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LC-HRMS Detection of Antibiotic Residues in Hospital Aquatic Systems in Patna, Bihar: An analytical cross-sectional study

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3. **Dr. Ramalingam Peraman:** Concept, design of study & protocol, data collection, analysis of samples, statistical analysis and preparation of Figures & Tables.
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Abstract

Background: Antibiotic contamination in aquatic environments is a growing global concern, particularly in low- and middle-income countries where wastewater treatment infrastructure is limited. Hospital effluents are recognized as hotspots for pharmaceutical residues, contributing to antimicrobial resistance (AMR) and ecological disruption. While metropolitan India has been studied, Bihar remains underexplored.

Objectives: This study aimed to detect and quantify residues of four commonly prescribed antibiotics, Amoxicillin, Ampicillin, Ciprofloxacin, and Tetracycline in hospital-linked water systems around Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS).

Methods: An analytical cross-sectional design was employed. Surface, ground and waste water samples were collected during the winter season. LC-HRMS (Orbitrap 240) was optimized to identify parent ions and diagnostic fragments, with results validated against standards and degradation profiles. The m/z ratios & area of antibiotics, ppm errors, relative abundance and fragmentation patterns were analyzed to assess persistence and transformation.

Results: Amoxicillin, Ampicillin, and Ciprofloxacin were consistently detected across all matrices, while Tetracycline appeared only as minor fragments. Groundwater showed disproportionately high Amoxicillin contamination, with diagnostic fragments (m/z 323, 318, 274) confirming extensive degradation. Wastewater contained diverse transformation products, including Ampicillin s-oxide and Ciprofloxacin skeletal fragments (m/z 261, 245, 174). Surface water exhibited moderate contamination dominated by β-lactams and fluoroquinolones. Diagnostic fragments provided clear evidence of incomplete degradation and persistence of bioactive residues.

Conclusions: This study provides the first robust evidence of hospital-driven antibiotic contamination in Bihar's aquatic environment. Hospital effluents contribute significantly to antibiotic contamination in aquatic environments, with groundwater functioning as the most vulnerable compartment. These findings highlight eco-pharmacological risks and underscore the urgent need for policy interventions, including effluent treatment plants, year-round monitoring, resistome mapping, and low-cost remediation technologies. Addressing hospital-driven contamination must be prioritized within India's AMR containment strategy to safeguard public health and ecosystem integrity.

Keywords: Anti-Bacterial Agents, Hospital waste, Water Pollutions, Antimicrobial resistance, Aquatic Environmentology, LC-HRMS

Main Text

INTRODUCTION

Antibiotic contamination in aquatic environments has emerged as a pressing global public health concern. The widespread use of antibiotics in human and veterinary medicine, coupled with inadequate disposal and treatment practices, has led to their persistent presence in water systems. Hospital effluents, pharmaceutical industries, aquaculture, and animal farming are recognized as major contributors to this contamination, releasing unmetabolized or partially metabolized antibiotics into sewage and surface waters. These residues often bypass conventional wastewater treatment plants, accumulate in ecosystems, and eventually infiltrate drinking water supplies, thereby exacerbating the risk of antimicrobial resistance (AMR) development (Emadikhiav, A. et al 2024, Cabanillas-Chirinos, L. A. et al 2025, Kümmerer, K. 2009).

The persistence of antibiotics in water systems is particularly alarming because even trace concentrations can exert selective pressure on microbial communities, fostering the emergence of antibiotic-resistant bacteria (ARB) and resistance genes (ARGs). Hospital wastewater is considered a hotspot for such contaminants, as it contains high loads of pharmaceuticals and resistant microorganisms. Studies have consistently demonstrated that traditional wastewater treatment methods are insufficient to eliminate these compounds, resulting in their continuous release into the environment (Michael, I., Rizzo, L. et al 2012, Verlicchi, P. et al 2012). This phenomenon has been described under the emerging discipline of Eco-pharmacology, which investigates the environmental impact of pharmaceutical active ingredients, including antibiotics and other pharmaceutical and personal care products (PPCPs) (Daughton, C. G., & Ternes, T. A. 1999).

Globally, PPCPs such as antibiotics, hormones, analgesics, and antidepressants have been detected in surface water, groundwater, and even drinking water. Their ecological consequences are well documented: diclofenac residues have been linked to vulture population declines, ethinyl estradiol has feminized male fish, and fluoxetine has altered shellfish spawning behaviour (Oaks, J. L. et al 2004). Such findings highlight the far-reaching implications of pharmaceutical pollution beyond human health, affecting biodiversity and ecosystem stability.

In low- and middle-income countries, including India, the challenge is compounded by limited wastewater treatment infrastructure. Hospital-linked water systems often discharge effluents directly into municipal sewage or natural water bodies without adequate monitoring or remediation. This not only contaminates local environments but also contributes to the global burden of AMR, which the World Health Organization has identified as one of the greatest threats to public health in the 21st century (Mendelson, M., & Matsoso, M. P. 2015).

To address these challenges, advanced analytical techniques are essential for detecting and quantifying antibiotic residues in complex environmental matrices. Liquid Chromatography coupled with High-Resolution Mass Spectrometry (LC-HRMS) has emerged as a powerful tool for this purpose, offering high sensitivity and specificity in identifying trace contaminants. The present study focuses on the detection of antibiotic contamination in hospital-linked water systems at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, employing LC-HRMS to provide robust evidence of pharmaceutical pollution and its potential implications for public health and environmental safety.

AIMS & OBJECTIVES

The primary aim of this study is to detect and quantify antibiotic residues and their degradation products using LC-HRMS in surface, ground, and waste water samples collected from hospital-linked water systems during winter season at Indira Gandhi Institute of Medical

Sciences (IGIMS), Patna, Bihar, with particular focus on areas in proximity to drug disposal hubs.

MATERIALS & METHODS

Study Setting: This study was conducted in Patna, the capital city of Bihar, located in eastern India. Patna is a rapidly growing urban centre with significant healthcare infrastructure, including the Indira Gandhi Institute of Medical Sciences (IGIMS), which serves as a major tertiary care hospital. The hospital generates substantial effluent due to its high patient load and extensive pharmaceutical usage, making it an ideal site for investigating antibiotic contamination in hospital-linked water systems.

Study Design: Analytical cross-sectional study using surface, ground and waste water samples from the proximity of IGIMS, Patna hospital area during winter season. Residues and degradation products of four antibiotics i.e. Ciprofloxacin, Amoxicillin, Ampicillin and Tetracycline were estimated in these water samples using LC-HRMS.

Sampling Sites and Categories: Primary site selected was within the IGIMS hospital area and its immediate surroundings. From this site, three categories of water samples were collected:

1. **Surface water**: obtained from nearby ponds and open water bodies.
2. **Groundwater**: collected from shallow tube wells in the vicinity.
3. **Wastewater**: sampled from hospital drainage systems and sewers.

Sampling Protocol: Sampling was performed during winter season using the grab sampling method, which involves collecting water samples at a single point in time. During each sampling session, 2-liter grab samples were collected from each water category once every eight hours, ensuring representative coverage.

Sample Collection, Storage, and Handling: Samples were collected in screw-capped amber glass bottles wrapped in silver foil to prevent photodegradation of antibiotics. Immediately after collection, samples were stored at temperatures below 4°C and transported to the analytical laboratory within 12–24 hours. This protocol minimized degradation and ensured sample integrity for subsequent analysis.

Selection of Antibiotics: Four antibiotics were selected for analysis based on prescription patterns at IGIMS, their metabolic fate in humans, environmental stability, and known ecological impacts:

1) Ciprofloxacin (Fluoroquinolone)
2) Amoxicillin (β-lactam, Penicillin group)
3) Ampicillin (β-lactam, Penicillin group)
4) Tetracycline (Tetracycline group)

Standards of these antibiotics were procured as active pharmaceutical ingredients (APIs) from certified pharmaceutical companies to serve as analytical references.

Study Phases: The investigation was conducted in three sequential phases.

1) **Phase I (Degradation profiling):** Standard solutions of each antibiotic (10 mg in 10 mL distilled water) were prepared, with pH adjusted to match the recorded values of the collected water samples. These solutions were stored under controlled conditions for 45 days to simulate environmental degradation.

2) **Phase II (Method optimization):** Concentrations of antibiotic residues and degradation products in the simulated samples were analyzed using optimized LC-HRMS protocols to establish retention times, mass-to-charge (m/z) ratios, and fragmentation patterns for each antibiotic and its degradation products. This step ensured accurate identification in environmental samples.
3) **Phase III (Environmental sample analysis):** Test water samples collected from IGIMS sites were processed using the pre-optimized LC-HRMS method. Antibiotic residues were identified and quantified by comparing retention times and m/z ratios with those of standards and degradation profiles.

Quality Control and Validation: To ensure reliability, all analyses were performed in triplicate. Blank samples (distilled water) were included to rule out contamination. Calibration curves were prepared using known concentrations of standards, and recovery experiments were conducted to validate extraction efficiency.

Analytical Methodology: Water sample analysis was performed using Liquid Chromatography coupled with High-Resolution Mass Spectrometry (LC-HRMS, Orbitrap 240) at the National Institute of Pharmaceutical Education and Research (NIPER), Hajipur, Bihar. LC-HRMS was chosen for its high sensitivity and specificity in detecting trace levels of antibiotics and their degradation products. LC-HRMS was operated in positive ion mode with nitrogen as nebulization and dissolution gas. Mass spectra were acquired in the 50–600 Dalton range, monitoring precursor and daughter ions for each antibiotic. Quantification was based on ion transitions, using Waters XTerra MS C18 column under isocratic conditions with three solvent systems. The m/z (mass-to-charge) ratios of test samples were matched with standards to confirm contamination levels along with identification of the retention time for each peak corresponding to the antimicrobial residues.

Data collection & analysis: LC-HRMS data was collected and tabulated in the Excel sheets. The m/z ratios and area of Antibiotics from different water samples were documented and matched the test samples with the standard samples. Calculated ppm errors (Mass accuracy) for the matched data to know the deviation of Test measurement from the Standard. Relative abundance (Based on Peak Area %) for each antibiotic was calculated based on peak area of each antibiotic relative to the total area of all detected peaks. Mass spectrometry (MS) fragmentation pattern was observed to confirm the identity of compounds by breaking the "parent" molecule into characteristic pieces. Interpreted the purity of test samples through looking at the ratio of Parent Ions (the intact molecule) to Fragment/Secondary Ions (broken or degraded pieces).

$$\boldsymbol{ppm\ error = \frac{Test\ m/z - Standard\ m/z}{Standard\ m/z}\ x\ 10^6}$$

RESULTS

Table 1: Antibiotics in Surface water (Standard & test samples)

	m/z ratio Std	m/z ratio Test	Area of Test	ppm error & its Status	Relative abundance	Mass spectrometry (MS) fragmentation patterns
Amoxicillin	494.1506	494.3755	-	-	37.9%	• Parent: m/z ≈ 366 $[M+H]^+$, • Major peak at m/z 323.25
	323.1059	323.2578	1586815080	+470.12 High		
	645.1704	645.5084	-	-		
iprof	558.4362	558.360	-	-	10.7%	• Parent: m/z ≈ 332 $[M+H]^+$ • Major peaks at m/z 330.22 & m/z 338.34
	484.4723	484.4719	-	-		

	330.3364	330.2265	255165988	-332.69 High		
	512.5029	512.5032	-	-		
	338.3415	338.3416	194143138	+0.30 Excellent		
	540.5343	540.5344	-			
	700.5171	700.5170	-			
	349.1500	349.2010	-			
Ampicillin	331.2167	331.2265	754058642	+29.59 Excellent	21.8%	• Parent: m/z ≈ 350 $[M+H]^+$ • Major peaks at m/z 331.22 & 299.20.
	299.1388	299.2003	157269325	+205.59 High		
Tetracycline	331.2167	331.1902	754058642	-80.01 Moderate	29.6%	• Parent: m/z ≈ 445 $[M+H]^+$ • Major Peaks at m/z 331.19 & 387.18
	387.2162	387.1800	487523710	-93.49 Moderate		

Std- Standard Water Sample, Test- Test Water Sample

Table 2: Antibiotics in Ground water (Standard & test samples)

	m/z ratio Std	m/z ratio Test	Area of Test	ppm error & its Status	Relative abundance	Mass spectrometry (MS) fragmentation patterns
Amoxicillin	293.1908	293.1909	297149979	+0.34 Excellent	90.1%	• Parent ion (m/z ~366.37 [M+H]+) • Major Peaks at m/z 318.30, 323.25, 274.27 & 338
	432.2802	432.2803	-	-		
	476.3062	476.3060	-	-		
	364.1288	364.2226	-	-		
	349.0851	349.2010	199515753	+331.99 High		
	366.1115	366.3727	876689444	+713.43 Very High		
	274.2738	274.2739	905034	+0.36 Excellent		
	318.2999	318.3001	14191383507	+0.63 Excellent		
	362.1165	362.3261	1756783946	+578.82 High		
	323.1059	323.2578	8468386790	+470.12 High		
	362.1165	362.2977	8841451	+500.39 Very High		
	554.1699	554.1755	-	-		
	338.3416	338.316	206568449	-75.66 Moderate		
	338.3416	338.3413	206551449	-88.67 Moderate		
Ciprofloxacin	388.3415	388.2539	-		1.7%	• Parent ion (m/z ~332 [M+H]+) • Major Peak at m/z 279.15
	349.1500	349.2010	-			
	279.1589	279.1590	485203370	+0.36 Excellent		
	540.5343	540.4253	-			
	316.2117	316.2844	-			
	484.4723	484.4721	-			
	512.5029	512.5023	-			
	684.1486	684.2015	-			
	540.5343	540.5345	-			
	388.3415	388.3413	-			
	700.5171	700.5171	-			
	415.2100	415.2112	-			
Ampicillin	349.0851	349.1831	383168997	+280.73 High	5.6%	• Parent ion (m/z ~349.18 [M+H]+) • Major Peaks at m/z 349.08, 331.22 & 299.20
	331.2167	331.2266	318930747	+29.89 Moderate		
	366.1115	366.1208	876689444	+25.40 Moderate		
	331.2167	331.1903	342112265	-79.70 Moderate		
	299.1388	299.2004	61088046	+205.92 High		

Tetracyclin	243.1829	243.1827	194323367	-0.82 Excellent	2.9%	• Parent ion (m/z ~445 [M+H]+) • Major Peaks m/z 387.17 & 243.18
	387.2162	387.1796	624570038	-94.52 Moderate		

Std- Standard Water Sample, Test- Test Water Sample

Table 3: Antibiotics in Waste water (Standard & Test samples)

	m/z ratio Std	m/z ratio Test	Area of Test	ppm error & its Status	Relative abundance	Mass spectrometry (MS) fragmentation patterns
Amoxicillin	190.0895	190.9792	-	-	60.9%	• Parent ion (m/z ~366.37 [M+H]+) • Major Peaks at m/z 316.21, 349.18 & 190.97
	349.0815	349.1835	-	-		
	316.2115	316.2119	4183313246	+1.26 Excellent		
Ciprofloxacin	302.3051	302.1963	-	-	10.7%	• Parent ion (m/z ~332 [M+H]+) • Major Peaks at m/z 261.13, 245.18, 245.02 & 174.11
	261.1120	261.1308	291750510	+71.99 Moderate		
	242.2841	242.0110	35494807	-1127.19 Poor		
	316.2117	316.2119	-	-		
	174.1277	174.1125	116119204	-87.29 Moderate		
	245.0275	245.1859	224493249	+646.46 Poor		
	437.1931	437.2357	-	-		
	302.2687	302.2115	66112498	-189.24 Moderate		
	363.2116	363.1500	-	-		
	349.1500	349.2008	-	-		
Ampicillin	393.1190	393.2099	70837646	+231.23 High	31.1%	• Parent ion (m/z ~349.18 [M+H]+) • Major Peaks at m/z 218.21, 349.20 & 393.20
	218.2113	218.2113	1416979024	0.00 Perfect		
	349.0850	349.2008	647713380	+331.73 High		
Tetracycline	265.1479	265.1548	268491902	+26.02 Good	12.5%	• Parent ion (m/z ~445 [M+H]+) • Major Peaks at m/z 265.15 & 331.19
	331.2167	331.0702	-	-		
	331.2167	331.1904	589455650	-79.40 Moderate		

Std- Standard Water Sample, Test- Test Water Sample

Figure 1: LC-HRMS chromatogram retention time and peak (Amoxicillin): Oxidation product present at 323 m/z ratio (Ground Water)

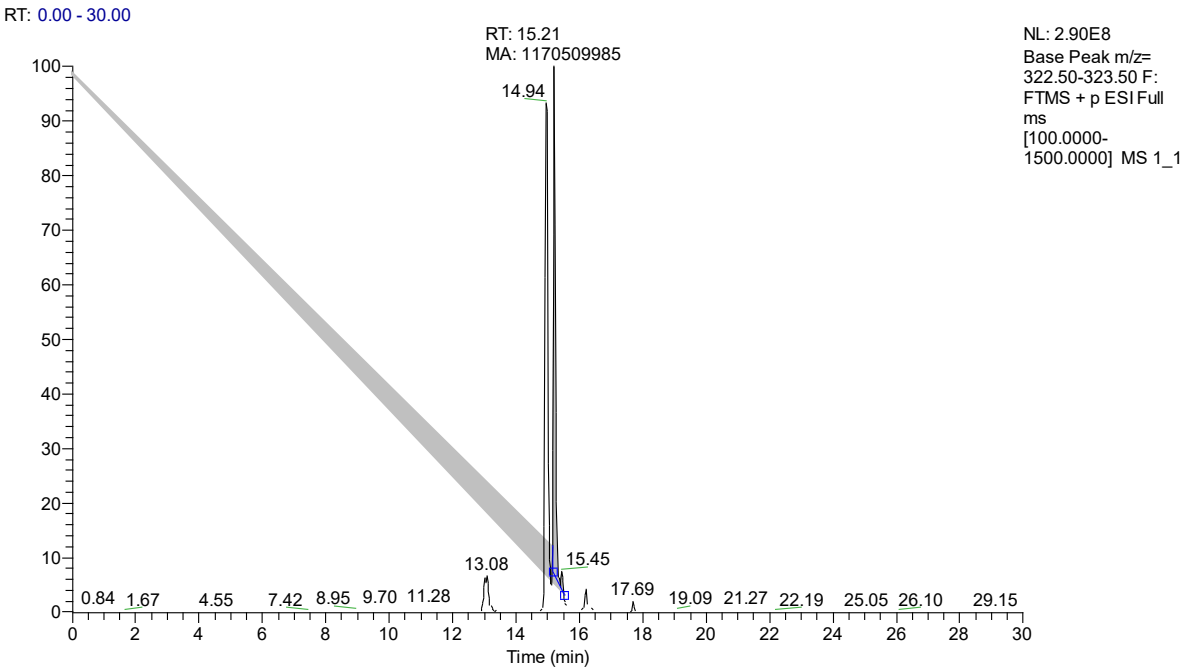


Figure 2: LC-HRMS chromatogram retention time and peak (Amoxicillin): Penilloic acid at 338 m/z ratio (Ground Water)

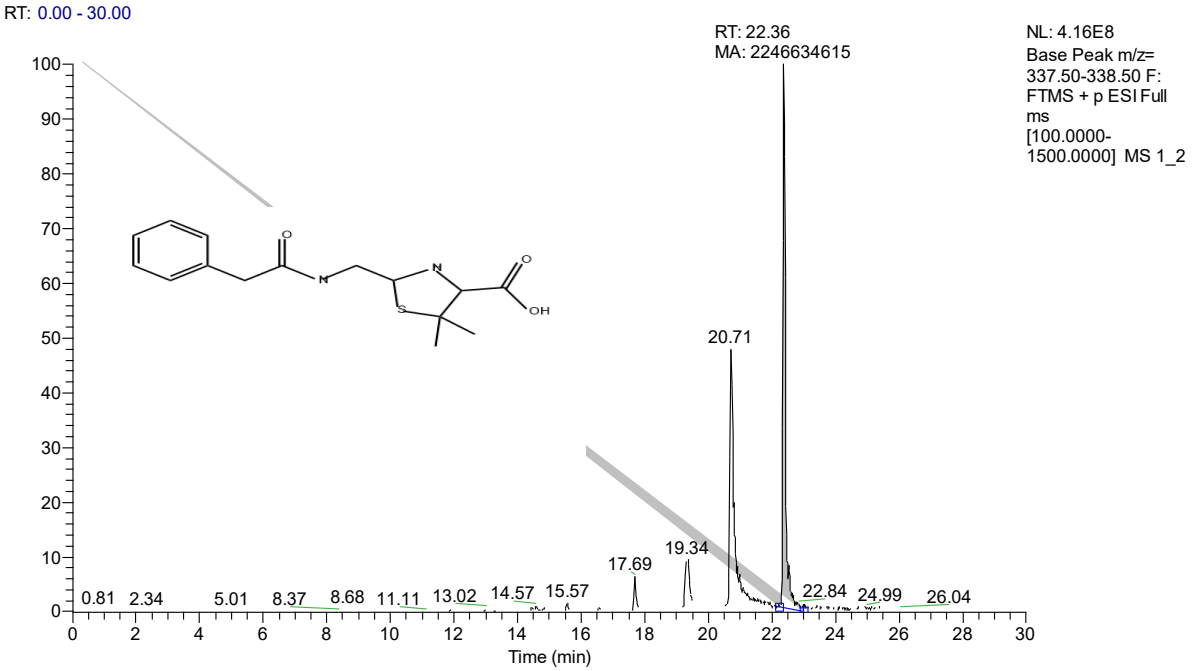


Figure 3: LC-HRMS chromatogram retention time and peak (Amoxicillin): Diketopiperazine ring at 366 m/z ratio (Ground Water)

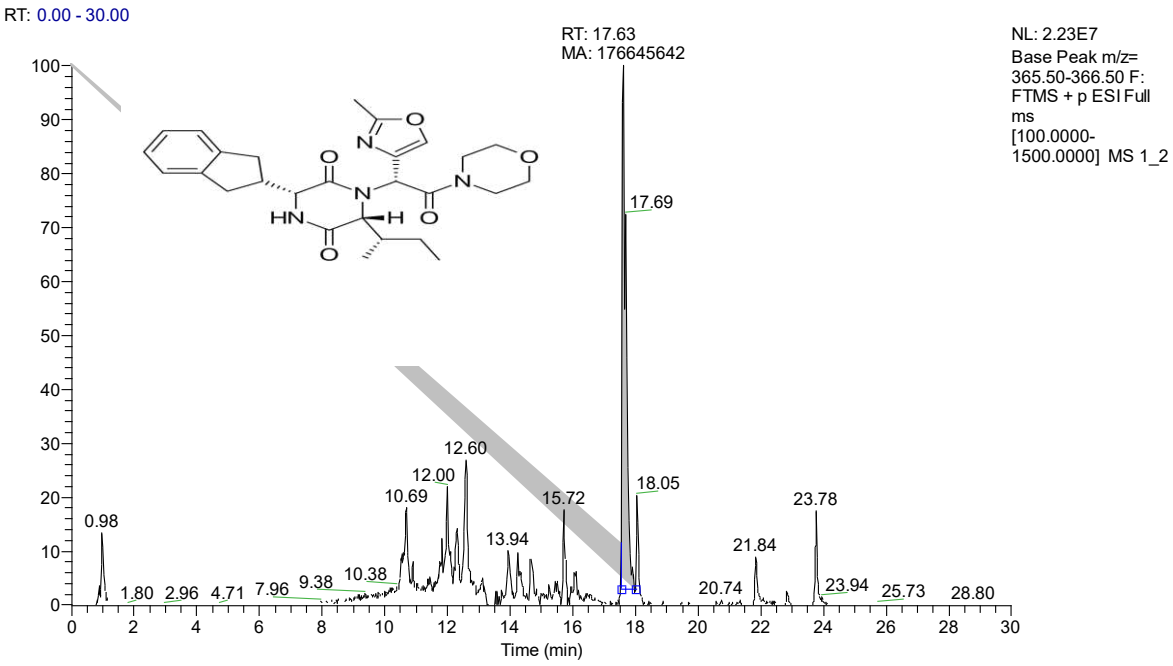


Figure 4: LC-HRMS chromatogram retention time and peak (Amoxicillin): Protonated molecular and potassium adduct at 554 m/z ratio (Ground Water)

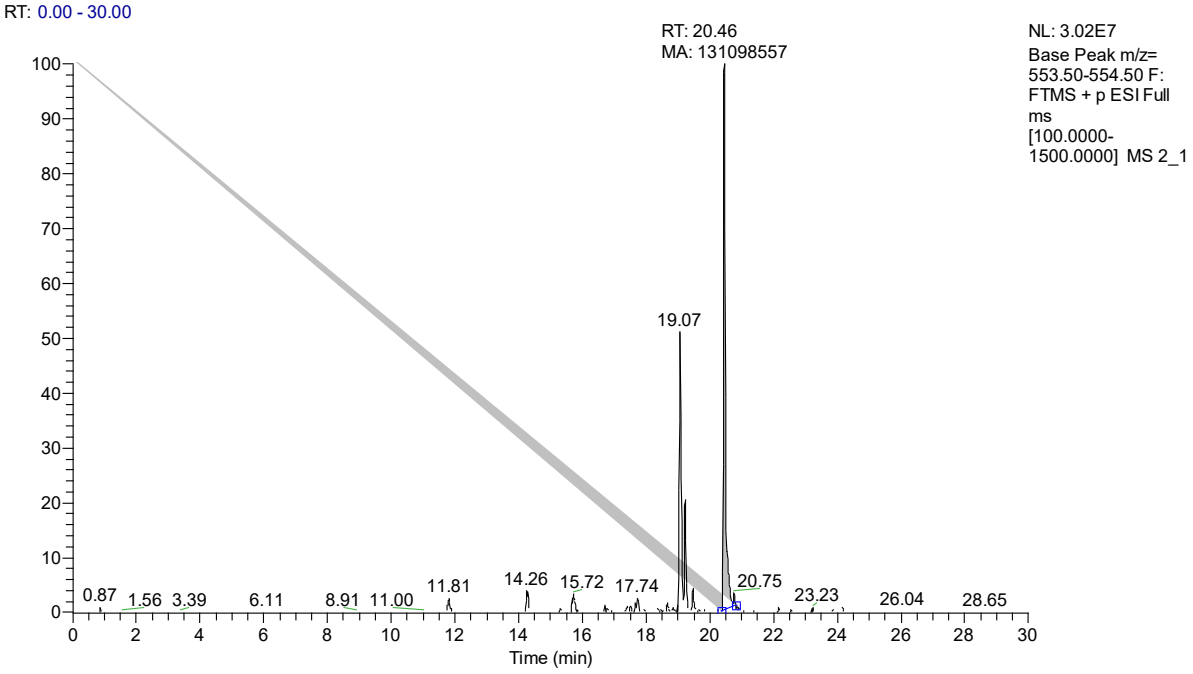


Figure 5: LC-HRMS chromatogram retention time and peak (Ciprofloxacin): Product of piperazine ring and defluorination/ amino group loss at m/z 363 (Waste water)

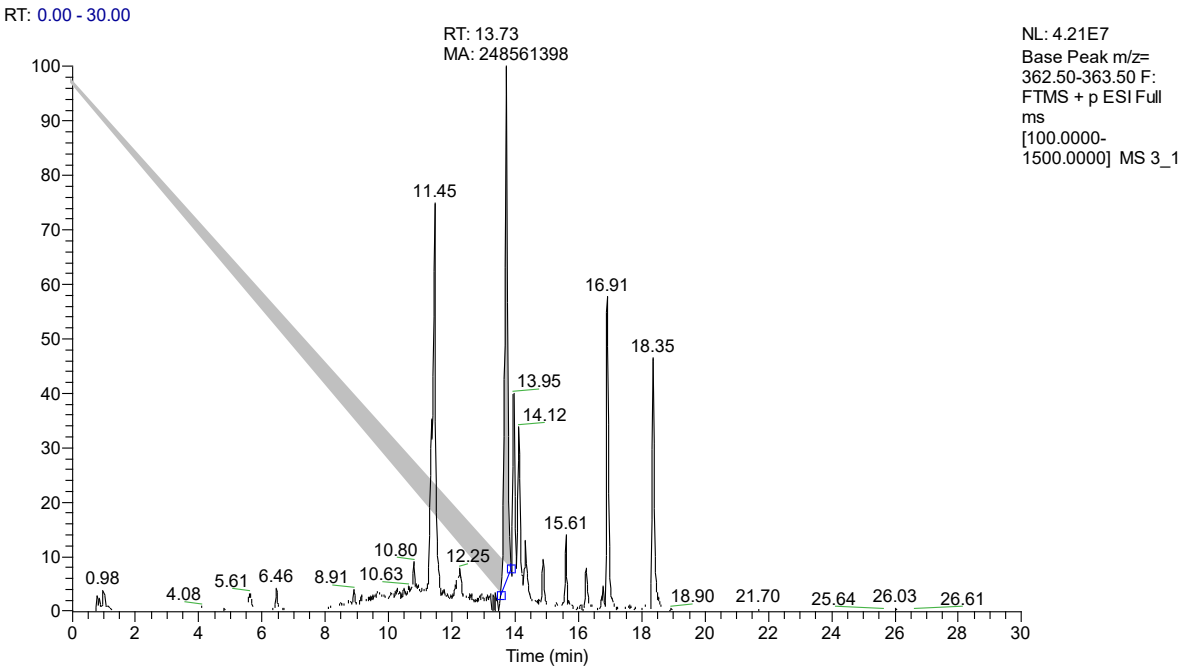


Figure 6: LC-HRMS chromatogram retention time and peak (Ciprofloxacin): Hydroxylation on quinolone ring at m/z 349 (Ground Water)

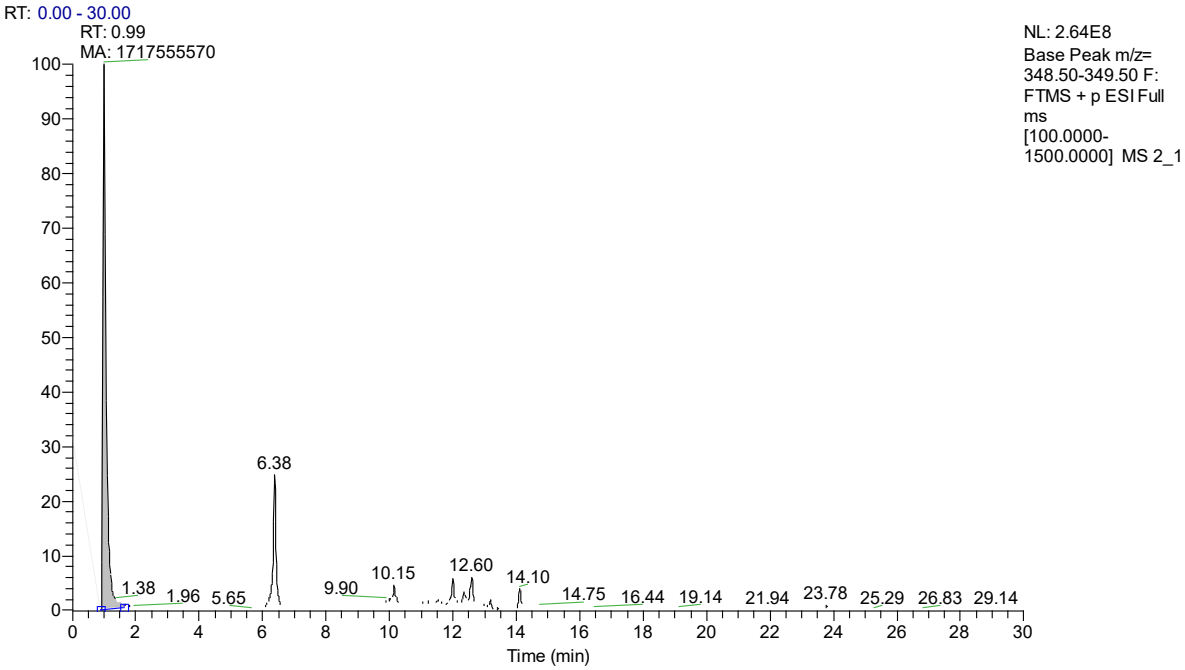


Figure 7: LC-HRMS chromatogram retention time and peak (Ciprofloxacin): Decarboxylation at m/z 279 (Ground Water)

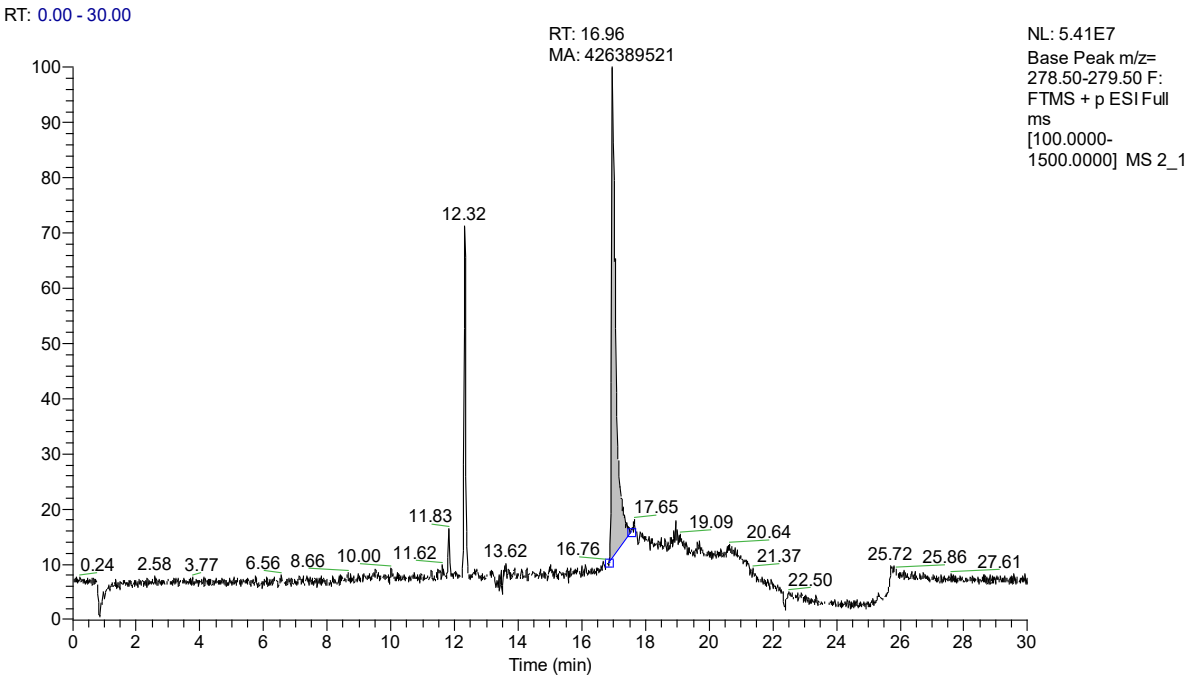


Figure 8: LC-HRMS chromatogram retention time and peak (Ciprofloxacin): Cleavage of the piperazine ring at m/z 261 (Waste water)

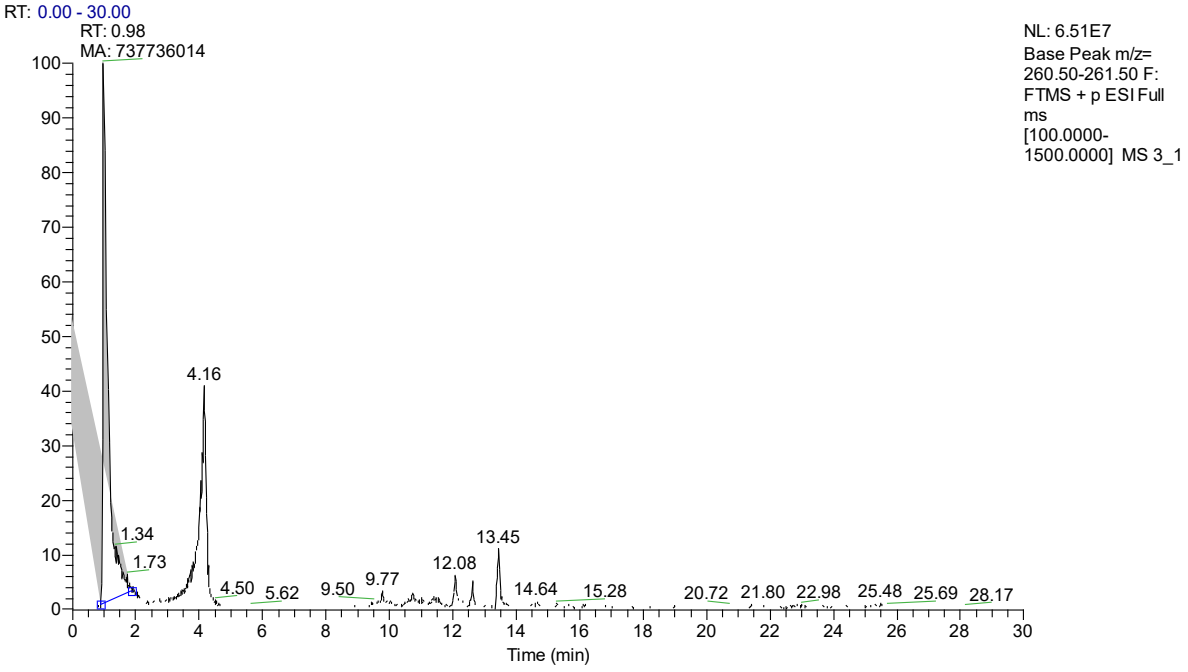


Figure 9: LC-HRMS chromatogram retention time and peak (Ciprofloxacin): Product of piperazine ring and defluorination/amino group loss at m/z 245 (Surface & Waste water)

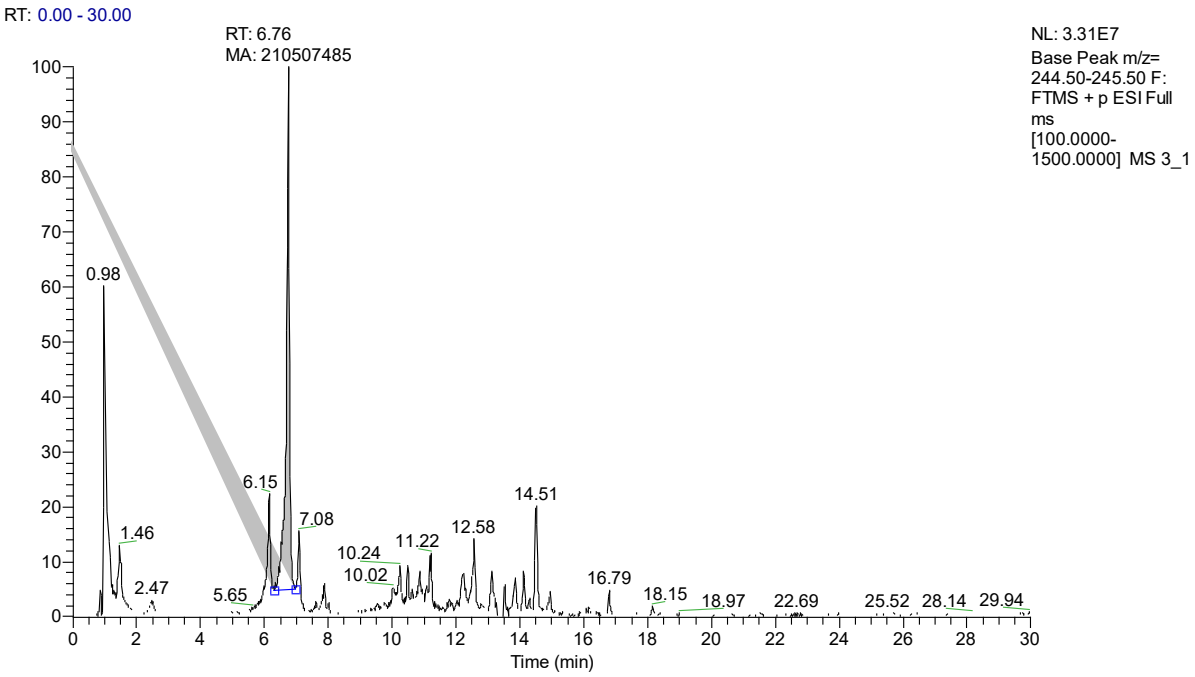


Figure 10: LC-HRMS chromatogram retention time and peak (Ciprofloxacin): Low molecular weight aliphatic acids and short-chain compounds at m/z 174 (Waste water)

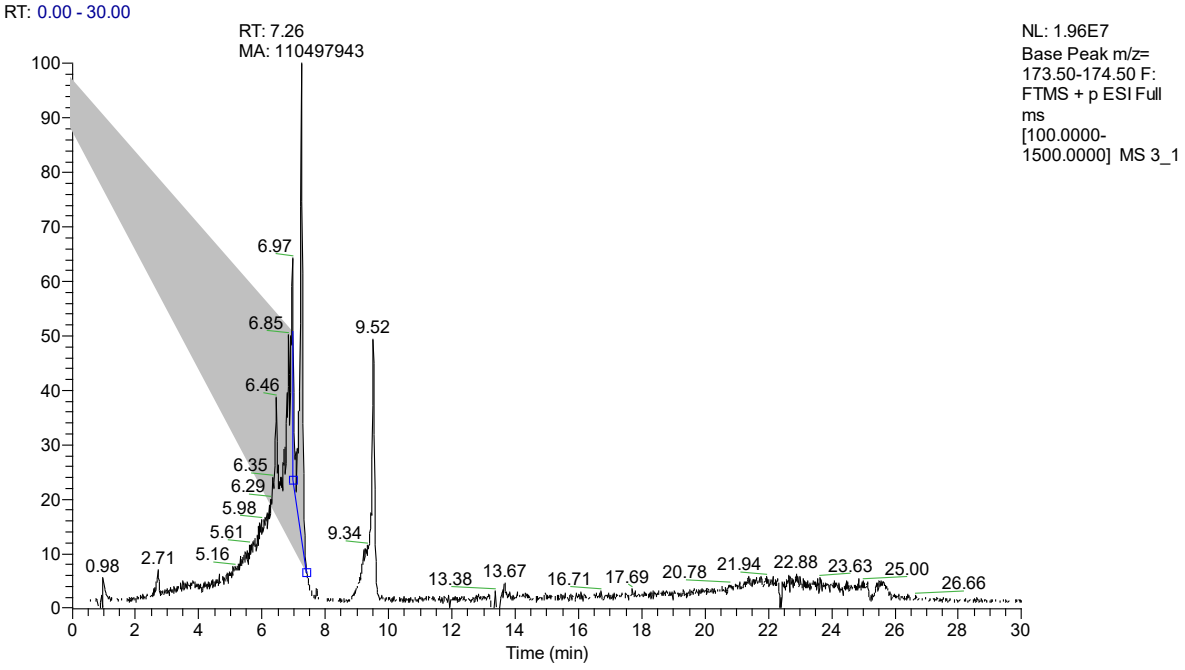


Figure 11: LC-HRMS chromatogram retention time and peak (Ampicillin): Ampicillin s-oxide at m/z 349 (Ground & Waste water)

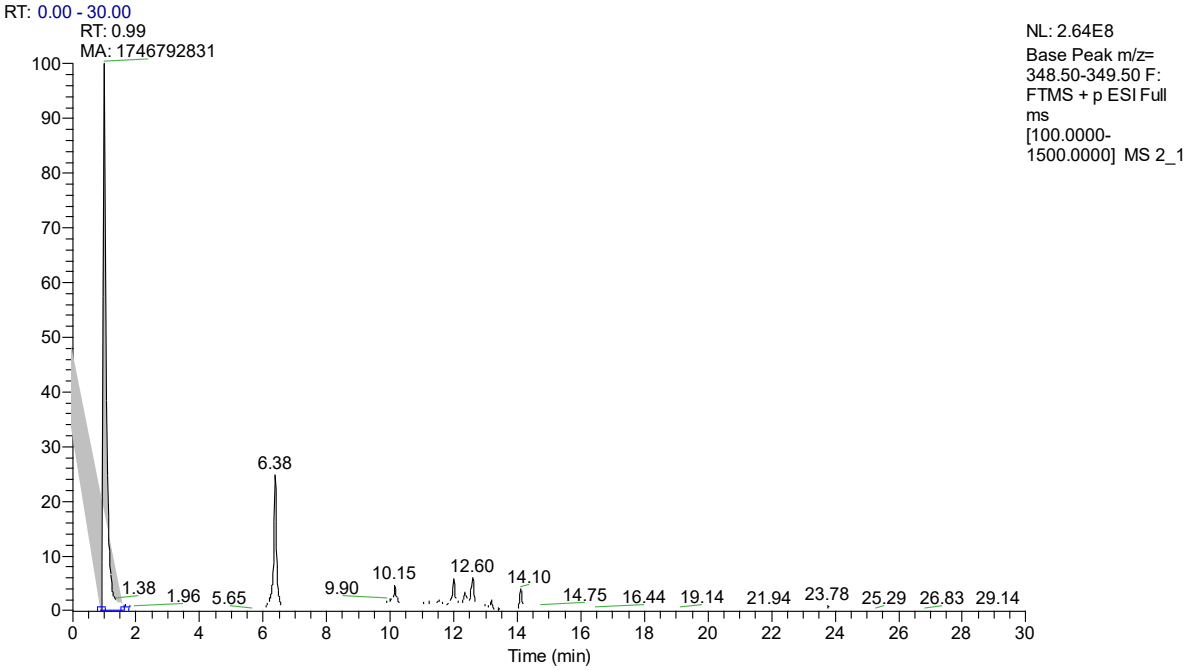


Figure 12: LC-HRMS chromatogram retention time and peak (Tetracycline): Small fragment to large fragment at m/z 387 (Surface & Ground water)

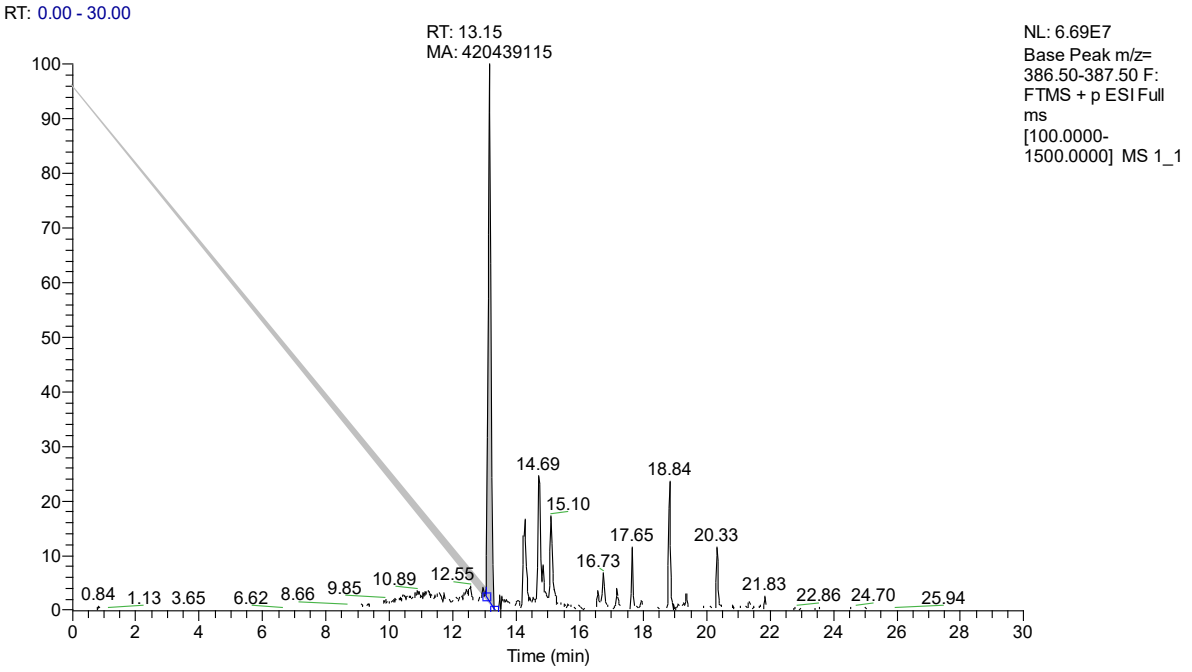
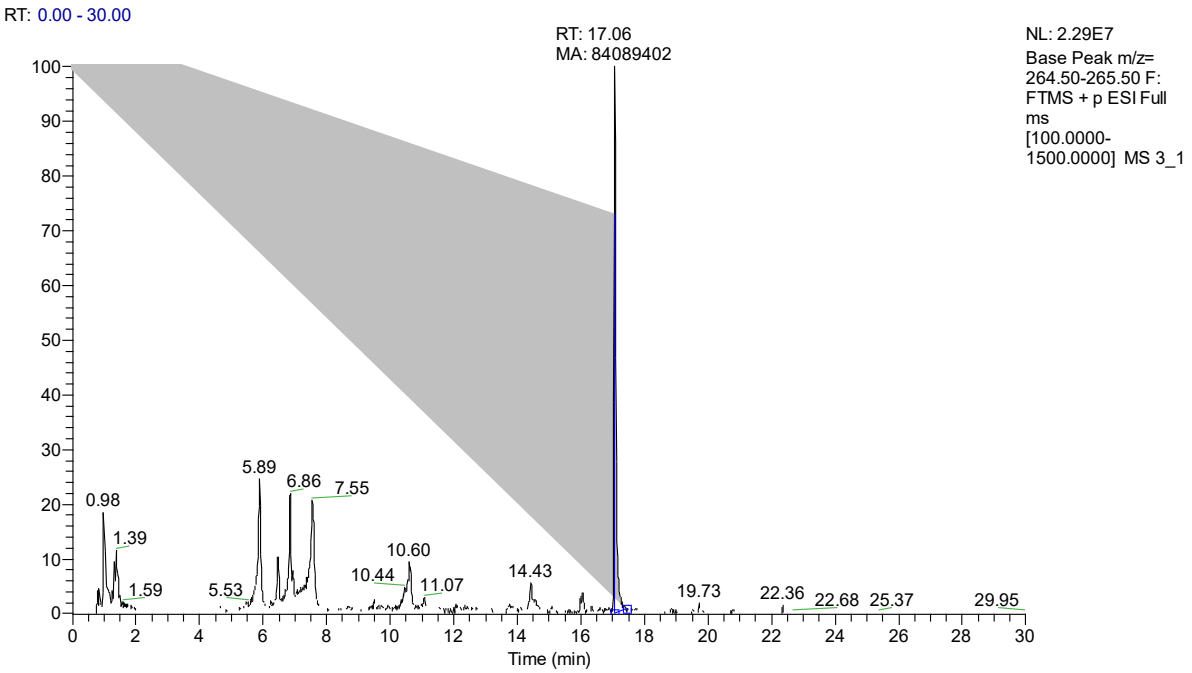


Figure 13: LC-HRMS chromatogram retention time and peak (Tetracycline): Smaller molecular weight carboxylic acid/ fragments at m/z 265 (Waste water)



Detection of Antibiotic Residues in Aquatic Samples

Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis confirmed the presence of multiple antibiotic residues in surface water, groundwater, and wastewater samples collected around IGIMS, Patna during the winter season. Among the four antibiotics investigated—Amoxicillin, Ampicillin, Ciprofloxacin, and Tetracycline—three (Amoxicillin, Ampicillin, Ciprofloxacin) showed consistent detection across matrices, while Tetracycline was largely absent, with only minor fragment peaks observed in isolated samples.

Surface water samples (Table 1) revealed significant contamination with β-lactams and fluoroquinolones. Amoxicillin was detected with a major peak at m/z 323.25, corresponding to an area of 1.58×10^9, while Ciprofloxacin showed strong signals at m/z 330.23 (area 2.55×10^8) and m/z 338.34 (area 1.94×10^8). Ampicillin was consistently detected at m/z 331.22 (area 7.54×10^8) and m/z 299.20 (area 1.57×10^8). Interestingly, Tetracycline residues were negligible, with only fragment peaks at m/z 387.18 (area 4.87×10^8) and m/z 331.19 (area 7.54×10^8), suggesting minimal contribution from agricultural runoff.

Groundwater samples (Table 2) demonstrated disproportionately high contamination, particularly with Amoxicillin. A strikingly large peak was observed at m/z 318.30, with an area of 1.41×10^{10}, indicating severe leaching into aquifers. Additional Amoxicillin signals were noted at m/z 323.25 (area 8.46×10^9) and m/z 362.32 (area 1.75×10^9). Ciprofloxacin residues were detected at m/z 279.15 (area 4.85×10^8), while Ampicillin showed multiple peaks, including m/z 366.11 (area 8.76×10^8) and m/z 331.22 (area 3.18×10^8). Tetracycline was again largely absent, with only minor fragments at m/z 243.18 (area 1.94×10^8) and m/z 387.17 (area 6.24×10^8).

Hospital wastewater samples (Table 3) exhibited complex contamination profiles with both parent compounds and degradation products. Amoxicillin was detected at m/z 316.21 with a large area of 4.18×10^9, while Ampicillin residues included m/z 218.21 (area 1.41×10^9) and m/z 349.20 (area 6.47×10^8). Ciprofloxacin degradation products were prominent, including cleavage of the piperazine ring at m/z 261.13 (area 2.91×10^8) and defluorination fragments at m/z 245.18 (area 2.24×10^8). Tetracycline residues were minimal, with a small fragment at m/z 265.15 (area 2.68×10^8).

LC-HRMS analysis also identified several transformation products, confirming partial degradation of parent antibiotics:

1) **Amoxicillin** (Figure 1 to 4)
   - Parent: m/z ≈ 366.37 [M+H]+
   - m/z 190.97: Smaller fragment from cleavage of the phenol-substituted side chain.
   - m/z 208: Opening of the beta-lactam ring and loss of the bicyclic system.
   - m/z 274.27: Indicates further carboxyl group loss.
   - m/z 316.21: Largest, clean peak; fragment after loss of CO_2 and H_2O ([M+H–CO_2–H_2O]+.
   - m/z 318.30: Sodium adduct [M+Na–CO_2] + or stable rearranged ion after side-chain loss.
   - m/z 323.25 (Major peak in Surface & Ground Water): Fragment from amoxicilloic acid (a hydrolysis product/ metabolite of amoxicillin. Clear degradation marker from β-lactam ring opening and CO_2 loss (Figure 1).
   - m/z 338: Fragment from Penilloic acid (Figure 2)
   - m/z 366: Diketopiperazine ring (Figure 3)
   - m/z 554: Protonated molecular and potassium adduct of Amoxicillin (Figure 4)
2) **Ciprofloxacin** (Figure 5 to 10)
   - Parent: m/z ≈ 332 [M+H]+
   - m/z 330.22 & m/z 338.34 (Major peak in Surface Water)
   - m/z 363: Product of piperazine ring and defluorination/amino group loss (Figure 5) (Waste Water)
   - m/z 349: Hydroxylation on Quinolone Ring (Figure 6) (Ground Water).
   - m/z 314: Typical loss of a water molecule (-H2O) (Surface Water)
   - m/z 288: Loss of the carboxyl group (-CO2) (Surface Water)
   - m/z 279.15 (Major Peak in Ground Water): Loss of cyclopropyl group and partial piperazine ring; represents the core quinolone structure after cleavage (Figure 7) (Ground Water).
   - m/z 261.13: Cleavage of the Piperazine Ring (Quinolone core fragments); formed by loss of cyclopropyl (–42 Da) and carboxyl (–44 Da) (Figure 8) (Waste Water).
   - m/z 245.18: Product of piperazine ring and defluorination/amino group loss (Figure 9) (Surface & Waste Water)
   - m/z 174.11: Small fragment from rupture of central aromatic rings. Low molecular weight aliphatic acids and short-chain compounds. (Figure 10) (Waste water).
3) **Ampicillin** (Figure 11)
   - Parent: m/z ≈ 349.18 [M+H]+
   - m/z 331.22 & 299.20 (Major peaks in Surface & Ground Water)
   - m/z 393.20 (Adduct): Ampicillin bound to formate or potassium during ionization.
   - m/z 349.20: Ampicillin s-oxide (Figure 11) (Ground & Waste Water)
   - m/z 331.22 (Dehydration): Common secondary peak from H_2O loss (Surface & Ground Water).
   - m/z 299.20: Loss of phenylglycine side chain; characteristic Ampicillin fingerprint (Surface & Ground Water).
   - m/z 218.21 (Perfect Match): Zero ppm error; stable thiazolidine + opened β-lactam core fragment (Waste Water).
   - m/z 192/174: Characteristic fragments from side-chain cleavage and dehydration (Surface Water).
   - m/z 160: Fragmentation of the thiazolidine ring (Surface Water).
   - m/z 106: Corresponds to the benzylamine group in ampicillin (Surface Water).
4) **Tetracycline** (Figure 12 & 13)
   - Parent: m/z ≈ 445 [M+H]+
   - Major Peaks at m/z 331.19 & 387.18 (Surface Water).

- m/z 427: Dehydration loss of -H2O (Surface Water)
- m/z 410: Loss of the amino group (-NH3) (Surface Water)
- m/z 387.17: Major fragment from loss of NH_3 and H_2O (Figure 12) (Surface & Ground Water).
- m/z 331.19: More complete fragment; loss of side-chain functional groups (amines, hydroxyls) (Surface & Waste Water).
- m/z 265.15: Mid-range fragment; loss of two rings from tetracycline's four-ring core. Common MS/MS diagnostic peak (Figure 13) (Waste Water).
- m/z 243.18: Low-mass fragment from four-ring cleavage; strong diagnostic peak confirming Tetracycline class (Ground Water).

Absence of Tetracycline

Across all matrices, tetracycline residues were negligible. Only minor fragment peaks were detected in surface and wastewater samples, with no significant signals in groundwater. This absence highlights the clinical prescription-driven contamination fingerprint of IGIMS, dominated by β-lactams and fluoroquinolones rather than veterinary antibiotics.

DISCUSSION

As per the findings mentioned in Tables 1-3 & Figures 1-13, Amoxicillin, Ampicillin, and Ciprofloxacin were consistently detected across all matrices. Groundwater contamination was disproportionately high, particularly for Amoxicillin. Wastewater contained multiple degradation products, indicating incomplete treatment. Tetracycline was largely absent, confirming hospital effluents as the primary contamination source. The persistence of active metabolites underscores the eco-pharmacological risk and potential for antimicrobial resistance selection. These finding were supported by the different other studies like for

A. Amoxicillin degradation products: Zhao, F. K. et al 2022, Shimadzu. (n.d.)., Fabregat-Safont, D. et al 2021, Frański, R. et al 2014, Deshpande, A. D. et al 2004 & Nägele, E. et al 2005.
B. Ciprofloxacin degradation products: Di Rocco, M. et al 2020, Fan, S. Et al 2024, Kovačević, B. et al 2014, El-Gamel, N. E. A. et al 2011 & Volmer, D. A. et al 1997.
C. Ampicillin degradation products: Zhang, L. et al 2014 & Nägele, E. et al 2005.
D. Tetracycline degradation products: Zhao, F. K. et al 2022, Zhang, L. et al 2014 & Cherlet, M. et al 2003.

Seasonal Persistence and the "Winter Effect"

The present study provides compelling evidence of antibiotic persistence in aquatic environments during the winter season in the Indo-Gangetic plains, specifically around the Indira Gandhi Institute of Medical Sciences (IGIMS), Patna. Winter in Bihar is characterized by low ambient temperatures (10°C-20°C), reduced solar radiation due to fog, and shorter daylight hours. These conditions collectively slow down both abiotic and biotic degradation processes. Antibiotics such as Ciprofloxacin, which are highly susceptible to photodegradation under UV radiation, remain chemically stable in the absence of intense sunlight. Similarly, β-lactams like Amoxicillin and Ampicillin undergo hydrolysis at higher temperatures; however, in colder conditions, the rate of hydrolytic ring-opening is significantly reduced, prolonging their environmental half-life (Kümmerer, 2009; Michael et al., 2013).

This "winter effect" essentially creates a preservative phase, allowing antibiotics to persist longer in surface water, wastewater drains, and shallow aquifers. The reduced microbial metabolic activity during colder months further limits biodegradation, compounding the persistence of these compounds (Sui et al., 2011). Consequently, winter contamination represents a critical seasonal vulnerability, extending the exposure window for aquatic microflora and increasing the risk of antimicrobial resistance (AMR) selection.

Comparative Analysis: Indian Scenario

Our findings resonate with earlier Indian studies but highlight a more concentrated impact in Patna. Mutiyar and Mittal et al 2014 reported Ciprofloxacin as a dominant contaminant in hospital-fed drains in New Delhi, though dilution in the Yamuna River reduced its environmental burden. In contrast, our study revealed disproportionately high Amoxicillin peak areas in groundwater (area of 1.41×10^{10}), suggesting severe contamination at the residential-hospital interface.

Studies in Ujjain (Diwan V et al. 2010, Diwan V et al. 2012, Diwan V et al. 2018) observed that hospital effluents contained high antibiotic loads, but surrounding groundwater remained relatively shielded. Our results contradict this, showing that Patna's high-water table and unlined "kutcha" drainage systems facilitate direct leaching of pharmaceutical-laden wastewater into shallow aquifers. This infrastructural vulnerability underscores the inadequacy of natural geological barriers in preventing contamination in urban hospital settings.

Furthermore, unlike Punjab and Haryana where tetracycline residues are common due to intensive livestock farming (Sarmah et al., 2006), our study found negligible tetracycline contamination. This absence confirms that the contamination fingerprint in Patna is driven by clinical prescriptions rather than agricultural runoff, with β-lactams and fluoroquinolones dominating due to their widespread use in tertiary care hospitals (Laxminarayan et al., 2016).

Global Perspective and Effluent Treatment Plant (ETP) Efficacy

Globally, hospital wastewater management varies significantly. In Europe, advanced oxidation processes (AOPs) and membrane bioreactors are routinely employed to pre-treat hospital effluents before discharge into municipal sewers, resulting in low antibiotic concentrations in rivers such as the Rhine and Danube (Kovalova et al., 2012; Verlicchi P et al., 2010, Verlicchi P et al., 2012, Verlicchi P et al., 2015). In contrast, our findings mirror those from Wuhan, China, and Lagos, Nigeria, where minimal remediation leads to high antibiotic loads in receiving waters (Sui et al., 2011; Awolusi et al., 2018; Ajibola et al. 2022).

The absence of a pharmaceutical-grade ETP at IGIMS exacerbates the problem. Our LC-HRMS analysis revealed transformation products such as Penilloic acid and Ampicillin s-oxide, indicating partial degradation. However, these metabolites retain biologically active scaffolds, continuing to exert ecotoxicological pressure (Kümmerer & Henninger, 2003). This incomplete degradation highlights the urgent need for dedicated hospital wastewater treatment infrastructure in India.

Eco-pharmacological Implications and AMR Selection

The Eco pharmacological implications of our findings are profound. Ciprofloxacin's Predicted No-Effect Concentration (PNEC) for resistance selection is as low as 64 ng/L (Bengtsson-Palme & Larsson, 2016). Given the massive peak areas detected in our samples, it is highly probable that groundwater concentrations exceed these thresholds. Chronic exposure of environmental bacteria to sub-therapeutic levels of Amoxicillin and Ciprofloxacin fosters the proliferation of antibiotic-resistant genes (ARGs) and mobile genetic elements (MGEs), effectively transforming groundwater into a "bioreactor" for multidrug-resistant (MDR) strains (Martinez, 2009; Wellington et al., 2013).

This creates a vicious cycle: hospital waste contaminates water, communities consume contaminated water, and patients return to hospitals with infections resistant to first-line therapies. Such a feedback loop accelerates the spread of AMR, undermining public health and increasing healthcare costs (World Health Organization. Global antibiotic resistance surveillance report 2025; O'Neill, 2016).

Public Health Consequences

The detection of Ampicillin and Ciprofloxacin in groundwater is particularly alarming because this water is frequently used for domestic purposes. Ingestion of sub-therapeutic antibiotic doses can disrupt the human gut microbiome, promote colonization by resistant pathogens, and increase the risk of treatment failure (Van Boeckel et al., 2014). From an ecological perspective, antibiotic residues exert chemical stress on aquatic ecosystems, reducing microbial biodiversity and altering nutrient cycling (Grenni et al., 2018).

Even degradation products such as Penilloic acid, though less potent antimicrobials, contribute to environmental toxicity by retaining chemical signatures that interfere with microbial community dynamics (Kümmerer, 2009). Thus, the contamination observed in Patna represents both a direct human health hazard and an ecological threat.

STRENGTHS OF THE STUDY

This study is the first analytical cross-sectional study to systematically investigate antibiotic contamination in hospital-linked water systems in Bihar, a region with limited prior Eco pharmacological data. Using advanced LC-HRMS for sensitive detection of parent drugs and transformation products at trace levels. By analyzing surface, ground, and wastewater, it provides a comprehensive view of contamination pathways. Penilloic acid, Ampicillin s-oxide, and Ciprofloxacin fragments, highlighting incomplete degradation and persistent ecotoxicological risk. High Amoxicillin loads in groundwater highlight serious public health risks, while winter conditions revealed prolonged antibiotic persistence.

LIMITATIONS OF THE STUDY

This study was limited to winter sampling, preventing year-round analysis of antibiotic persistence across summer and monsoon seasons. Its scope was restricted to the IGIMS hospital area, which may not represent wider contamination patterns across Patna or other urban centres. Only four antibiotics were examined, excluding other commonly prescribed drugs e.g., cephalosporins, macrolides.

FUTURE DIRECTIONS OF THE STUDY

Future work should expand sampling across all seasons to capture year-round persistence of antibiotics under varying climatic conditions. Broader drug coverage, including cephalosporins and macrolides, will provide a more complete contamination fingerprint. Integrating metagenomic resistome mapping will directly link chemical residues with microbial resistance. Hydrogeological modelling can clarify aquifer vulnerability, while pilot interventions using low-cost treatment technologies should be tested for hospital effluents. Policy frameworks must mandate pharmaceutical-grade effluent treatment plants, and community health surveillance should monitor exposure impacts, ensuring both environmental safety and public health protection.

CONCLUSIONS

This study provides clear evidence of hospital-driven antibiotic contamination in Bihar's aquatic environment, with Amoxicillin, Ampicillin, and Ciprofloxacin consistently detected across water matrices. Groundwater showed the highest Amoxicillin burden, reflecting direct leaching into shallow aquifers. Identification of metabolites such as Penilloic acid and Ampicillin s-oxide highlights incomplete degradation and persistent ecotoxicological risk.

The absence of tetracycline confirms a clinical prescription-driven contamination fingerprint, while winter conditions prolonged antibiotic persistence. These findings

underscore urgent need for effluent treatment plants, year-round monitoring, and resistome mapping to safeguard public health and ecosystem stability.

CLINICAL TRIAL NUMBER: Not Applicable

DECLARATION: All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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