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Method Validation and Kinetic Investigation of Ciprofloxacin Mutarotation by Polarimetry

Lalasaheb Kashid

Postgraduate Research Center, Department of Chemistry, Vidya Pratishthan's, Arts, Science and Commerce College, Baramati, Pune-413133, Maharashtra, India
Corresponding Author Email : *lmkashid[at]gmail.com*

Abstract: ***A reliable and rapid polarimetric method was developed for the estimation of Ciprofloxacin Hydrochloride in bulk and pharmaceutical dosage forms. Standard and sample solutions were prepared in distilled water, and the method was validated according to ICH guidelines. Linearity was demonstrated in the range of 0.4–4.0 mg/mL with a correlation coefficient (r^2) of 0.9930. The accuracy of the method was confirmed with a mean recovery of 99.56 ± 0.28%. Precision studies showed %RSD values below 2% for both intra- and inter-day measurements, indicating excellent reproducibility. The method showed good robustness with minimal variation in optical rotation due to sample positioning. Sensitivity was established with LOD and LOQ values of 0.2795 mg/mL and 0.8472 mg/mL, respectively. The validated method was successfully applied to the kinetic study of ciprofloxacin mutarotation in acidic medium at constant temperature. Overall, the method is simple, accurate, precise, and suitable for routine quality control and kinetic investigations.***

Keywords: Ciprofloxacin, Method Validation, Polarimetry, Kinetic Mutation

1. Introduction

Ciprofloxacin is a second-generation fluoroquinolone antibiotic with broad-spectrum activity against Gram-positive and Gram-negative bacteria [1, 2]. It inhibits bacterial DNA gyrase and topoisomerase IV, essential enzymes for DNA replication [3, 4]. Due to its clinical relevance, accurate quantification in pharmaceuticals and biological fluids is crucial [5]. HPLC is widely accepted for ciprofloxacin analysis owing to its precision and sensitivity [6–8]. UPLC has improved throughput and resolution in newer studies [9, 10]. Spectrophotometric methods offer simpler, low-cost alternatives using ion-pair, oxidation, or charge-transfer reactions [11–13].

Colorimetric assays based on methyl orange and Ce (IV) reagents are efficient for ciprofloxacin in dosage forms [14, 15]. Simultaneous drug estimation via dual-wavelength methods has been achieved in fixed-dose combinations [16, 17]. Despite analytical advancements, the stereochemical dynamics of ciprofloxacin, particularly mutarotation, are underexplored. Mutarotation involves the change in optical rotation due to interconversion between stereoisomeric forms in solution [18]. It affects solubility, stability, and bioavailability key factors in drug formulation [19]. Ciprofloxacin contains chiral centers, and in acidic environments such as the stomach, it may undergo configurational changes via protonation [3, 18]. Polarimetry is a classical optical technique that measures such rotation and provides real-time insight into chiral behavior [19, 20]. Though widely used in sugar chemistry, its pharmaceutical applications remain limited. This study focuses on the development and validation of a polarimetric method to investigate the kinetics of ciprofloxacin mutarotation in dilute hydrochloric acid. The work aims to establish a relationship between acid concentration and the rate of mutarotation, enhancing understanding of ciprofloxacin's chiral stability.

2. Materials and Methods

Instrumentation

The optical rotation measurements for the mutarotation study of ciprofloxacin were carried out using a digital Polarimeter (Model EQ 801). This instrument is equipped with a 4-digit LED display and allows measurement over a range of ±200° with a resolution of 0.01° and an accuracy of **±0.1°**. The EQ 801 model operates without the need for a darkroom or sodium lamp by utilizing a monochromatic light source of 620 nm with infinite lamp life. Detection is achieved through an electronic sensor, ensuring high repeatability of 0.1°. Sample measurements were conducted using 10 cm of Teflon tubes, depending on the concentration and volume of the ciprofloxacin solution. The instrument operates on 230V AC ±10% at 50 Hz, and calibration was performed using standard reference solutions. This setup enabled precise monitoring of changes in optical rotation over time, indicating the mutarotation behavior of ciprofloxacin.

Preparation of Working Standard Solutions

A standard stock solution of ciprofloxacin was prepared in water using a 100 mL volumetric flask. Appropriate dilutions were made with distilled water to obtain the desired concentrations. The optical rotation of these solutions was measured at a constant temperature using a digital polarimeter.

Experimental Procedure

Optical rotation measurements were performed using the EQ 801 digital polarimeter. A 25 mL solution of ciprofloxacin was mixed with varying concentrations of hydrochloric acid in a stoppered reagent bottle. The mixture was thoroughly shaken to ensure homogeneity, and the polarimeter tube was rinsed and filled with this solution. The initial optical rotation, denoted as α_0, was recorded. Subsequent readings, representing α_t, were taken at regular intervals of 10 minutes to monitor changes in optical activity over time. To determine the final optical rotation, $\alpha\infty$, the remaining solution was transferred to a stoppered conical flask, heated

to 60°C for 5 minutes, then cooled to room temperature (approximately 5–10 minutes), and measured again using the Polarimeter.

Kinetics and Thermodynamics Study

The kinetics of ciprofloxacin mutarotation were investigated using a thermostat bath with moderate temperature control, acknowledging potential experimental errors. Samples were collected at fixed time intervals to study hydrogen ion–catalyzed mutarotation with hydrochloric acid concentrations ranging from 0.2000 to 2.000 M, prepared from a 5.000 M stock solution. The reaction started by mixing ciprofloxacin with the acid solutions, and optical rotation measurements were taken every five minutes. The effect of temperature on mutarotation was assessed at 298 K, 308 K, and 318 K. Rate constants were calculated using the Infinite Time and Guggenheim methods. Important thermodynamic parameters including Gibbs free energy (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°) were also evaluated.

3. Results and Discussion

Method Validation

The method was validated by assessing linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. Multiple blank readings were taken before adding ciprofloxacin standards. LOD was determined by adding low drug concentrations until a detectable response above the blank was achieved. Precision was evaluated through intra-assay and inter-assay repeatability using repeated measurements within a day and across different days. Accuracy was confirmed by recovery studies, where known amounts of ciprofloxacin were added to samples, and the percentage recovery was calculated.

Linearity

Calibration curves were prepared by plotting the angle of rotation against ciprofloxacin concentration, showing linearity in the range of 0.4 to 4.0 mg/mL as shown in figure 1. Least squares linear regression analysis gave the mean regression equation: y = -8.988x + 0.095 with a correlation coefficient $\mathbf{r^2}$ = 0.9930. Here, *y* represents the angle of rotation, *a* is the slope, *b* the intercept, and *x* the concentration (mg/mL). The results indicate an excellent correlation between the angle of rotation and drug concentration within the tested range.

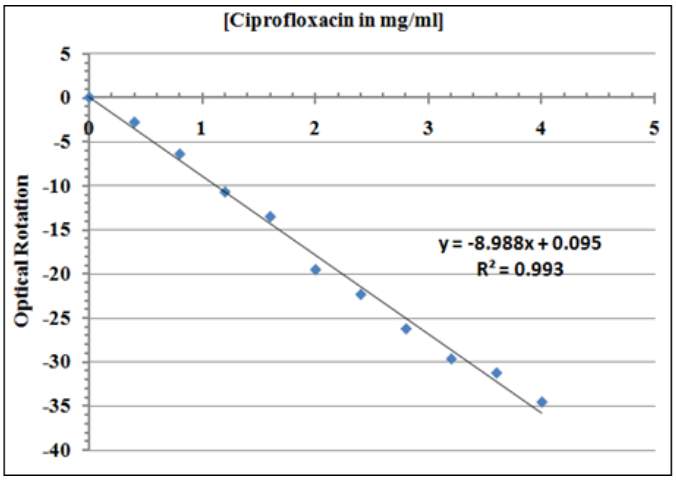

Figure 1: Linearity curve of ciprofloxacin

Accuracy

Accuracy refers to the closeness between the experimental results and the true values. It was evaluated by calculating the relative error between measured and added concentrations. Recovery studies were performed by adding three different concentrations of ciprofloxacin (1.6, 2.8, and 4.0 mg/mL). The actual ciprofloxacin content was calculated using the regression equation (y = -8.988x + 0.095). The recoveries obtained were 101.0 ± 2.42%, 108 ± 1.62%, and 99.56 ± 0.28%, respectively. These results, shown in Table 1, demonstrate good recoveries at all concentrations, confirming the accuracy of the method.

Table 1: Mean values for recovery of ciprofloxacin standard solution (n = 3)

No. of Expt.	Amount Added (mg/mL)	Optical Rotation	Amount Found (µg/mL)	Recovery %	% Recovery ± SD (RDS)
1	1.6	-14.1	1.5793	98.708	101.0 ± 2.44 (2.42%)
		-14.4	1.6127	100.794	
		-14.8	1.6572	103.576	
2	2.8	-25.2	2.8143	100.511	101.8 ± 1.65 (1.62%)
		-25.4	2.8366	101.306	
		-26	2.9033	103.69	
3	4	-35.8	3.9937	99.841	99.56 ± 0.27 (0.28%)
		-35.6	3.9714	99.285	
		-35.7	3.9825	99.563	

Precision

The precision of the method was evaluated at three levels: repeatability, intra-day precision, and inter-day precision across different concentrations of ciprofloxacin. Repeatability was assessed by measuring the angle of rotation of a 2.8 mg/mL ciprofloxacin HCl solution six times and calculating the %RSD. Intra-day precision involved analyzing six replicate standard solutions prepared at different times within a single day, and inter-day precision was determined by analyzing the same concentration of drug solution across three consecutive days using the proposed Polarimeter method.

Table 2: Results of Intraday precision for ciprofloxacin

[Ciprofloxacin] (mg/mL)	Replicate No.	Optical Rotation
2.8	1	-25.3
2.8	2	-25.5
2.8	3	-25.2
2.8	4	-25.4
2.8	5	-25.45
2.8	6	-26

Intraday Precision

The intraday precision of ciprofloxacin was evaluated by assessing the repeatability of the developed method. A standard solution of ciprofloxacin at 2.8 mg/mL was prepared in six replicates at different times within the same day. The optical rotation values for these six measurements were recorded, and the %RSD was calculated as 1.09%, indicating good precision (Table 2).

Interday Precision

Interday precision was assessed by preparing and analyzing the ciprofloxacin solution of the same concentration (2.8 mg/mL) at different times across three consecutive days. Optical rotation readings from six replicates each day were recorded, and the %RSD was found to be 1.74%, which is within the acceptable limit of less than 2%, confirming the precision of the method (Table 3).

Table 3: Results of Interday precision for ciprofloxacin

[Ciprofloxacin] (mg/mL)	Optical Rotation (1 day)	Optical Rotation (2 day)	Optical Rotation (3 day)	Average %RSD
2.8	-25.3	-25.8	-25.1	
2.8	-25.5	-25.9	-25.3	
2.8	-25.2	-25.4	-25.4	
2.8	-25.4	-25.6	-25.8	
2.8	-25.45	-25.15	-25.9	
2.8	-26	-26.9	-26.35	
%RSD	1.09	2.35	1.8	1.74

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) are indicators of the sensitivity of the developed method and the Polarimetry equipment employed for the analysis of ciprofloxacin. These parameters were calculated based on the standard deviation (σ) of the response and the slope (S) of the calibration curve, in accordance with the International Council for Harmonisation (ICH) guidelines, using the signal-to-noise ratio method.

The calculations were based on the following equations:

$$\mathbf{LOD = 3.3 \times (\sigma/S)}$$
$$\mathbf{LOQ = 10 \times (\sigma/S)}$$

The LOD and LOQ for ciprofloxacin were found to be **0.2795 mg/mL** and **0.8472 mg/mL**, respectively. These results, confirm that the method and instrumentation exhibit high sensitivity.

Robustness

The robustness of the developed polarimetric method was evaluated by assessing the effect of sample placement within the Polarimeter. Ciprofloxacin samples at three different concentrations were analyzed by positioning the Polarimeter tube at three distinct locations along the path of light: extreme left, center, and extreme right. The angle of rotation was recorded for each position, and the percentage relative standard deviation (%RSD) was calculated to determine intra-day variability. The results, presented in Table 4, showed low %RSD values for the angle of rotation across all positions, indicating that minor variations in sample placement did not significantly affect the measurement. This confirms the excellent robustness of the developed polarimetric method for the quantification of ciprofloxacin.

Table 4: Data for Robustness of the Polarimetric Method for Ciprofloxacin Analysis

[Ciprofloxacin] (mg/mL)	Extreme Left	Center	Extreme Right	Angle of Rotation (α)	Average % RSD
1.6	-14.1	-14.4	-14.8	-14.43 ± 0.3511	1.4466
2.8	-25.2	-25.4	-26	-25.53 ± 0.4163	
4.0	-35.8	-35.6	-35.7	-35.70 ± 0.1000	

Kinetics of Mutarotation of Ciprofloxacin

The kinetics of mutarotation of ciprofloxacin in acidic medium were investigated using the Polarimetry technique at a constant ciprofloxacin concentration and constant temperature (25 °C). The influence of hydrochloric acid concentration on the rate of mutarotation was studied over the range of 0.2 M to 2.0 M HCl. Figure 2 to 6 illustrates a representative plot of log(a/(a-x) versus time and log (a-x) verses time for ciprofloxacin at 25 °C. The observed decrease in optical rotation with increasing time indicates that the compound undergoes structural rearrangement, consistent with a pseudo-first-order kinetic process. This behavior is characterized by an exponential decay curve, confirming that mutarotation proceeds as a time-dependent reaction influenced by acid concentration. The results suggest that HCl acts as a catalytic medium, enhancing the rate of mutarotation through protonation, which facilitates the interconversion of isomeric forms of ciprofloxacin.

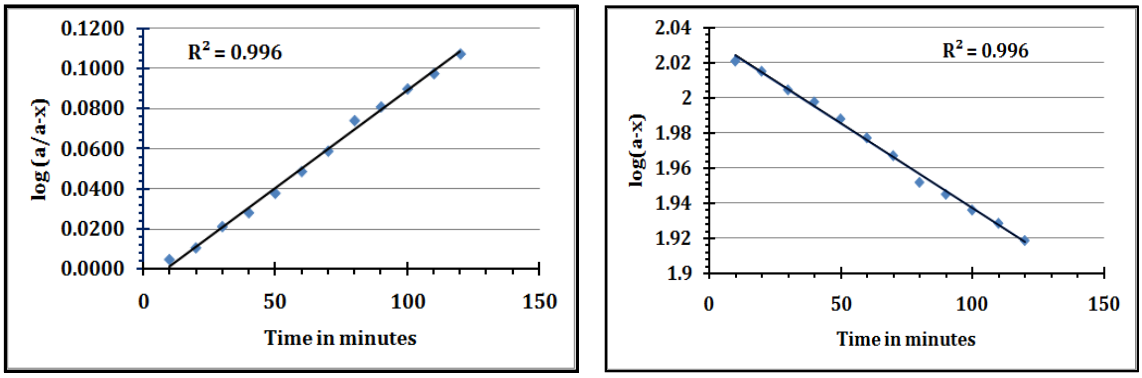

Figure 2: Mutarotation rate constant of ciprofloxacin in 0.2M HCl

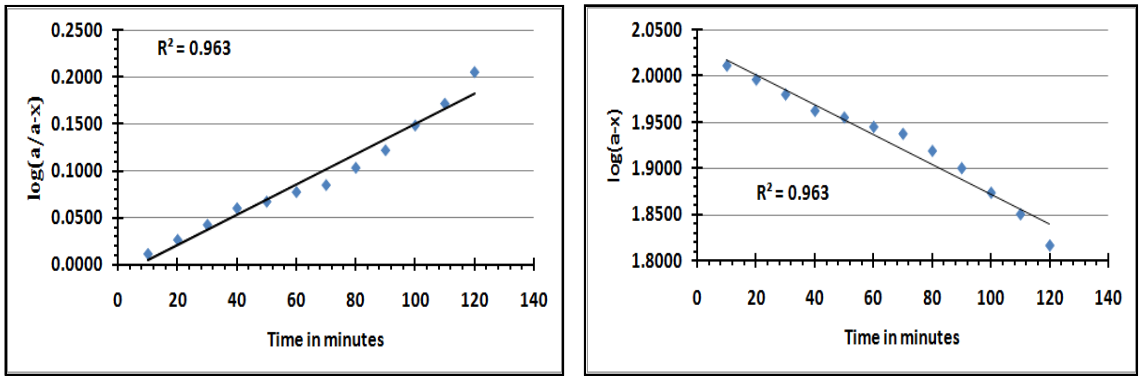

Figure 3: Mutarotation rate constant of ciprofloxacin in 0.4M HCl

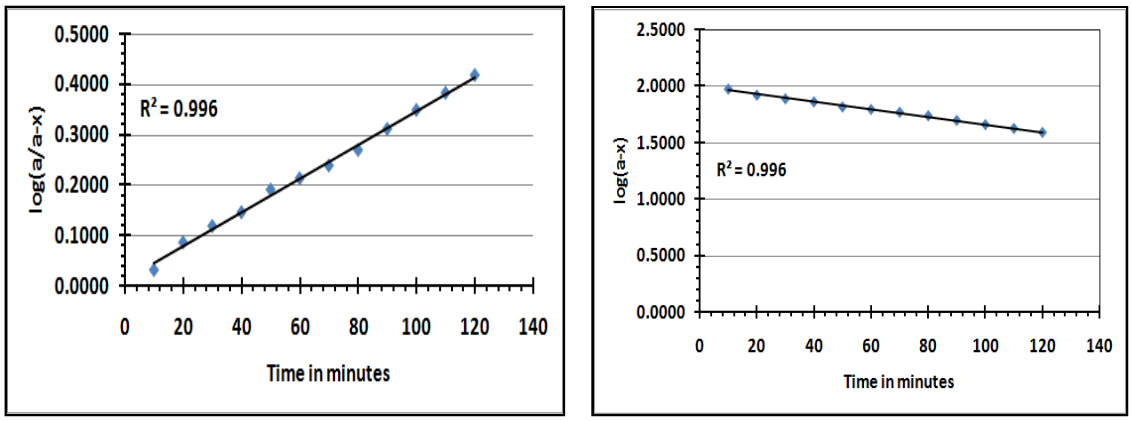

Figure 4: Mutarotation rate constant of ciprofloxacin in 0.8M HCl

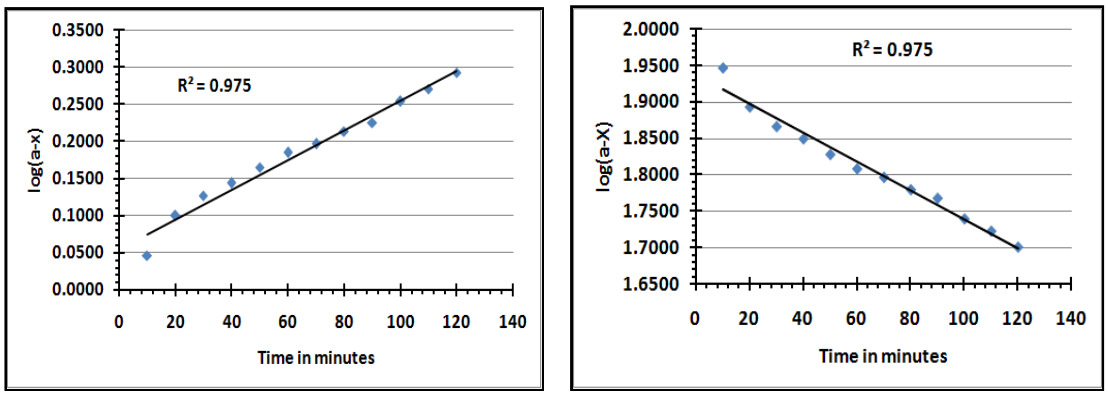

Figure 5: Mutarotation rate constant of ciprofloxacin in 1.5M HCl

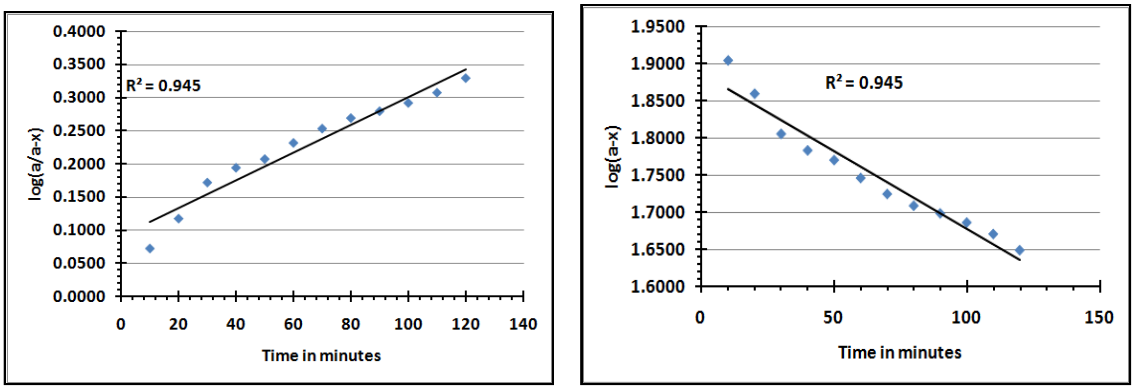

Figure 6: Mutarotation rate constant of ciprofloxacin in 2.0M HCl

Table 5: Rate constant (K) for the mutarotation ciprofloxacin at concentration of HCl

Conc. of HCl (M)	Regression Equation	Slope $\times 10^{-2}$	Correlation Coefficient	Rate Constant K (min^{-1})
0.2	y = 0.097x - 0.832	0.097	0.996	2.234×10^{-3}
0.4	y = 0.161x - 1.145	0.161	0.963	3.708×10^{-3}
1.5	y = 0.199x + 5.530	0.199	0.975	4.583×10^{-3}
2	y = 0.209x + 9.064	0.209	0.945	4.813×10^{-3}

Thermodynamic Calculations of the Ciprofloxacin Mutarotation Reaction

Thermodynamic parameters are essential for predicting the effect of temperature on acid-catalyzed mutarotation reactions such as that of ciprofloxacin. Among these, Gibbs free energy change (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°) are of particular importance. The thermodynamic constants ΔH° and ΔS° were determined from the Van't Hoff plot, using the following relation:

$$\ln K = \frac{\Delta H^\circ}{R}\frac{1}{T} + \frac{\Delta S^\circ}{R}$$

A plot of **ln K vs. 1/T** was constructed from the equilibrium constant values obtained at different temperatures. From the **slope** and **intercept** of this plot, the values of **ΔH°** and **ΔS°** were calculated.

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

$$\Delta G^\circ = -RT \ln (K)$$

The Gibbs free energy change (ΔG°) was then determined using the relation:

- K is the equilibrium constant
- R is the universal gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$)
- T is the temperature in Kelvin
- ΔG°, ΔH° are in J/mol; ΔS° is in J/mol·K

The calculated thermodynamic parameters are presented in Table 6, 7 and Table 8

The positive value of enthalpy change (ΔH° = +8.7119 kJ/mol**)** confirms that the mutarotation of ciprofloxacin in HCl is an endothermic process. The positive entropy change (ΔS° = +19.41 J/mol·K) indicates an increase in disorder, likely due to the interconversion between different conformational or tautomeric forms during mutarotation. Figure 7 shows the effect of temperature on angle of rotation of ciprofloxacin in HCl at25°C and 35°C

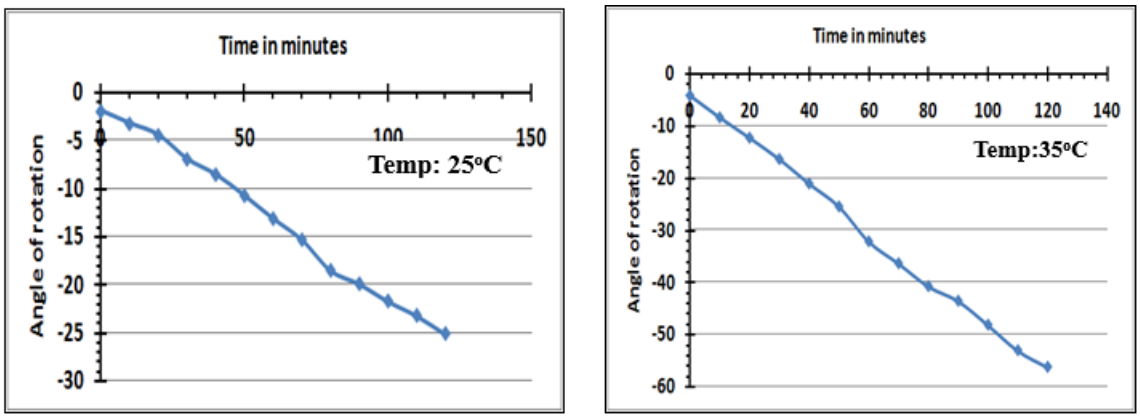

Figure 7: Effect of temperature on angle of rotation of ciprofloxacin in HCl at25°C and 35°C

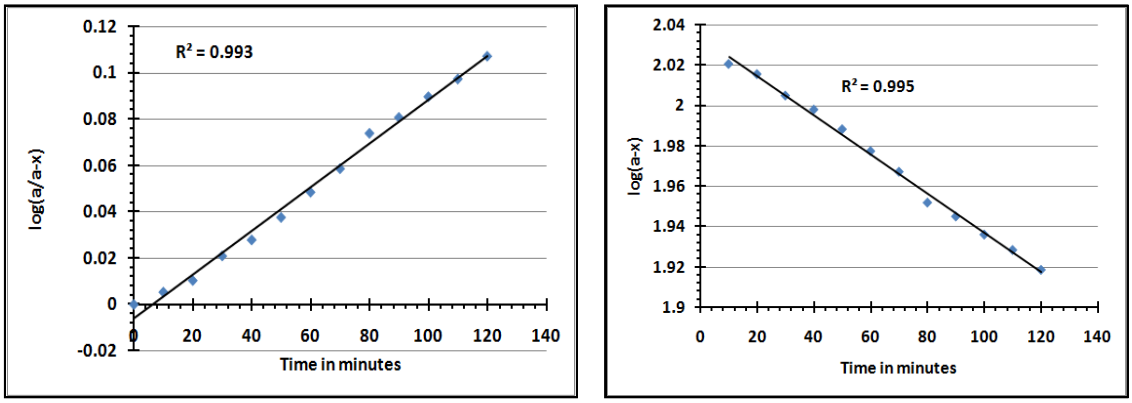

Figure 8: Mutarotation rate constant of ciprofloxacin at 25°C in HCl

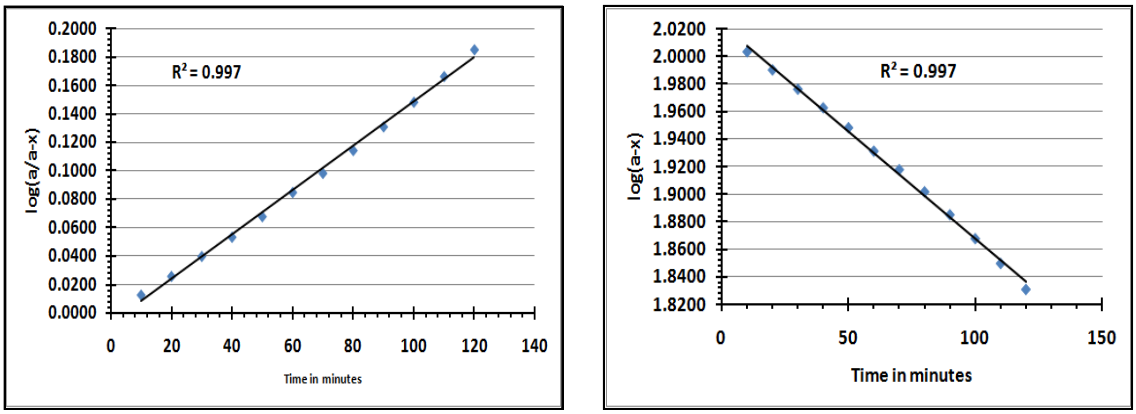

Figure 9: Mutarotation rate constant of ciprofloxacin at 35°C in HCl

Table 6: Rate constant (K) for the mutarotation ciprofloxacin at different temperature

Temp. in K	Regression Equation	Slope ×10⁻²	Correlation Coefficient R²	Rate Constant K (min⁻¹)
298	y = 0.096x - 0.804	0.096	0.995	2.211 × 10⁻³
308	y = 0.155x - 0.736	0.155	0.994	3.570 × 10⁻³
318	y = 0.295x - 3.339	0.295	0.996	6.794 × 10⁻³

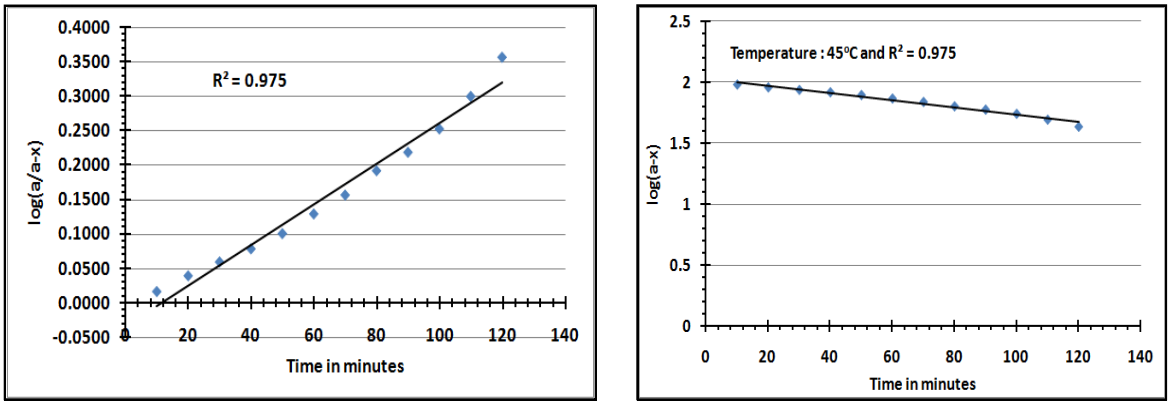

Figure 10: Mutarotation rate constant of ciprofloxacin at 45°C in HCl

Table 7: Thermodynamic parameters of mutarotation ciprofloxacin

T (K)	Log(K/T)	(1/T) ×10⁻³	(Slope) ×10⁻³	Intercepts ×10⁻³	ΔG° (J mol⁻¹)	ΔH° (kJ mol⁻¹)	ΔS° (kJ mol⁻¹)
298	-5.1296	3.3557	-0.455	1.014	-5.7799	8.7119	19.41
308	-4.9359	3.2467					
318	-4.6703	3.1446					

The energy of mutarotation of ciprofloxacin in hydrochloric acid was evaluated by studying the rate constant (K) at three different temperatures, as illustrated in Table 9. These rate constants were used to calculate the logarithm of K and the reciprocal of temperature (1/T), which were then plotted to generate the Arrhenius plot. From the linear regression of this plot, the slope was determined to be –0.429 × 10³. This value was applied in the Arrhenius equation to calculate the activation energy (Ea), which was found to be 8.21 kJ mol⁻¹. The relatively low activation energy indicates that the mutarotation process of ciprofloxacin in an acidic medium is kinetically feasible and proceeds with minimal energy input.

Table 8: Energy of mutarotation of ciprofloxacin in HCl

Temperature (K)	K (min^{-1})	Log K	(1/T) $\times 10^{-3}$	(Slope) $\times 10^{-3}$	Ea (kJ mol^{-1})
298	2.211×10^{-3}	-2.6554	3.3557	-0.429	8.2141
308	3.570×10^{-3}	-2.4473	3.2467		
318	6.794×10^{-3}	-2.1679	3.1446		

4. Conclusions

Modern polarimetric methods play a crucial role in analyzing chiral pharmaceutical compounds. Since about 25% of drugs exist as racemates or diastereomers, their stereochemistry significantly impacts efficacy and safety. Ciprofloxacin, being optically active, was analyzed using a newly developed and validated polarimetric method. The method met ICH guidelines for linearity, accuracy, precision, robustness, LOD, and LOQ, showing excellent linearity (R^2 = 0.9930), accuracy (99.56 ± 0.28%), and precision (%RSD < 2%). It is non-destructive, sensitive, and demonstrated good solution stability. The method was successfully applied to study the mutarotation kinetics of ciprofloxacin in acidic media. Results indicated pseudo-first-order kinetics, with a decrease in optical rotation over time. Thermodynamic analysis revealed an endothermic process with increased entropy. These findings confirm that the method is suitable for routine analysis. It is reliable for kinetic and thermodynamic investigations of optically active drugs.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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