area ratios of extracted samples near the low to high QC levels with the peak area ratios of extracted blank matrix spiked with 11 antibiotics postextraction.

Reproducibility. The intermediate precision of the test method was determined across different analysts when compared with the repeatability study.

Repeatability. Precision was determined by measuring quality control pools prepared at the lower LOQ, the approximate midpoint of calibration range, and approximately 85% of the upper LOQ.

Green Assessment of the Developed Method by GAPI. The developed UFLC-MS/MS analytical method for the simultaneous quantification of antibiotic residues in surface, ground, and pharmaceutical wastewater samples in South India (M.III) was compared with two other existing methods by (M.I)—Meritxell et al. (2006)[26] and M.II—Lacey et al. (2008)[27] to determine which method was more environmentally friendly. A total of 15 parameters were taken into consideration for the green assessment.[32]

Assessment of the Practicality of the Method by BAGI. The following primary characteristics are taken into consideration for evaluating the applicability of an analytical method by the BAGI metric tool.

1. The steps of the analytical determination
2. The sample preparation phase
3. Both steps

The steps of the analytical determination are represented by attributes 1−3 (Table S3), the sample preparation phase by attributes 4 and 5 (Table S3), and both steps by attributes 6−10 (Table S3).[33]

RESULTS AND DISCUSSION

Simultaneous quantification of antibiotics in single method analysis, in terms of ionization, sample extraction suitability with possible antibiotics, and chromatography, is very challenging. Several trial and hit methods were followed for chromatography optimization to bring all compounds' peak within the shortest run time and advanced instrumentation. Possibilities were tried to estimate more antibiotics within a single LC−MS run with a multiple transition monitoring method. The UFLC-MS/MS is an advance methodology and is more sensitive (i.e., individual analyte detection limits shown in ng/L). The procedure was linear between 2.0 and 1000.0 ng/mL.

System Suitability. On every day of the study period, a system suitability test was conducted to verify the detector's reaction to the analyte before the analytical batch run.

Selectivity. Drug and internal standard retention times were recorded without the presence of any interfering endogenous chemical peak (Figure S2).

Linearity. Regression analysis was used to establish linearity between the peak area and concentration. The linearity of the method in the solvent was evaluated at various ranges for different antibiotics with a coefficient of variation of 0.995 or higher for all of the analytes. Linearity R^2 was found to be >0.9. The linearity graphs of all 11 antibiotics are given in Figure S3 and Table 3.

LOQ and LOD. Detection limits obtained are reported in Table 3. LOQ ranged from 2.22 to 2.494 ng/L, and LOD ranged from 0.4419 to 0.4988 ng/L for wastewater, surface water, and groundwater.

Specificity. No interference was detected at each target compound's mass transition within ±2.5% of the retention time, indicating good specificity. The chromatograms of 11 antibiotic samples are listed in Figure S4.

RSD and Recovery Studies. Relative standard deviation ranges from 0.56 to 3.50%. Recoveries range from 57.62 to

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Table 3. Quantitation Detection Linearity, LOD, LOQ, and Volume Determined for Target Antibiotics by UFLC-MS/MS

analyte	linearity (R^2)	LOD (ng/L)	LOQ (ng/L)	volume (mL)
amikacin	0.9973	0.4689	2.245	10
bacitracin	0.9988	0.4724	2.362	10
ciprofloxacin	0.9965	0.4988	2.494	10
ceftazidime	0.9984	0.4648	2.324	10
cefaperazone	0.9979	0.4444	2.222	10
clindamycin	0.9988	0.4436	2.218	10
erythromycin	0.9945	0.4419	2.269	10
imipenem	0.9984	0.4544	2.272	10
piperacillin	0.9956	0.4548	2.274	10
tazobactum	0.9971	0.4456	2.222	10
sulbactum	0.9956	0.4444	2.222	10

85.27 for wastewater, surface water, and groundwater, which are tabulated in Table 4.

Table 4. Recoveries and RSD for LC−MS/MS Monitoring

s. no	drugs	RSD (%)	recoveries
1	amikacin	3.50	60.37
2	bacitracin	2.14	78.26
3	ciprofloxacin	1.56	71.46
4	ceftazidime	2.14	67.34
5	cefaperazone	3.12	57.62
5	clindamycin	2.72	85.27
7	erythromycin	2.57	82.46
8	imipenem	2.21	69.35
9	piperacillin	1.96	61.05
10	tazobactum	0.56	63.57
11	sulbactum	3.46	62.57

Reproducibility and Repeatability. Repeatability ranges from 1.84 to 6.59% for wastewater, surface water, and groundwater. Reproducibility results range from 1.56 to 7.35%. Matrix effect for each antibiotic was estimated by spiking low- and high-quality control samples in matrix (e.g, environmental sample-specific) and extracted using optimized procedure. Calibration curve samples were prepared in neat samples. Both calibrators and matrix-spiked quality control samples were extracted as per the optimized extraction method. Calibration standards and matrix QC samples were analyzed via LC-MS/MS. Average % nominal accuracy was reported as ion suppression/ion enhancement for each of the antibiotics. The outcomes are tabulated in Table 5.

Optimal declustering potential (DP), entrance potential (EP), collision energies (CEs), collision cell exit potential, typical retention time (RT), and selected reaction monitoring (SRM) conditions are examples of analyte-based metrics (CXP) in the MS/MS mode and are shown in Table 6 for every component and the usual product ions produced in these circumstances. The MRM transition for antibiotics were amikacin-350.3/105.9 (m/z), bacitracin −711.5/198.9 (m/z), ciprofloxacin −331.6/230.9 (m/z), ceftazidime −547.2/467.9 (m/z), cefaperazone −644.1/114.9 (m/z), clindamycin −427.2/379.0 (m/z), erythromycin −362.1/318.2 (m/z), imipenem −299.5/141.8 (m/z), piperacillin −540.1/397.9 (m/z), tazobactam −299.2/253.0 (m/z), and sulbactum −235.1/139.8 (m/z). Dwell time range: 200 ms. DP ranges from 13 to 128 V. EP ranges from 10 V. CXP ranges from 15 V. CE ranges from −11 to 46 V, and RT ranges from 0.98 to 2.57 min for wastewater, surface water, and groundwater.

Antibiotic Estimation. Quantification of antibiotics was performed by the UFLC-MS/MS method, and the amounts of antibiotics present in various water samples are given in Table 7. The highest value for influent water observed ranged from 95.744 ng/L for bacitracin to 2.414 ng/L for ciprofloxacin, effluent water ranges from 2.013 ng/L for piperacillin to 3.251 ng/L for erythromycin, surface water ranges from 2.313 for erythromycin to 6.697 for amikacin, and ground water ranges from 2.003 ng/L for amikacin to 54.971 ng/L for clindamycin-(Figure S5).

Table 7 summarizes the results, which indicated that 11 of the 15 target drugs were identified. Ten antibiotics in influent samples, 4 antibiotics in effluent samples, 7 antibiotics in surface water, and 8 antibiotics in groundwater samples were detected; however, their concentrations were above the LOQ, and those of only 3 drugs (amikacin, ciprofloxacin, and ceftazidime) were below the LOQ. Two antibiotics (bacitracin and clindamycin) were present in the influent samples collected. One antibiotic (clindamycin) was present in the surface water samples collected. The absence of clindamycin and bacitracin in effluent samples suggests that these substances were eliminated throughout the treatment procedure. But clindamycin is present in surface and groundwater (5.4 and 55.0 ng/L, respectively). Also, bacitracin (2 ng/L) was detected in groundwater. Over the course of the treatment, ceftazidime at very low levels rose, while the concentrations of amikacin, erythromycin, and piperacillin in

Table 5. Reproducibility of the Method and Ion Enhancement due to Matrix

		% RSD (n = 3)		
s. no	drugs	reproducibility	repeatability	% ion suppression/ion enhancement (%)
1	amikacin	7.17	6.59	87
2	bacitracin	2.68	1.84	104
3	ciprofloxacin	3.84	3.95	108
4	ceftazidime	7.35	6.26	89
5	cefaperazone	6.12	5.93	93
6	clindamycin	3.84	2.84	97
7	erythromycin	3.57	3.73	93
8	imipenem	6.36	4.83	112
9	piperacillin	4.90	3.67	90
10	tazobactum	1.56	4.85	94
11	sulbactum	4.67	4.93	105

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Table 6. RT and SRM Conditions for Antibiotics by UFLC-MS/MS

compounds	time (m sec)	precursor ion (m/z)	product ion (m/z)	dwell time (ms)	DP (V)	EP (V)	CXP (V)	CE (V)	RT (min)
amikacin	200	350.3	105.9	200	128	10	15	30	0.79
bacitracin	200	711.5	198.9	200	95	10	15	30	2.41
ciprofloxacin	200	331.6	230.9	200	110	10	15	46	0.98
ceftazidime	200	547.2	467.9	200	−85	10	15	−35	1.74
cefaperazone	200	644.1	114.9	200	−85	10	15	−11	1.73
clindamycin	200	427.2	379.0	200	13	10	15	27	2.41
erythromycin	200	362.1	318.2	200	80	10	15	30	1.77
imipenem	200	299.5	141.8	200	120	10	15	37	1.98
piperacillin	200	540.1	397.9	200	52	10	15	23	1.87
tazobactam	200	299.2	253.0	200	−54	10	15	−30	1.80
sulbactum	200	235.1	139.8	200	−31	10	15	−17	2.57

Table 7. Concentrations of Antibiotics (ng/L) in Waste Water, Surface Water, and Groundwater

		waste water			
s. no	antibiotic	influent water	effluent water	surface water	ground water
1	amikacin	0.013−8.424	0.414−2.313	0.011−6.697	<0−2.003
2	bacitracin	0.011−95.744	ND	ND	<0−6.567
3	ciprofloxacin	0.081−2.414	ND	0.016−5.449	ND
4	ceftazidime	ND	<0−2.413	0.3−2.739	ND
5	cefaperazone	0.012−6.697	ND	ND	0.2−3.419
6	clindamycin	0.014−24.748	ND	0.014−5.405	0.3−54.971
7	erythromycin	0.016−7.202	0.012−3.251	0.059−2.313	0.4−6.697
8	imipenem	0.012−7.242	ND	ND	0.018−3.251
9	piperacillin	0.016−8.434	0.018−2.013	ND	0.1−2.424
10	tazobactum	0.1−3.251	ND	0.6−3.251	0−2.414
11	sulbactum	0.011−5.449	ND	0.019−2.971	0.2−3.251

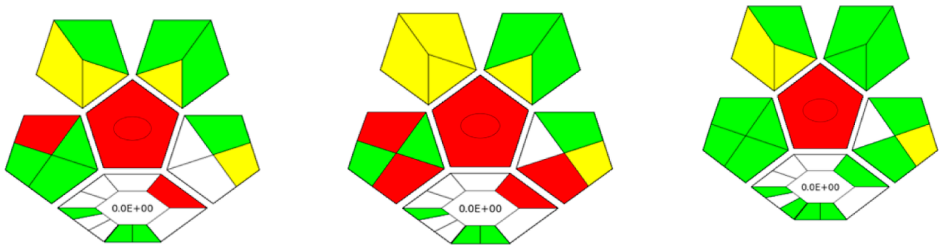

Figure 3. Comparison of the developed method's green profile with that of reported methods using GAPI tools.

effluent samples remained lowered. Ciprofloxacin, cefaperazone, imipenem, tazobactum, and sulbactum seemed to be totally eliminated (Table 7). Ceftazidime is not detected in the influent sample, but it is present in effluent and surface water. The compounds may be present in the influent as conjugated metabolites that are cleaved to liberate the current molecule during the treatment process, hence the absence of the compounds in the influent. The concentrations of amikacin, erythromycin, and sulbactum in surface water and groundwater remain decreased. Cefaperazone and imipenem were detected in groundwater, but the concentrations were below the LOQ. Tazobactum and sulbactum were also detected in surface and groundwater, but the concentrations were below the LOQ.

The concentrations of 9 antibiotics (amikacin, bacitracin, cefaperazone, clindamycin, erythromycin, imipenem, piperacillin, tazobactum, and sulbactum) in influent water were found to be above the LOQ. However, the concentrations of ciprofloxacin and ceftazidime were found to be below the LOQ. In effluent water, the concentrations of ceftazidime and erythromycin were above the LOQ, while the concentrations of the other 9 antibiotics (amikacin, bacitracin, cefaperazone, clindamycin, ciprofloxacin, imipenem, piperacillin, tazobactum, and sulbactum) were below the LOQ; surface water showed the highest LOQ for 6 antibiotics(amikacin, ciprofloxacin, ceftazidime, clindamycin, tazobactum, and sulbactum) and low LOQ for 5 antibiotics (bacitracin, cefaperazone, erythromycin, imipenem, and piperacillin). In groundwater, 8 antibiotic samples (bacitracin, cefaperazone, clindamycin, erythromycin, imipenem, piperacillin, tazobactum, and sulbactum) were detected, with concentrations above the LOQ, while amikacin, ciprofloxacin, and ceftazidime showed concentrations below the LOQ.

GAPI Assessment. The current study uses the GAPI tool, which consists of pictograms representing 15 different characteristics, to evaluate the MRM system's greenness in surface, ground, and pharmaceutical wastewater samples in South India (M.III.). Following sample collection, extraction, and cleanup to final quantification by the equipment, these characteristics were applied and contrasted with two previously used techniques in surface, wastewater treatment plant influent, and effluent samples (M. I, M. II). GAPI-guided comparison of the developed method's green profile to that of

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the current techniques for residue analysis in surface water, groundwater, and pharmaceutical wastewater samples is mentioned in Table S4 and Figure 3. The developed method M.III analyzed 11 antibiotics in a 2.5 min single run, whereas M.I analyzed 29 antibiotics in 20 min run method, and M.II analyzed 20 antibiotics in 55 min run time. The research indicates that compared to the other techniques in the study, the developed method (M.III) is safer and much greener in terms of sample preparation, reagents and solvents, instruments, and preanalytical procedure (Table 8).

Table 8. Characteristics of the GAPI and a Comparison of the Proposed Technique with the Current Techniques for Antibiotic Residue Analysis in Surface Water, Groundwater, and Pharmaceutical Wastewater Samples in South India

indexing parameters	Meritxell et al. (2006)	Lacey et al. (2008)	proposed method
Sample Preparation			
collection	green	green	green
preservation	red	red	green
transport	green	green	green
storage	red	red	yellow
type of method	red	red	red
scale of extraction	yellow	yellow	yellow
solvents/reagents used	yellow	yellow	yellow
additional treatments	yellow	green	green
Reagents and Solvents			
amount	yellow	yellow	yellow
health hazard	green	green	green
safety hazard	green	green	green
Instrumentation			
energy	white	white	white
occupational hazard	green	green	green
waste	yellow	white	yellow
waste treatment	red	white	green
Method Type			
types of analysis	qualitative and quantitative		
Pre-Analysis Processes			
yields	not applicable	not applicable	not applicable
temperature/time	red	red	green
Relation to Green Economy			
number of rules met	not applicable	not applicable	not applicable
Instrumentation			
technical setup	not applicable	not applicable	green
Workup and Purification			
end products workup, purification	not applicable	not applicable	not applicable
yield	not applicable	not applicable	not applicable

BAGI Assessment. Table S3 and Figure 4 present a comparative analysis between the methodologies employed in the current research and those in previous studies of a similar nature. The table illustrates that the approaches utilized in the present research are more advanced than those employed by earlier researchers. Within the BAGI tools, a dark blue shade corresponds to 10 points, blue to 7.5 points, light blue to 5 points, and white to 2.5 points. Consequently, our findings reveal that the practicality of the current analytical methods surpassed that of prior research, with a score of 62.5, compared to 57.5 (Meritxell et al., 2006) and 60.0 (C. Lacey et al., 2007) for the earlier methodologies.

CONCLUSIONS

The sustainability of the environment and public health are seriously threatened by antibiotic residues in the environment. It is imperative to prioritize rigorous monitoring and regulatory measures to control and reduce antibiotic residues. Hence, we could safeguard against antibiotic resistance and ensure the long-term efficacy of antibiotics. As an initiative to address this global challenge, an advanced SPE-UFLC-MS/MS technique was established and validated for the simultaneous detection of 11 antibiotic residues out of 55 samples from several therapeutic classes with various physical and chemical properties. For all antibiotic residues from wastewater (influent and effluent), surface water, and groundwater samples, a single SPE method by UFLC-MS/MS in both negative and positive negative ionization modes was used. The nanogram per liter range corresponded to the individual analyte detection limits for the overall technique. This demonstrates how well this method works for checking the pharmaceutical contaminated wastewater, surface water, and groundwater.

The greenness and practicality of the developed method were assessed using GAPI and BAGI tools. According to GAPI index parameters, the developed method is far greener than the other techniques. Our findings reveal that the practicality of the developed method surpassed that of prior research. BAGI makes it simple to assess the effectiveness of various analytical techniques and to pinpoint a method's strong and weak aspects in terms of application and practicality. We conclude that the chemical community will start to trust and comply with the BAGI metric instrument in addition to receiving attention.

Nine to ten pharmaceutical residues were simultaneously determined using water and acetonitrile as mobile phase by Bui von et al. 2021[30] and Mostafa et al. 2018[24] and the flow rate was 12−15 mL/min and 0.6 mL/min, respectively, whereas in the developed method, 11 antibiotics were quantified, and 0.5 mL/min is the flow rate. The unique aspects of the novel developed method are the shorter run time, improved sensitivity, and advanced instrumentation, i.e., UFLC-MS/

Figure 4. BAGI index pictograms for three different analytical methods.

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MS, which offers the capability for high sample throughput. The proposed method could achieve the quantification within 2.5 min. By using this method, samples were separated with sufficient accuracy, precision, specificity, linearity, and sensitivity. Hence, the proposed green method could be successfully employed in the simultaneous quantification of antibiotic residues in aqueous samples.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c08566.

> Green assessment of the developed method by GAPI, assessment of the practicality of the method by BAGI, therapeutic class, structure and CAS no value for chosen analytes, target compounds and their physio-chemical properties, BAGI parameters and comparison between the existing method and developed method for residue analysis in surface, ground and pharmaceutical wastewater samples in South India, characteristics of the GAPI and a comparison of the proposed technique with the current techniques for residue analysis in surface water, ground water, and pharmaceutical wastewater samples in South India, flowchart of SPE, typical blank chromatograms for erythromycin and ciprofloxacin antibiotics, linearity graphs, chromatograms , and concentrations of antibiotics (ng/L) in wastewater, surface water, and groundwater (PDF)

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K.S. study design; K.S. sampling; K.S., K.S., data analysis; K.S. funding; K.S., K.S. manuscript drafting; all authors provided helpful recommendations and comments and approved the final version of the manuscript.

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Notes

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■ REFERENCES

(1) Grujić, S.; Vasiljević, T.; Laušević, M. Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography-ion trap-tandem mass spectrometry. *J. Chromatogr. A* **2009**, *1216* (25), 4989−5000.

(2) Larsson, D. J.; de Pedro, C.; Paxeus, N. Effluent from Drug Manufactures Contains Extremely High Levels of Pharmaceuticals. *J. Hazard. Mater.* **2007**, *148* (3), 751−755.

(3) Kümmerer, K. Antibiotics in the Aquatic Environment - a Review - Part I. *Chemosphere* **2009**, *75* (4), 417−434.

(4) Diwan, V.; Tamhankar, A. J.; Khandal, R. K.; Sen, S.; Aggarwal, M.; Marothi, Y.; Iyer, R. V.; Sundblad-Tonderski, K.; Stålsby-Lundborg, C. Antibiotics and Antibiotic-Resistant Bacteria in Waters Associated with a Hospital in Ujjain, India. *BMC Publ. Health* **2010**, *10* (1), 414.

(5) Kummerer, K. Significance of Antibiotics in the Environment. *J. Antimicrob. Chemother.* **2003**, *52* (2), 317.

(6) Voolaid, V.; Jõers, A.; Kisand, V.; Tenson, T. Co-Occurrence of Resistance to Different Antibiotics among Aquatic Bacteria. *BMC Microbiol.* **2012**, *12* (1), 225.

(7) Heidari, M.; Kazemipour, M.; Bina, B.; Ebrahimi, A.; Ansari, M.; Ghasemian, M.; Amin, M. M. A Qualitative Survey of Five Antibiotics in a Water Treatment Plant in Central Plateau of Iran. *J. Environ. Public Health* **2013**, *2013*, 1−9.

(8) World Health Organization. Department of Essential Drugs and Medicines Policy. *Bundesgesundheitsblatt Gesundheitsforsch.Gesundheitsschutz*; Springer, 2005; Vol. *48*, pp 221−231.

(9) Koczura, R.; Mokracka, J.; Jabłońska, L.; Gozdecka, E.; Kubek, M.; Kaznowski, A. Antimicrobial Resistance of Integron-Harboring Escherichia Coli Isolates from Clinical Samples, Wastewater Treatment Plant and River Water. *Sci. Total Environ.* **2012**, *414*, 680−685.

(10) Xi, C.; Zhang, Y.; Marrs, C. F.; Ye, W.; Simon, C.; Foxman, B.; Nriagu, J. Prevalence of Antibiotic Resistance in Drinking Water Treatment and Distribution Systems. *Appl. Environ. Microbiol.* **2009**, *75* (17), 5714−5718.

(11) Hösl, J.; Gessner, A.; El-Najjar, N. Liquid Chromatography-Tandem Mass Spectrometry for the Quantification of Moxifloxacin, Ciprofloxacin, Daptomycin, Caspofungin, and Isavuconazole in Human Plasma. *J. Pharm. Biomed. Anal.* **2018**, *157*, 92−99.

(12) Peris-Vicente, J.; Peris-García, E.; Albiol-Chiva, J.; Durgbanshi, A.; Ochoa-Aranda, E.; Carda-Broch, S.; Bose, D.; Esteve-Romero, J. Liquid Chromatography, a Valuable Tool in the Determination of Antibiotics in Biological, Food and Environmental Samples. *Microchem. J.* **2022**, *177*, 107309.

(13) Kim, H. Y.; Baldelli, S.; Märtson, A. G.; Stocker, S.; Alffenaar, J.-W.; Cattaneo, D.; Marriott, D. J. E. Therapeutic Drug Monitoring of the Echinocandin Antifungal Agents: Is There a Role in Clinical Practice? A Position Statement of the Anti-Infective Drugs Committee of the International Association of Therapeutic Drug

Monitoring and Clinical Toxicology. *Ther. Drug Monit.* **2022**, *44* (1), 198−214.
(14) Decosterd, L. A.; Mercier, T.; Ternon, B.; Cruchon, S.; Guignard, N.; Lahrichi, S.; Pesse, B.; Rochat, B.; Burger, R.; Lamoth, F.; Pagani, J.-L.; Eggimann, P.; Csajka, C.; Choong, E.; Buclin, T.; Widmer, N.; André, P.; Marchetti, O. Validation and Clinical Application of a Multiplex High Performance Liquid Chromatography − Tandem Mass Spectrometry Assay for the Monitoring of Plasma Concentrations of 12 Antibiotics in Patients with Severe Bacterial Infections. *J. Chromatogr. B* **2020**, *1157*, 122160−160.
(15) (a) Zhang, M.; Moore, G. A.; Chin, P. K.; Everts, R.; Begg, E. J. Simultaneous Determination of Cefalexin, Cefazolin, Flucloxacillin, and Probenecid by Liquid Chromatography−Tandem Mass Spectrometry for Total and Unbound Concentrations in Human Plasma. *Ther. Drug Monit.* **2018**, *40* (6), 682−692. (b) Xie, F.; Colin, P.; Van Bocxlaer, J. Zwitterionic hydrophilic interaction liquid chromatography-tandem mass spectrometry with HybridSPE-precipitation for the determination of intact cisplatin in human plasma. *Talanta* **2017**, *174*, 171−178.
(16) Sime, F. B.; Roberts, M. S.; Roberts, J. A.; Robertson, T. A. Simultaneous determination of seven β-lactam antibiotics in human plasma for therapeutic drug monitoring and pharmacokinetic studies. *J. Chromatogr. B* **2014**, *960*, 134−144.
(17) Piestansky, J.; Cizmarova, I.; Mikus, P.; Parrak, V.; Babiak, P.; Secnik, P.; Secnik, P.; Kovac, A. An Ultra-High-Performance Liquid Chromatography−Tandem Mass Spectrometry Method for Simultaneous Determination of 4 β-Lactam Antibiotics, Tazobactam, and Linezolid in Human Plasma Samples. *Ther. Drug Monit.* **2022**, *44* (6), 784−790.
(18) Zander, J.; Maier, B.; Suhr, A.; Zoller, M.; Frey, L.; Teupser, D.; Vogeser, M. Quantification of Piperacillin, Tazobactam, Cefepime, Meropenem, Ciprofloxacin and Linezolid in Serum Using an Isotope Dilution UHPLC-MS/MS Method with Semi-Automated Sample Preparation. *Clin. Chem. Lab. Med.* **2015**, *53* (5), 781.
(19) Paal, M.; Zoller, M.; Schuster, C.; Vogeser, M.; Schütze, G. Simultaneous Quantification of Cefepime, Meropenem, Ciprofloxacin, Moxifloxacin, Linezolid and Piperacillin in Human Serum Using an Isotope-Dilution HPLC−MS/MS Method. *J. Pharm. Biomed. Anal.* **2018**, *152*, 102−110.
(20) Ferrari, D.; Ripa, M.; Premaschi, S.; Banfi, G.; Castagna, A.; Locatelli, M. LC-MS/MS Method for Simultaneous Determination of Linezolid, Meropenem, Piperacillin and Teicoplanin in Human Plasma Samples. *J. Pharm. Biomed. Anal.* **2019**, *169*, 11−18.
(21) Mortensen, J. S.; Jensen, B. P.; Zhang, M.; Doogue, M. Preanalytical Stability of Piperacillin, Tazobactam, Meropenem, and Ceftazidime in Plasma and Whole Blood Using Liquid Chromatography−Tandem Mass Spectrometry. *Ther. Drug Monit.* **2019**, *41* (4), 538−543.
(22) Chan, C.; Wai, H. K.; Wu, P.; Lai, S.; Chan, O. S.; Tun, H. M. A Universal LC-MS/MS Method for Simultaneous Detection of Antibiotic Residues in Animal and Environmental Samples. *Antibiotics* **2022**, *11*, 845−917.
(23) Botero-Coy, A. M.; Martínez-Pachón, D.; Boix, C.; Rincón, R.; Castillo, N.; Arias-Marín, L.; Manrique-Losada, L.; Torres-Palma, R.; Moncayo-Lasso, A.; Hernández, F. 'An investigation into the occurrence and removal of pharmaceuticals in Colombian wastewater. *Sci. Total Environ.* **2018**, *642*, 842−853.
(24) Mostafa, A.; Shaaban, H.; Alasmry, K.; Alshammari, A.; Alsuwaigh, R.; Aga, O. Development and validation of SPE-LC-MS method for simultaneous determination of selected pharmaceuticals in hospital wastewater. *J. Anal. Pharmaceut. Res.* **2018**, *7* (6), 611−615.
(25) Beatriz, B.; Raquel de O, V.; Mariana M, F.; Victor, C.; Allan M, J.; Josiane Mmf, S.; Eric L, D.; Patrício Guillermo-Peralta, Z.; Roberto, P. HPLC-MS/MS Method for Quantification of Pharmaceuticals in Subtropical Rivers and Water Treatment Plants in Brazil. *J. Environ. Sci. Public Health.* **2020**, *04* (04), 390−408.
(26) Lacey, C.; McMahon, G.; Bones, J.; Barron, L.; Morrissey, A.; Tobin, J. M. An LC-MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. *Talanta* **2008**, *75* (4), 1089−1097.
(27) Gros, M.; Petrović, M.; Barceló, D. Development of a multiresidue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta* **2006**, *70* (4), 678−690.
(28) Mirzaei, R.; Yunesian, M.; Nasseri, S.; Gholami, M.; Jalilzadeh, E.; Shoeibi, S.; Bidshahi, H. S.; Mesdaghinia, A. An optimized SPE-LC-MS/MS method for antibiotics residue analysis in ground, surface and treated water samples by response surface methodology- central composite design. *J. Environ. Health Sci. Eng.* **2017**, *15*, 21.
(29) Moslah, B.; Hapeshi, E.; Jrad, A.; Fatta-Kassinos, D.; Hedhili, A. Pharmaceuticals and illicit drugs in wastewater samples in north-eastern Tunisia. *Environ. Sci. Pollut. Res.* **2018**, *25* (19), 18226−18241.
(30) Van Hoi, B.; Vu, C.; Phung-Thi, L. A.; Thi Nguyen, T.; Nguyen, P. T.; Mai, H.; Le, P. T.; Nguyen, T. H.; Thanh Duong, D.; Nguyen Thi, H.; et al. Determination of Pharmaceutical Residues by UPLC-MS/MS Method: Validation and Application on Surface Water and Hospital Wastewater. *J. Anal. Methods Chem.* **2021**, *2021*, 1−12.
(31) *Guidance for Industry: Bioanalytical Method Validation*; US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research: Rockville, Md, USA, 2001.
(32) Płotka-Wasylka, J.; Wojnowski, W. Complementary green analytical procedure index (Complex GAPI) and software. *R. Soc. Chem.* **2021**, *23*, 8657−8665.
(33) Manousi, N.; Wojciech, W.; Płotka-Wasylka, J.; Victoria, S. Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality. *Green Chem.* **2023**, *25*, 7598.

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