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RP-HPLC-Based Quantification of Ciprofloxacin in Active and Pharmaceutical Preparations

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ABSTRACT

A straightforward and highly sensitive isocratic reversed-phase HPLC technique has been developed for the detection and quantification of ciprofloxacin in pharmaceutical formulations, which has been validated following ICH guidelines. This study introduces a straightforward, cost-effective HPLC technique that delivers accurate results, a low limit of quantification (LOQ), and reduced analysis time. The separation process was performed on a C18 reversed-phase column, using a mobile phase of acetonitrile and water in a ratio of 80:20 and with a pH adjustment of 2.7 achieved by the addition of 85% phosphoric acid. Ultraviolet detection occurred at 275.0 nm, with a flow rate of 1.7 mL/min at ambient temperature. Statistical evaluations of the precision, both inter- and intra-day, showed that the method is highly accurate and precise, with a correlation coefficient of 0.9999 or higher. The detection limit was 500 ng/mL, and the recovery was found to range between 99.01% and 101.19%. This method is reliable for routine analysis in pharmaceutical and clinical laboratories and can also be used for the measurement of ciprofloxacin in human serum.

Keywords: Human serum, Ciprofloxacin, Dosage form, RP-HPLC (reverse phase high-performance chromatography)

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Introduction

Ciprofloxacin hydrochloride **(Figure 1)**, a commonly used quinolone [1], is available in two forms: as a hydrochloride monohydrate for oral administration [2] and as a lactate for parenteral use [3]. It appears as a slightly yellow to light yellow crystalline powder, which dissolves in water and is moderately soluble in methanol [4]. The drug is efficiently absorbed and rapidly eliminated from the body, with a half-life of three to five hours under normal conditions. A polymeric formulation of ciprofloxacin demonstrates increased diffusivity and a prolonged release rate compared to the ciprofloxacin salt [5].

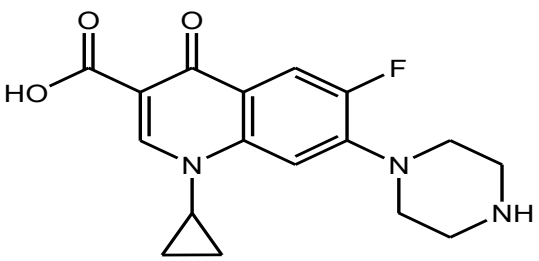

Figure 1. Ciprofloxacin

A range of analytical methods has been developed for the quantification of ciprofloxacin in various biological samples and pharmaceutical forms, including tablets, injectables, gels, and eye drops [6-12]. One of the proposed methods for ciprofloxacin detection in influenza vaccines uses an acetonitrile-water-phosphoric acid mobile phase, with triethylamine adjusting the pH to 3, and separation achieved at a flow rate of 0.6 mL/min, with ultraviolet detection at 280 nm [7]. Another study combined ciprofloxacin with other quinolones, employing a mobile phase consisting of acetonitrile and water (50:50, v/v), with a pH of 2.9 adjusted by phosphoric acid, utilizing propylparaben as the internal standard at a flow rate of one mL/min [8]. Additionally, a liquid chromatography approach was proposed for concurrent determination of ciprofloxacin and rosuvastatin in serum and formulations, with methanol-water as the mobile phase (90:10 v/v), a flow rate of one mL/min, and detection at 255 nm [9]. An HPLC-UV method was also suggested for measuring ciprofloxacin in plasma with a mobile phase of phosphate buffer (pH 2.7) and acetonitrile (77:23, v/v), using a detection wavelength of 277 nm [10, 13-15].

This study introduces a straightforward, cost-effective HPLC technique that delivers accurate results, a low limit of quantification (LOQ), and reduced analysis time. The method utilizes readily accessible chemicals, solvents, and an internal standard, making it suitable for routine application in pharmaceutical and clinical laboratories [16-20].

Materials and Methods

Materials and reagents

Ciproxin™ 250 mg tablets (manufactured by Bayer Pakistan Pvt. Ltd., Karachi) were sourced from a local pharmacy, while mebeverine hydrochloride, used as the internal standard, was kindly provided by AGP (Private) Limited, Karachi. De-ionized water was prepared in the laboratory from double-distilled water, and all solvents used were HPLC grade from Merck, Germany. The chromatographic setup consisted of a Shimadzu LC-10 AT VP pump and an SPD-10 AV VP UV-visible detector, both from Shimadzu Corporation, Japan. The separation process was performed using a µ Bondapak 125A C-18 10 µm column.

Analytical procedure

To prepare the solutions, ten mg of ciprofloxacin hydrochloride and mebeverine hydrochloride (internal standard) were each dissolved in water separately in one hundred mL volumetric flasks, yielding an initial concentration of 100 ppm for both. For the stock solution, 10 mL of the 100 ppm ciprofloxacin solution was transferred to a one hundred mL volumetric flask and diluted to a concentration of 10 ppm using water. Working solutions of various concentrations were then prepared from this stock.

A range of dilutions from 0.50 to 3.0 ppm was prepared by transferring 5, 10, 15, and 30 mL of the stock solution into separate one hundred mL volumetric flasks, which were filled to the mark with water. To each of these solutions, 1 mL of the internal standard solution was added, resulting in a final concentration of 5 ppm for the internal standard. Six distinct concentrations were analyzed for each sample.

For intra-day precision, each concentration was analyzed four times on the same day. The average value for each concentration was used to calculate the % RSD. The method exhibited good repeatability for both compounds within a single day and across alternate days. Low RSD values indicated that the method is reliable for the individual analysis of each drug.

Results and Discussion

Multiple approaches for ciprofloxacin estimation have been documented [6-12], however, the method introduced in this study is straightforward, validated per ICH (International Conference on Harmonization) standards [21], and meets all the required parameters. The chromatographic analysis revealed that the method is specific, as no interference was observed from the internal standard, confirming its selectivity.

The analysis was carried out under isocratic conditions using an 80:20 mixture of acetonitrile and water, with the pH adjusted to 2.7 using 85% phosphoric acid. A flow rate of 1.7 mL/min was employed, and all experiments were conducted at room temperature with detection at 275.0 nm. The retention times of ciprofloxacin and the

internal standard were found to be 1.52 and 2.7 minutes, respectively. This method was found to be linear (r2 = 0.9999), demonstrating high accuracy (% RSD > 2%), precision, specificity, and sensitivity, with detection and quantification limits of 120 ng/mL and 500 ng/mL, respectively.

Discussion

Linearity and regression analysis

The method proved to be linear across a concentration range of 0.5 to 3.0 ppm, with calibration curves exhibiting a correlation coefficient of no less than 0.9999 **(Table 1)**.

Table 1. Statistical regression characteristics of the method

	Day 1	Day 2	Day 3	Day 4
Correlation coefficient (R^2)	0.9999	0.9999	0.9999	0.9999
Standard error of estimate	0.0041	0.0012	0.0036	0.0011
Standard error	0.0038	0.0011	0.0034	0.0011
Intercept	-0.0014	0.0005	0.0006	-0.0000
P-value	0.0000	0.0000	0.0000	0.0000
Slope	1	1	1	1

Accuracy

The accuracy of the method was evaluated following the guidelines set by ICH [21]. As shown in **Table 2**, the recovery data confirms that the method is reliable for the accurate determination and quantification of ciprofloxacin.

Table 2. Recovery of ciprofloxacin in dosage form

Serial No.	*Conc. ppm	The peak area of the sample	% Recovery	mg/tablet	Conc.
1	0.5	70964	99.229	248.073	0.4961
2	1	141618	99.012	247.532	0.9901
3	1.5	215867	100.61	251.54	1.5092
4	2	289465	101.19	252.975	2.0238
5	2.5	356674	99.748	249.37	2.4937
6	3	430762	100.38	250.974	3.0116

*Concentration

Precision

The method's precision was evaluated following the ICH guidelines [21]. This assessment involved testing repeatability, as well as performing intra-day precision checks on the same day, utilizing six distinct concentrations of ciprofloxacin. The relative standard deviation (RSD) values, as shown in **Table 3**, reflect the reliability and consistency of the method's performance.

Table 3. Intra and inter-day variations in the analysis of ciprofloxacin hydrochloride

Serial	Concn	Area under curve					Standard deviation	Relative standard deviation	% Recovery	Recovered concentration
		Time								
No.	ppm	8:00	11:00	14:00	17:00	Mean				
Day 1										
1	0.5	71563	71502	71488	71426	71494.75	56.22	0.0008	99.97	0.4999
2	1	143011	142820	141983	141992	142451	541.44	0.0038	99.59	0.996
3	1.5	214549	214371	214052	214364	214334	206.57	0.001	99.9	1.4985
4	2	287117	287217	286659	287009	287000.5	242.99	0.0008	100.38	2.0066
5	2.5	357283	357212	357110	356991	357149	127.03	0.0004	99.88	2.497
6	3	429201	429228	428967	428964	429090	144.19	0.0003	100	3

Day 2										
1	0.5	73892	73659	73610	73707	73717	123.2	0.0017	100.23	0.5012
2	1	147457	146831	147325	147013	147156.5	285.91	0.0019	100.04	1.0005
3	1.5	221113	220219	220413	220453	220549.5	389.32	0.0018	99.96	1.4995
4	2	294128	293496	294428	294338	294097.5	420.24	0.0014	99.97	1.9995
5	2.5	368339	367657	368009	368173	368044.5	291.35	0.0008	100.09	2.5023
6	3	442114	440367	441428	441093	441250.5	726.3	0.0016	100	3
Day 3										
1	0.5	81029	81001	80994	80983	81001.75	19.62	0.0002	99.99	0.5
2	1	162116	162453	161357	161637	161890.8	488.58	0.003	99.92	0.9992
3	1.5	243246	243179	242996	243004	243106.3	125.74	0.0005	100.03	1.5005
4	2	325561	325229	325006	324811	325151.8	321.87	0.001	100.34	2.0069
5	2.5	405332	402961	405075	404883	404562.8	1083.56	0.0027	99.88	2.4971
6	3	486229	486634	485630	485690	486045.8	475.75	0.001	100	3
Day 4										
1	0.5	83802	83714	83696	83755	83741.75	47.15	0.0006	100.24	0.5012
2	1	167003	167394	166469	166845	166927.8	383.12	0.0023	99.9	0.9991
3	1.5	250119	250964	250328	250419	250457.5	360.27	0.0014	99.93	1.499
4	2	334094	334267	334064	334562	334246.8	228.42	0.0007	100.02	2.0005
5	2.5	418229	417886	417931	417829	417968.8	178.45	0.0004	100.06	2.5016
6	3	501009	501439	500884	501634	501241.5	353.52	0.0007	100	3

System suitability

To ensure system suitability, the performance of the system was assessed by examining the symmetry of the ciprofloxacin and internal standard (mebeverine hydrochloride) peaks. The analysis also involved determining the column's theoretical plate count (which should exceed 2000) and evaluating the resolution between the drug and internal standard peaks.

Specificity

Specificity was validated by successfully separating ciprofloxacin from the internal standard. The HPLC analysis clearly distinguished the two components, showing no interference from other peaks, as evidenced by the chromatogram in **Figure 2**, where all extraneous signals were absent.

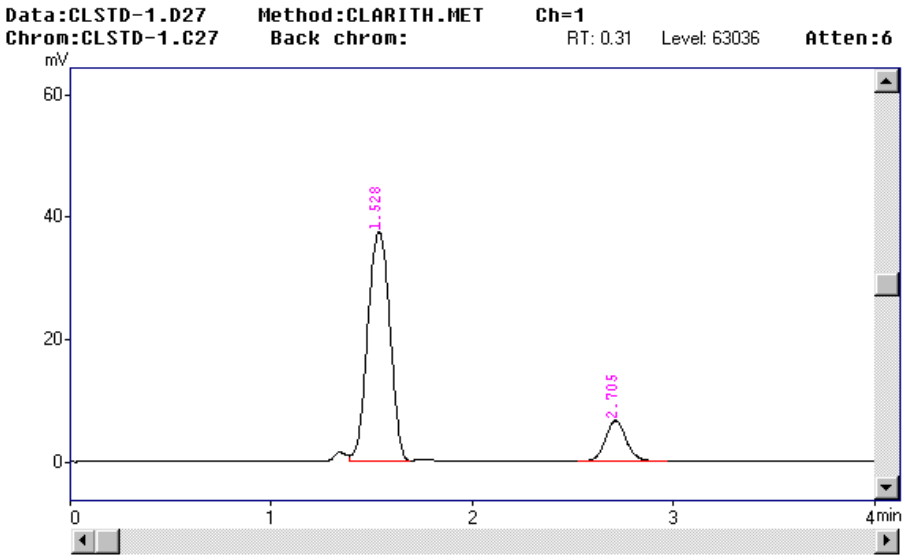

Figure 2. Representative chromatogram of ciprofloxacin and internal standard

Quantification limit

The quantification limit refers to the lowest analyte concentration that can be reliably measured with adequate precision and accuracy under the experimental conditions employed. In this study, the quantification limit was determined to be 500 ng/mL.

Detection limit

The detection limit indicates the smallest analyte concentration that can be detected but not necessarily quantified under the experimental conditions used. For this method, the detection limit was found to be 120 ng/mL.

Conclusion

The method described in this research demonstrated excellent performance in accurately quantifying ciprofloxacin molecules. The recoveries from the pharmaceutical formulations were in agreement with the declared values, with no interference from excipients in the ciprofloxacin measurement. The method, which involves minimal calculation, is efficient, reliable, and fast, making it well-suited for routine analysis with a short analysis time, ensuring consistent application of the calibration models.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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