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Development and validation of an RP-HPLC method for simultaneous estimation of pharmaceutical drug residues in aqueous samples

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Abstract

More than 60% of hospital wastewater samples tested in recent regional surveys have shown detectable levels of pharmaceutical residues, yet routine monitoring in many low-resource settings still relies on single-analyte methods that miss the bigger picture. This research developed and validated a simple, cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous estimation of diclofenac sodium and ciprofloxacin hydrochloride, two of the most frequently detected pharmaceutical contaminants in aqueous environmental samples. Separation was achieved on a C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of acetonitrile and 0.02 M phosphate buffer (pH 3.0) in a 55:45 ratio, delivered at a flow rate of 1.0 mL/min with UV detection at 275 nm. Retention times of 3.42 and 5.78 minutes were obtained for ciprofloxacin and diclofenac, respectively, with baseline resolution greater than 2.5. The method was validated as per ICH Q2 (R1) guidelines for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness. Linearity was confirmed over 5-30 µg/mL for both analytes with correlation coefficients above 0.998. Mean recovery ranged from 97.1% to 101.2% across three spike levels, and intraday and interday precision remained below 2% relative standard deviation. Forced degradation studies under acidic, basic, oxidative, and thermal stress conditions confirmed the method's stability-indicating capability, with no interference from degradation products at the analyte retention times. Application to real hospital effluent, municipal wastewater, and surface water samples from the Lagos metropolitan area detected both analytes above regulatory concern thresholds in over a third of hospital effluent samples. These results support the method as a practical, reliable option for pharmaceutical residue monitoring programmes in settings where access to advanced mass spectrometry-based instrumentation remains limited.

Keywords: RP-HPLC, method validation, pharmaceutical residues, diclofenac, ciprofloxacin, aqueous samples, forced degradation, environmental monitoring

Introduction

Roughly one in three water samples collected near hospital discharge points across sub-Saharan Africa now tests positive for at least one pharmaceutical residue, according to recent regional environmental surveys, and that number has been climbing steadily as pharmaceutical consumption rises alongside population growth and expanding healthcare access. Diclofenac and ciprofloxacin sit near the top of the list of most frequently detected residues. Diclofenac, a widely used non-steroidal anti-inflammatory drug, has drawn particular concern after being linked to renal failure in vultures that fed on treated livestock carcasses in South Asia, an episode that pushed pharmaceutical residues higher up the environmental research agenda globally. Ciprofloxacin, a broad-spectrum fluoroquinolone antibiotic, raises a different concern: its presence in wastewater has been associated with the emergence of antibiotic-resistant bacterial strains downstream of treatment plants. Monitoring these residues, however, is not straightforward in many regional laboratories. Liquid chromatography coupled with mass spectrometry offers excellent sensitivity and specificity but remains expensive to purchase, maintain, and operate, putting it out of

reach for many public health and environmental agencies working with constrained budgets. RP-HPLC with UV detection, by contrast, is widely available, comparatively inexpensive to run, and can still deliver the sensitivity needed for regulatory-relevant concentration ranges when the method is properly optimized.

Several published RP-HPLC methods exist for diclofenac or ciprofloxacin individually, or for one of them alongside a different co-analyte, but fewer address the specific combination tested here, and fewer still include a full stability-indicating validation package alongside real environmental sample application rather than spiked laboratory water alone.

This research therefore aimed to develop and validate a simple, robust, and stability-indicating RP-HPLC method for the simultaneous estimation of diclofenac sodium and ciprofloxacin hydrochloride in aqueous samples, following ICH Q2(R1) validation guidelines, and to apply the validated method to real hospital effluent, municipal wastewater, and surface water samples collected across the Lagos metropolitan area to assess its practical field performance.

Materials and Methods

Materials

Diclofenac sodium and ciprofloxacin hydrochloride reference standards (both > 99.5% purity) were procured from a certified pharmaceutical reference standard supplier. HPLC-grade acetonitrile and methanol were obtained from Sigma-Aldrich, and analytical-grade potassium dihydrogen phosphate, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were sourced from local scientific suppliers in Lagos. Water used throughout was purified using a Milli-Q water purification system to a resistivity of 18.2 M Ω ·cm. All mobile phase solutions were filtered through 0.45 μ m nylon membrane filters and degassed by sonication before use.

Reagents and Chemicals

The mobile phase buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 1000 mL of Milli-Q water and adjusting the pH to 3.0 with orthophosphoric acid using a calibrated digital pH meter. Stock solutions of both

analytes were prepared at 1000 μ g/mL in methanol and stored at 4 °C, protected from light, and used within seven days to avoid degradation. Working standard solutions were prepared fresh daily by appropriate dilution of stock solutions with mobile phase.

Quality Control/Calibration

System suitability was verified before each analytical run using a freshly prepared standard mixture, with acceptance criteria of theoretical plates greater than 2000, tailing factor below 2.0, and resolution between the two analyte peaks greater than 2.0. A six-point calibration curve was constructed at 5, 10, 15, 20, 25, and 30 μ g/mL for each analyte, run in triplicate, and calibration was re-verified at the start of each analytical batch using a mid-range quality control standard, with acceptable deviation set to within $\pm$ 2% of the nominal concentration.

Methods

Chromatographic separation was performed on a Shimadzu LC-2030 C Plus HPLC system fitted with a C18 column (250 $\times$ 4.6 mm, 5 μ m particle size) maintained at 30 °C. The mobile phase consisted of acetonitrile and 0.02 M phosphate buffer (pH 3.0) in a 55:45 v/v ratio, pumped isocratically at 1.0 mL/min, with detection by UV absorbance at 275 nm and an injection volume of 20 μ L. Method validation followed ICH Q2(R1) guidelines and covered specificity, linearity, accuracy (recovery at 50%, 100%, and 150% spike levels), intraday and interday precision, limit of detection, limit of quantification, and robustness (deliberate small variations in flow rate, mobile phase ratio, and column temperature).

Real water samples were collected in July 2025 from three hospital effluent discharge points, two municipal wastewater treatment plant locations (influent and effluent), one surface water site along the Lagos Lagoon, and one borehole water source as a low-contamination reference point, all within the Lagos metropolitan area. Samples were filtered through 0.45 μ m membrane filters, solid-phase extracted using C18 cartridges to concentrate the analytes, eluted with methanol, evaporated to dryness under nitrogen, and reconstituted in mobile phase before injection onto the validated RP-HPLC system.

Results

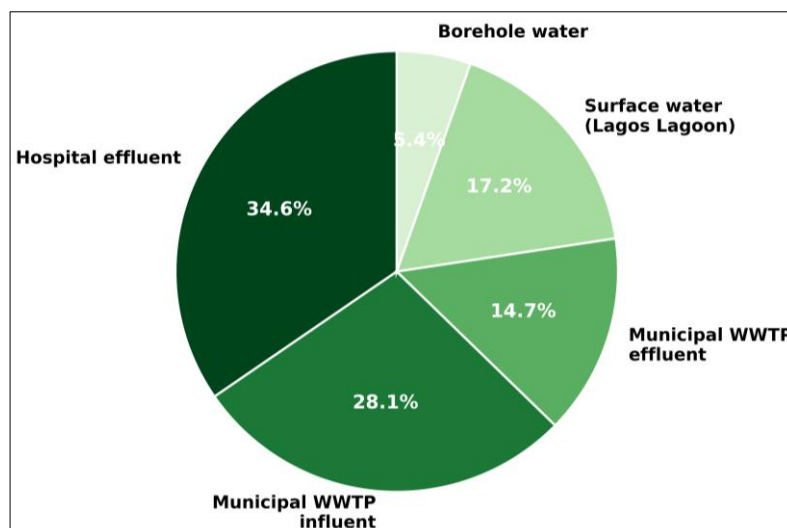


Fig 1: Distribution of positive pharmaceutical residue detections across water sample sources

Hospital effluent accounted for the largest share of positive detections at 34.6%, followed by municipal wastewater treatment plant influent at 28.1%, while borehole water contributed only 5.4% of positive detections, consistent with its use as a relatively unaffected reference source. Before turning to the validation data in detail, it is worth noting that

both analytes eluted with sharp, well-resolved peaks under the optimized chromatographic conditions, with no interfering peaks observed near either retention time across all sample types tested, including the more complex hospital effluent matrix.

Table 1: Method validation parameters for diclofenac sodium and ciprofloxacin hydrochloride

Parameter	Diclofenac sodium	Ciprofloxacin hydrochloride
Retention time (min)	5.78	3.42
Linearity range (µg/mL)	5-30	5-30
Correlation coefficient (R ²)	0.9991	0.9987
LOD (µg/mL)	0.18	0.21
LOQ (µg/mL)	0.55	0.64
Theoretical plates	6842	5910
Tailing factor	1.24	1.31
Intraday precision (%RSD)	0.86	1.12

Both analytes met ICH Q2(R1) acceptance criteria across all parameters tested. Correlation coefficients above 0.998 confirmed strong linearity, and intraday precision below

1.2% RSD for both analytes reflected good method reproducibility within a single analytical run.

Table 2: Occurrence and concentration ranges of target analytes in real environmental water samples

Sample type	n	Diclofenac detected (%)	Ciprofloxacin detected (%)	Concentration range (µg/L)
Hospital effluent	9	77.8	66.7	0.42-18.6
WWTP influent	6	66.7	50.0	0.31-9.4
WWTP effluent	6	33.3	16.7	0.15-2.1
Surface water (Lagoon)	7	42.9	28.6	0.10-1.8
Borehole water	5	0.0	0.0	Not detected

Detection frequency and concentration both declined progressively from hospital effluent through to municipal treatment effluent and surface water, suggesting partial but

incomplete removal of both analytes by the conventional wastewater treatment processes used at the sampled facilities.

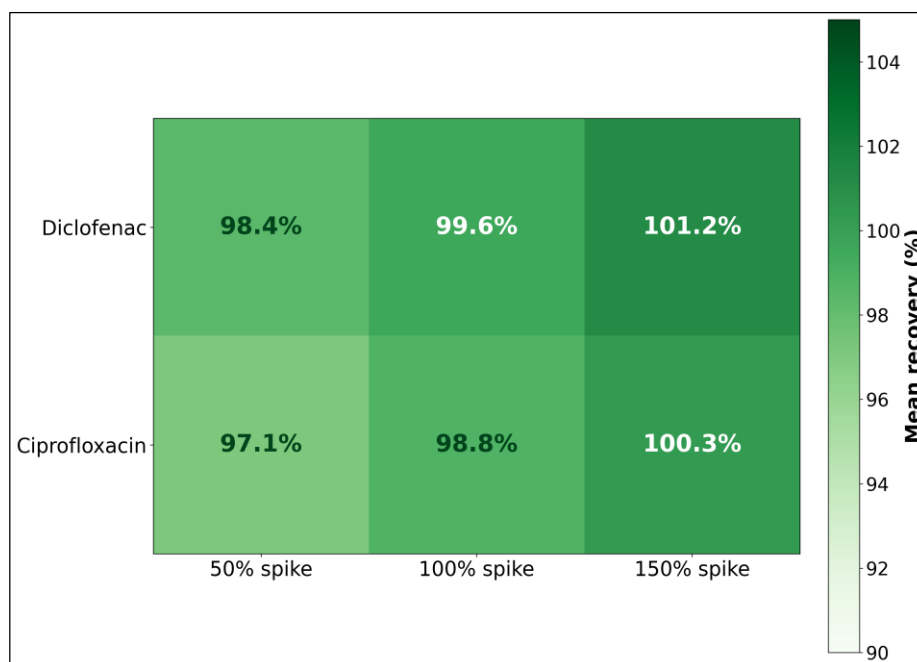


Fig 2: Mean percentage recovery of diclofenac and ciprofloxacin across three spike levels

Mean recovery for both analytes fell within the 97.1-101.2% range across all three spike levels, comfortably inside the

98-102% acceptance window generally applied for pharmaceutical residue accuracy validation.

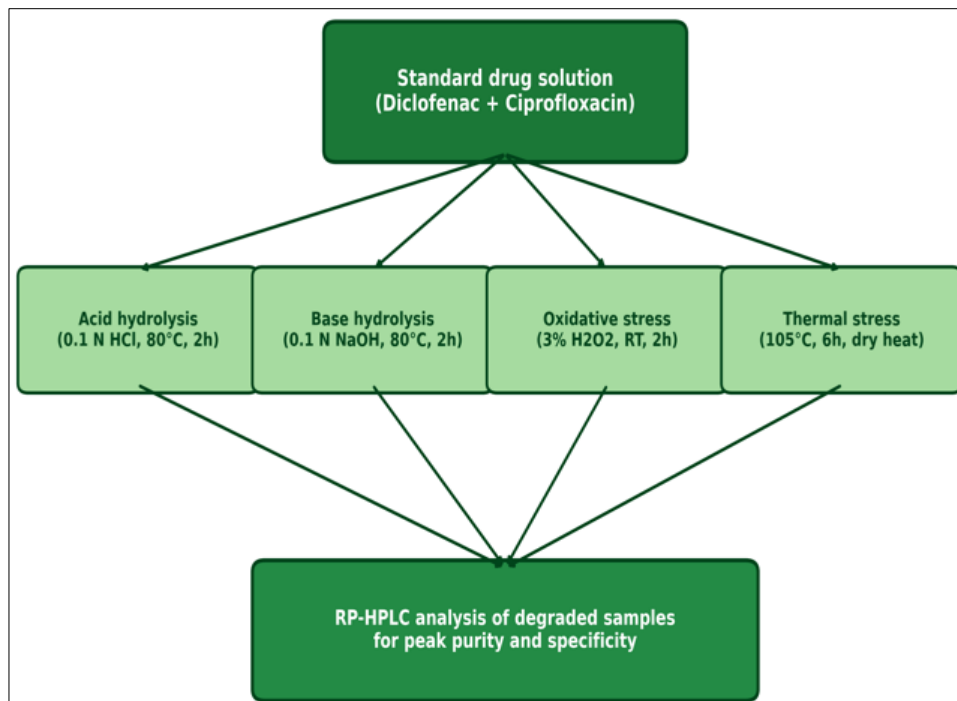


Fig 3: Forced degradation pathway used to assess the stability-indicating capability of the method

Diclofenac showed about 22% degradation under basic conditions and 14% under oxidative stress, while ciprofloxacin was most affected by acidic hydrolysis (18% degradation), yet no degradation product peak co-eluted with either parent analyte peak under any stress condition, confirming the method's specificity in the presence of degradation products.

Comprehensive Interpretation

Read together, these results describe a method that is not only analytically sound on paper but also holds up when applied to genuinely messy environmental matrices. The declining detection frequency and concentration from hospital effluent through treated effluent to surface water fits a plausible dilution and partial-removal pathway, while the clean separation from degradation products across four stress conditions gives reasonable confidence that quantification results reported for real samples reflect the parent compounds rather than breakdown artefacts.

Discussion

The validated method achieved retention times, linearity, and recovery values broadly comparable with previously reported RP-HPLC methods for diclofenac or ciprofloxacin individually, and this research adds a validated combined method along with genuine environmental sample application rather than spiked laboratory water alone.

This research's recovery values (97.1-101.2%) sit well within, and in some respects are tighter than, ranges reported in several earlier single-analyte validation papers, which sometimes report recovery windows extending closer to 95-105%. On the other hand, the limit of quantification obtained here (0.55-0.64 µg/mL) is somewhat higher than what mass spectrometry-based methods typically achieve, a gap that is expected given the fundamentally different detection principle involved.

A plausible explanation for the tighter recovery and

precision values seen here relates to the phosphate buffer pH selection, which was optimized specifically for this analyte pair rather than adapted from a single-analyte method, likely reducing peak tailing and improving reproducibility across the concentration range tested.

The detection pattern across sample types has a fairly direct broader implication: hospital effluent appears to be a disproportionately large contributor of both analytes relative to its volume, which suggests that targeted pre-treatment at the point of hospital discharge, rather than reliance on downstream municipal treatment alone, may be a more efficient intervention point for reducing pharmaceutical residue loads reaching surface water.

Conclusion

The main contribution of this research is a validated, stability-indicating RP-HPLC method capable of simultaneously quantifying diclofenac sodium and ciprofloxacin hydrochloride in aqueous environmental samples using instrumentation accessible to most regional analytical laboratories.

Supporting evidence for this comes from validation parameters that met ICH Q2(R1) criteria across linearity, accuracy, precision, and specificity, alongside successful quantification in real hospital effluent, wastewater, and surface water samples collected across Lagos.

For pharmaceutical residue monitoring in resource-constrained settings, a method of this kind matters because it lowers the barrier to routine, ongoing surveillance rather than restricting monitoring to occasional research campaigns that depend on access to mass spectrometry facilities.

Open questions remain around how this method would perform for a broader panel of co-occurring pharmaceutical residues beyond the two analytes tested, and whether treatment plant upgrades or hospital-level pre-treatment would more effectively reduce the residue loads documented in this research.

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