Quantitation of Ciprofloxacin by RP-HPLC from Active and Dosage Formulations

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ABSTRACT

A simple, sensitive isocratic reversed-phase HPLC method for detecting and quantifying ciprofloxacin in pharmaceutical dosage forms has been developed and validated according to ICH guidelines. In the method, the separation was achieved on a C_{18} reversed-phase column with a mobile phase acetonitrile-water at a ratio of (80:20). The pH was adjusted to 2.7 using 85% of phosphoric acid with ultraviolet detection at 275.0 nm. A flow rate of 1.7 mL/minute was used at room temperature. The method's statistical evaluation was scrutinized through inter and intra-day precision assays, and it was found satisfactory through high accuracy and precision with a correlation coefficient of at least 0.9999. The quantification limit was 500 ng/mL, while this method's recovery was observed in the range of 99.01–101.19% and has the potential to be used for routine analysis in pharmaceuticals and clinical laboratories. The present method could also be useful to determine ciprofloxacin in human serum.

Key Words: *Ciprofloxacin, RP-HPLC (Reverse Phase High-Performance Chromatography), Dosage form, Human serum*

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INTRODUCTION

Ciprofloxacin hydrochloride **(Figure 1)**, the most frequently used quinolone [1], is available for oral use as hydrochloride monohydrate [2] and parenteral use as lactate [3]. It is slightly yellowish to light yellow crystals or crystalline powder, dissolved in water, and somewhat soluble in methanol [4]. It is thoroughly absorbed and is immediately excreted from the body under typical conditions with an elimination half-life of 3 to 5 hours. A polymer combination of ciprofloxacin showed more diffusivity and an extended-release rate connected to ciprofloxacin salt [5].

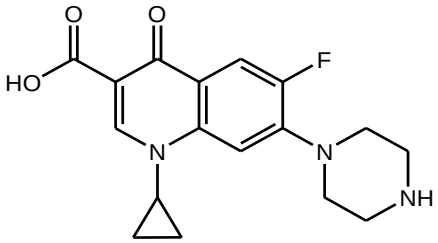

Figure 1. Ciprofloxacin

Several analytical techniques have been published to analyze ciprofloxacin in biological fluids and various pharmaceutical dosage forms (tablets, injectables, suppositories, gels, eye drops, etc.) [6-12]. A direct and sensitive HPLC method for determining ciprofloxacin in the influenza vaccine has been described [7] employing a mobile phase of acetonitrile/water/phosphoric acid. Its pH was adjusted to pH 3 with triethylamine. The separation was obtained at a flow rate of 0.6 $mLmin^{-1}$ at 280 nm. A method was mentioned for ciprofloxacin with other

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quinolones using a mobile phase consisting of water-acetonitrile (50:50, v/v) with a pH of 2.9 adjusted with phosphoric acid. Different wavelengths were applied at a flow rate of 1 mLmin^{-1}, while propylparaben was used as an internal standard [8]. A liquid chromatographic method was also reported for ciprofloxacin's concurrent determination with rosuvastatin in formulations and human serum. The separation was achieved using a mobile phase of methanol-water at a ratio of 90:10 v/v. The flow rate was 1 mLmin^{-1}, the pH of the mobile phase was pH 3, and drugs were monitored at 255 nm [9]. Similarly, an HPLC–UV method was described for the determination of ciprofloxacin in human plasma with a phosphate buffer (pH 2.7) and acetonitrile (77:23, v/v), and the drug was detected at 277 nm [10, 13-15].

The suggested task deals with the precise analysis of ciprofloxacin using a straightforward, moderate-cost, and less time-consuming HPLC method with significant accuracy and good LOQ (Limit of detection) value. The technique contains readily available chemicals, solvents, and an internal standard suitable for routine analysis [16-20].

MATERIALS AND METHODS

Material and reagents

Ciproxin™ 250 mg tablets (Bayer Pakistan Pvt. Ltd. Karachi) were purchased from the local pharmacy, while mebeverine HCl (internal standard) was a kind gift from AGP (Private) Limited Karachi. De-ionized water was prepared in the lab from double distilled water, while all other solvents were HPLC grade (Merck Germany). Shimadzu Corporation Japan's chromatographic system consisted of an LC-10 AT VP pump and SPD-10 AV VP UV-visible detector. The separation was achieved on the µ Bondapak 125A C-18 10 µm column.

Analytical procedure

10 mg of ciprofloxacin hydrochloride and internal standard (mebeverine hydrochloride) were dissolved in water in a 100 mL volumetric flask separately, with the same solvent. The concentrations of these immediate solutions were 100 ppm. For the stock solution preparation, 10 mL of ciprofloxacin (100 ppm) was taken in a 100 mL volumetric flask with water. The concentrations of these stock solutions were 10 ppm, from which standard working solutions of desired concentrations were prepared.

Different dilutions ranging from 0.50, 1.0, 1.50,..3.0 ppm were prepared from the stock solution of ciprofloxacin. For this purpose, 5, 10, 15,…..30 mL of stock solutions were pipetted out in different 100 mL volumetric flasks and made up to the mark with the same solvent (water) to have the required concentrations of 0.50, 1.0, 1.50, . . . 3.0 ppm, respectively, and in all solutions, added 1 mL of the internal standard (primary solution) added as internal standard became 5 ppm of final concentration. Six different concentrations of each sample were analyzed. For intra-day precision, different concentrations of every compound were investigated on a similar date and injected four times. The mean value of each concentration was used to calculate %RSD. Generally, satisfactory repeatability of the outcome in one day and alternate days was observed. Lower RSD values showed that the method is reliable to analyze both drugs separately.

RESULTS AND DISCUSSION

Many methods have already been reported for estimations of ciprofloxacin [6-12] and but the present method is straightforward and is validated according to ICH (International Conference on Harmonization) guidelines [21]. All of the parameters were found under the limits of ICH guidelines, and the chromatogram of the analyte revealed that the method was specific and no peak of internal standard interfered, and can be considered selective.

The analysis was conceded in isocratic conditions using a mobile phase acetonitrile-water (80:20), while pH 2.7 was adjusted using phosphoric acid (85%). The flow rate of 1.7 mLminutes^{-1} was used, and all experiments were performed at room temperature at 275.0 nm. The retention time of both the drug and internal standard were 1.52 and 2.7 minutes, respectively. The method was found linear (r^2=0.9999), accurate (%RSD>2%), precise, specific, and sensitive (DL= 120 ngmL^{-1} and QL=500 ngmL^{-1}).

Discussion

Linearity and regression analysis

The present method is linear over a range of 0.5 to 3.0 ppm, and all the calibration curves have a correlation coefficient value of at least 0.9999 **(Table 1)**.

Table 1. Statistical regression characteristics of 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

Accuracy was performed according to the guidelines published by ICH [21]. The recovery data expressed in

Table 2 showed that the method is accurate for determining and quantifying ciprofloxacin.

Table 2. Recovery of Ciprofloxacin in dosage form

Serial No.	*Conc. ppm	Peak area of 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

Like accuracy, the method's precision was also performed using the same guidelines [21].

The precision was examined regarding repeatability, and intra-day precision was analyzed on the same day using six ciprofloxacin concentrations. **Table 3** summarizes the relative standard deviation (RSD).

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

System suitability is the parameter that assists in checking the behavior of the system and was evaluated by analyzing the symmetry of the ciprofloxacin, internal standard (mebeverine hydrochloride), peaks, theoretical

plates of the column (>2000), and the resolution between the peaks of internal standard and drugs.

Specificity

Specificity is ensured by the separation of the internal standard and ciprofloxacin. The technique demonstrated a resolution between the internal standard and ciprofloxacin. HPLC chromatogram showed that extraneous peaks by adding an internal standard are the baseline resolved from the parent analyte **(Figure 2)**.

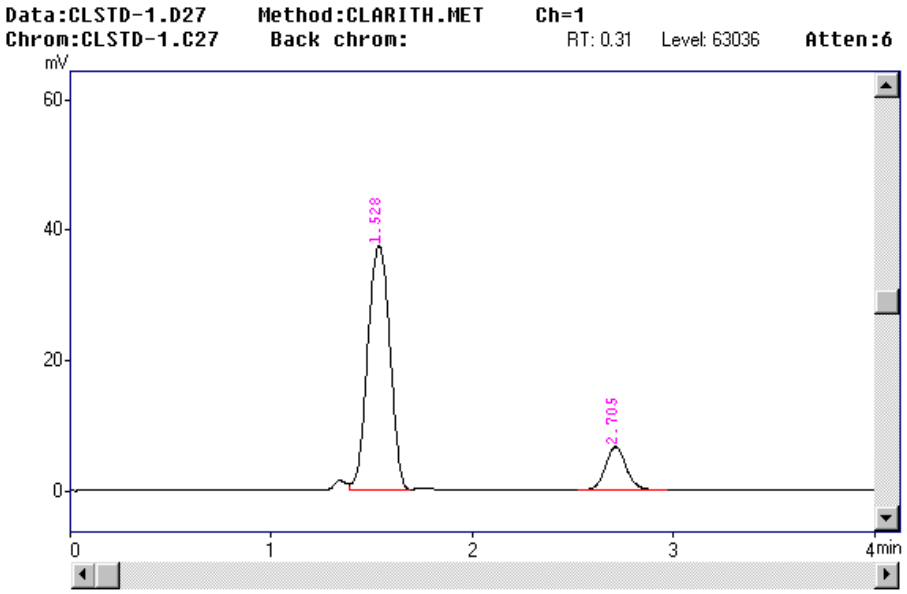

Figure 2. Representative chromatogram of ciprofloxacin and internal standard

Quantification limit

The quantification limit is the lowest concentration of an analyte in a sample determined under sufficient precision and accuracy using the stated experimental condition. The limit of quantification of the developed method was found to be 500 $ngmL^{-1}$.

Detection limit

The lowest detected concentration of an analyte had not necessarily been quantitated under the stated experimental conditions. The detection limit was found to be 120 $ngmL^{-1}$ of the developed method.

CONCLUSION

The proposed method offered an excellent outcome of the quantitative resolution of the drug molecules. The sample recoveries from the formulation were good in agreement and, with the label claims, suggested no interference of excipients in ciprofloxacin estimation. The recommended method with very simple mathematical content is more reliable and fast. The analysis time robustly supports us in relating these calibration models for regular investigation.

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