

DEVELOPMENT AND VALIDATION OF HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR CIPROFLOXACIN BY QUALITY BY DESIGN APPROACHSHRADDHA V TATHE^{1*}, MORESHWAR P MAHAJAN¹, RASHMI G PINJARKAR², ARUN M KASHID²¹Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmacy, Narhe, Pune, Maharashtra, India. ²Department of Pharmaceutical Quality Assurance, Sinhgad Institute of Pharmacy, Narhe, Pune, Maharashtra, India. Email: tatheshraddha09@gmail.com

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ABSTRACT

Objective: The aim of this paper is to create a new, systematic high-performance thin-layer chromatography (HPTLC) method for ciprofloxacin that is based on quality by design (QbD).

Methods: The mobile phase was chloroform: IPA: H₂O: Formic Acid (2:7:0.5:0.5V/V), and the chromatographic separation was performed on aluminum-backed silica gel 60 F254 plates. Ciprofloxacin was detected using UV light at 278nm. In factor screening studies, a 3-factor 17-run standard 3-level factorial design was used, and a Box-Behnken design was used to optimize HPTLC experimental parameters for obtaining anticipated chromatographic conditions. The basic method parameters were tested to understand risk assessment. Three independent parameters, such as saturation time, band duration, and migration distance, were chosen and analyzed based on the risk assessment to see if these three parameters influenced the responses. For ciprofloxacin, the method produces a compact and well-resolved band at R_f = 0.40 0.02. In the linear regression analysis performed on ciprofloxacin, the regression coefficient was found to be r² = 0.996.

Results: According to the International Council on Harmonization (ICH) guidelines, it was validated for validation parameters such as accuracy, precision, robustness, the limit of detection, and the limit of quantification. The proposed method for ciprofloxacin determination was found to be straightforward, precise, reliable, stable, and sensitive.

Conclusion: The QbD method produced a more robust method that can generate accurate, high-quality, and reliable data during the process, and it can be effectively used in the routine inspection of Ciprofloxacin in the tablets dosage form.

Keywords: Ciprofloxacin, High-performance thin-layer chromatography, Quality by design, Box-Behnken Design.

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INTRODUCTION

Ciprofloxacin is a type of antibiotic. Ciprofloxacin is a carboxylic acid that is made up of 1-cyclopropyl-6-fluoro-4-oxo-7-piperazine-1-ylquinoline-3-carboxylic acid. Bayer A.G. patented ciprofloxacin in 1983, and it was first used in 1987 [1,2]. Ciprofloxacin is classified as an important medication by the World Health Organization. Ciprofloxacin is classified as essential for human medicine by the WHO. Ciprofloxacin was a generic drug that was available. With over 6 million prescriptions written in 2018, ciprofloxacin was the 109th most widely prescribed drug in the United States. The FDA has approved Ciprofloxacin for urinary tract infections, sexually transmitted infections, bone, and joint infections [3,4].

It's a fluoroquinolone antibiotic with a wide range of action. Ciprofloxacin works against both gram-positive and gram-negative bacteria, inhibiting cell division by inhibiting a type II DNA gyrase and topoisomerase IV, which are needed to distinguish bacterial DNA. Ciprofloxacin can be taken orally or intravenously [5,6]. At therapeutic doses, ciprofloxacin's side effects are minor and often consist of gastrointestinal disturbances including nausea and diarrhea [7]. Non-aqueous titration, UV spectrophotometry, colorimetry, high-performance liquid chromatography, HPLC with mass spectroscopy, thin-layer chromatography, gas chromatography, and other methods were used to evaluate it. Non-aqueous titration, UV spectrophotometry, colorimetry, high-performance liquid chromatography, HPLC with mass spectroscopy, thin-layer chromatography, gas chromatography, and capillary electrophoresis were all used to evaluate it [8,9].

In this study, Box-Behnken design was used for the optimization of chromatographic analysis of the HPTLC analysis. This design was decided due to its adaptability to change or include or cross out any parameter at any time when our work is ongoing. This work aimed to develop and validate a quality by a design-based efficient method using HPTLC (Fig. 1) [10].

METHODS**Chemical and reagents**

Cipla LTD offered a free sample of Ciprofloxacin (Mumbai, India). HiMedica Laboratories PVT LTD was also purchased chloroform (ARGrade) from (India, Mumbai, LTD) E. Merck PVT LTD provided HPTLC plate silica gel 60 F254 (Mumbai India).

Instrumentation

Methanol was used to prewash HPTLC plates, which were then triggered in a 120°C oven for 10 minutes before chromatography. The production was carried out in a 20 cm × 10 cm twin through glass chamber with chloroform, isopropyl alcohol, water, and formic acid (2:7:0.5:0.5 v/v) as the mobile step and a development distance of 80 mm after 10 minutes of saturation with mobile vapor. The chromatography plates were dried with an air dryer after growth, and for the current analytical analysis, a CAMAG (Muttentz, Switzerland) HPTLC device with a CAMAG LINOMAT sample applicator, a Hamilton syringe (Bonduz, Switzerland), and a CAMAG TLC Scanner with Wincats software was used.

Preparation of standard stock solution

The study was to make the stock solution, dissolve 5 mg in 10 ml methanol to obtain a concentration of 500l.

Selection of analytical wavelength

The bands were scanned over a range of 400-200 nm after chromatographic development, and a wavelength of 278 nm was reached, which was used to detect method development and validation.

Method development as per experimental design

The Ishikawa fishbone diagram was created using Microsoft Excel at the start of the risk assessment plan to structure the risk operation plan for the factors that influence the system [11,12]. Critical analytical attributes (CAAs) 17 were studied using a standard 3-factorial design to determine the critical system parameters/critical process parameters that primarily affect the CAAs (saturation time, band length, migration distance) [13,14]. Using the software suggested in Table 1, the design matrix enrolled considered factors and their low, medium, and high levels. The experimental design runs were analyzed for the influence of the study factors on the CAAs.

CMPs, particularly saturation time, band length, and migration distance, were studied at three levels, namely low, medium, and high, using the box-Behnken design. According to the Box-Behnken design, a design matrix consists of 17 experimental runs. The mathematical software Design-Expert version 11 was used to evaluate all of the findings.

RESULTS AND DISCUSSION

Optimization and data analysis

Design-expert software version 11 was used to optimize data analysis by choosing a quadratic model for detecting retardation factor. For CAAs (as given in Fig. 2), the 3D response surface plot was observed, as well as the product of the model's analysis of variance (ANOVA) for the Rf value of the ciprofloxacin chromatogram. The model's estimation of

the Rf value was statistically important, as shown by a probability value of less than 0.05. For the Rf value responses, all quadratic terms were found to be meaningful.

With a saturation time of 10 minutes, a band length of 0.4mm, and a migration distance of 80 cm, the numerical optimization indicated optimal conditions (Table 2 and Figs. 2-4).

Method validation

According to the International Council of Harmonization (ICH) guidelines, the existing method was validated for linearity, accuracy (percent recovery), precision, robustness, the limit of detection (LOD), and the limit of quantification (LOQ).

Linearity

For ciprofloxacin, the typical calibration curve was plotted in a concentration range of 50–300 ng per band, respectively. The coefficient

Table 1: Design matrix as per Box-Behnken for optimization of the HPTLC method

Run	(A) Saturation time (min)	(B) Band length (mm)	(C) Migration distance (cm)
1	10	4	70
2	5	8	80
3	15	8	80
4	5	6	70
5	15	6	90
6	15	6	70
7	10	6	80
8	5	6	90
9	15	4	80
10	10	6	80
11	10	6	80
12	5	4	80
13	10	8	70
14	10	6	80
15	10	4	90
16	10	6	80
17	10	8	90

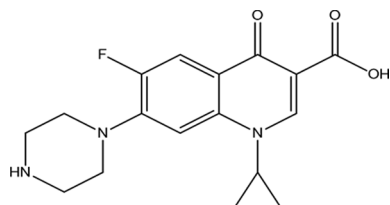


Fig. 1: Ciprofloxacin structure

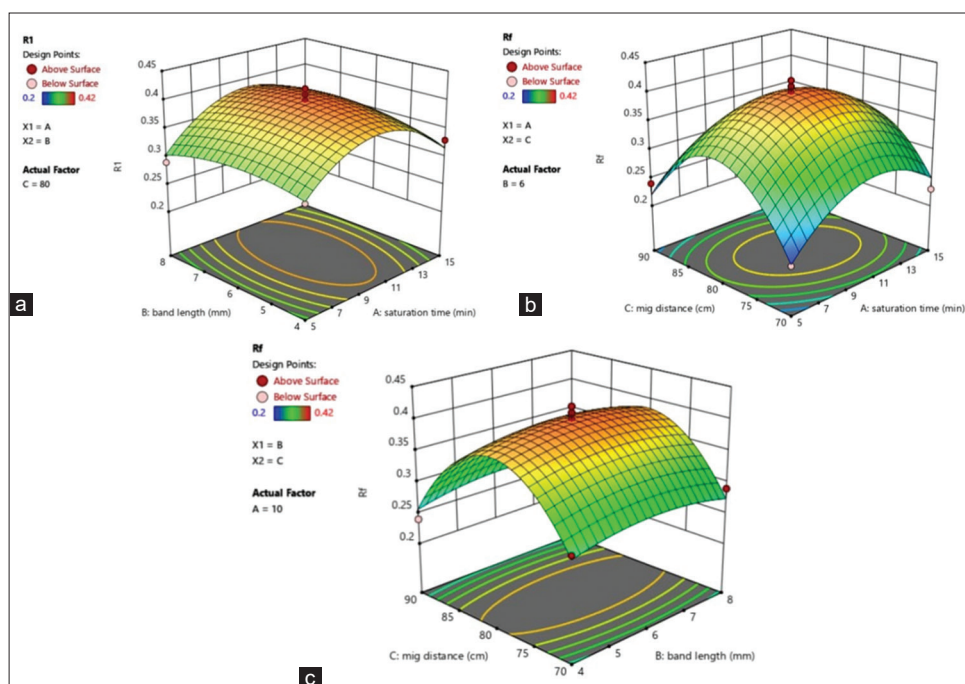


Fig. 2: 3D response surface plots of Ciprofloxacin (a) showing of band length and saturation time on the rf value, (b) the influence of migration distance and saturation time on rf value, (c) the influence of migration distance and band length on the rf value

values for the used linearity spectrum were strong ($r^2=0.996$). For ciprofloxacin, the linear regression equations were found to be $y=769.969x+533.278$. The linearity calibration curves are shown in Fig. 5.

Precision

The %RSD values for ciprofloxacin intra-day were found to be 0.38, respectively. The % RSD values for ciprofloxacin inter-day were found to be 1.33, respectively (<2).

Robustness

The standard deviation of areas was calculated for each condition and %RSD was less than 2%. The result of robustness is shown in the following Table 3.

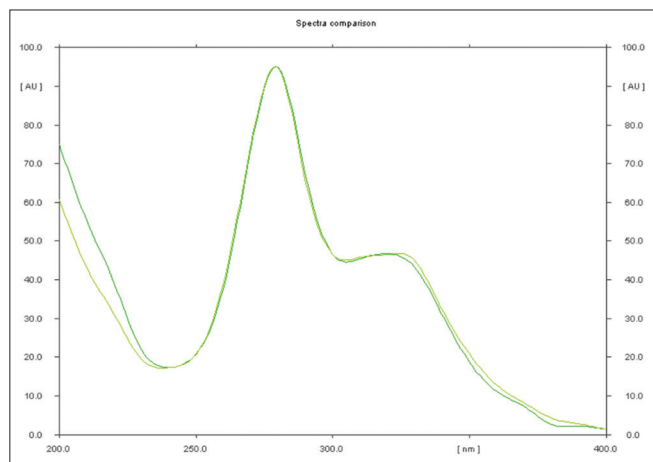


Fig. 3: Overlaid spectra of ciprofloxacin

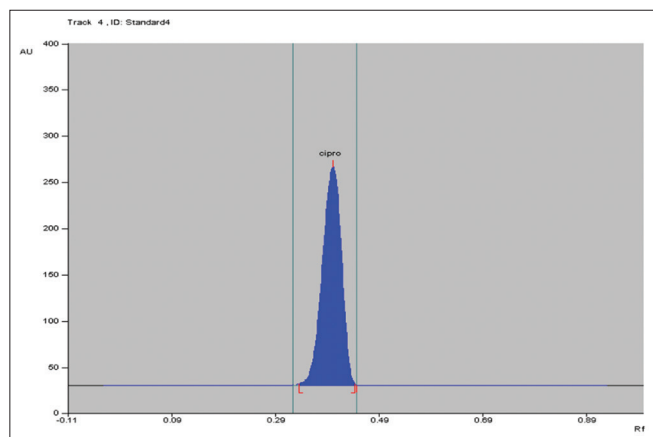


Fig. 4: Typical chromatogram of ciprofloxacin

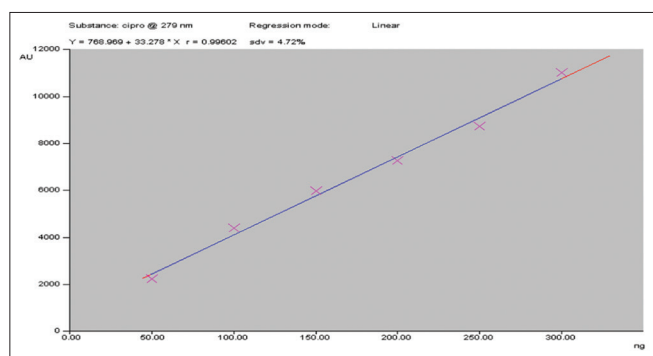


Fig. 5: Linearity of ciprofloxacin

Accuracy

The experiment's precision was determined by spotting a drug standard solution over the pre-analyzed sample solution. The recovery research was conducted at three different levels: 80%, 100%, and 120 percent. Table 4 displays the outcome.

Limit of detection and limit of quantification

The developed method's LOD and LOQ for ciprofloxacin were found to be 24.98 and 75.69 ng per band, respectively, indicating the proposed method's sensitivity. Table 5 displays the LOD and LOQ data.

Table 2: Results of analysis of ANOVA (ciprofloxacin)

Responses	SS	DF	MS	F-value	p-value	Results
Rf	0.0823	9	0.0091	22.67	0.0002	Significant

RF: Retention factor, SS: Sum of squares, DF: Degree of freedom, MS: Mean square

Table 3: Robustness study

Robustness	%RSD
Mobile phase composition (± 0.1 ml)	0.78
Saturation time (± 5 min)	1.17
Development to scanning (± 5 min)	0.99
Volume of the mobile phase (± 1 ml)	0.28
Spotting to development (± 5 min)	1.16

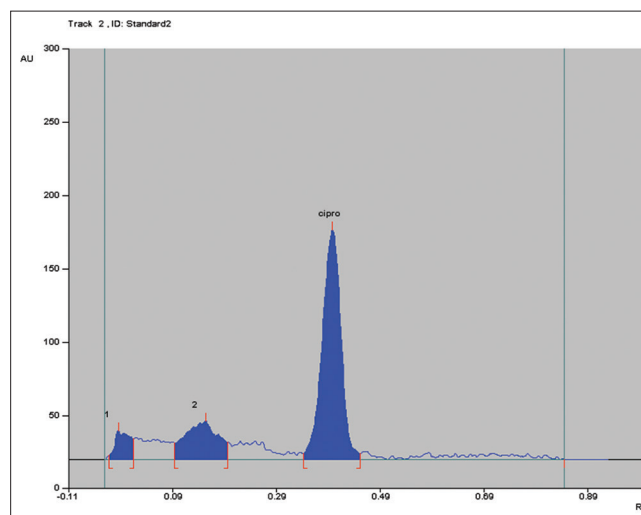


Fig. 6: Densitogram of alkali (0.1N NaOH) treated sample.

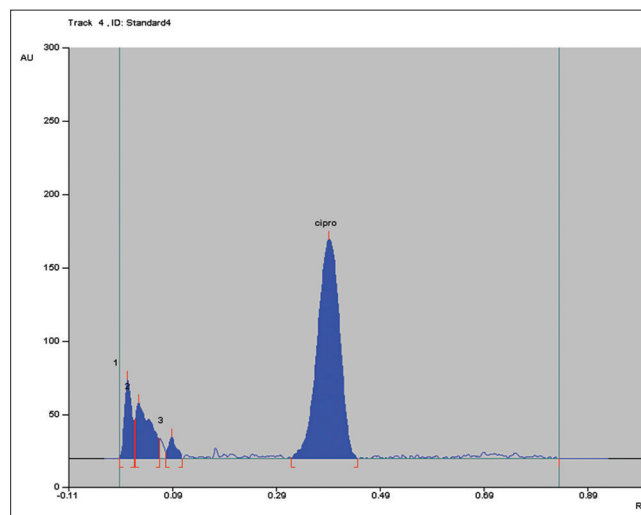


Fig. 7: Densitogram of the sample exposed to heat

Table 4: Recovery data for ciprofloxacin

Drug	Recovery level [%]	Initial amount [mg]	Initial contact. [ng per band length]	Amount added [mg]	Amount of drug recovered	% Recovery
Ciprofloxacin	80	5	200	4	3.93	98.25
	100	5	200	5	5.03	100.6
	120	5	200	6	6.05	100.83

Table 5: Summary of linear regression and validation data

Parameters	Ciprofloxacin
Linearity range [ng per band]	50–300 ng per band
Linear regression equation	$Y = 769.969x + 533.278$
Slope	769.969
Y-intercept	533.278
Correlation coefficient	0.996
Limit of detection (ng per band)	24.98
Limit of Quantification (ng per band)	75.69
System precision (% RSD)	0.48
Intra-day (% RSD)	0.38
Inter-day (% RSD)	1.33

Table 6: Observation table

Conditions	Degradation (%)
0.1N NaOH (room temp for ½ h)	6.45%
0.1 N HCL (room temp for ½ h)	8.39%
3% H ₂ O ₂ (room temp for ½ h)	6.96%
H ₂ O (1/2 h)	9.43%
Dry heat 40°C for 30 min	7.28%
UV light for 24 h 254 nm	8.29%

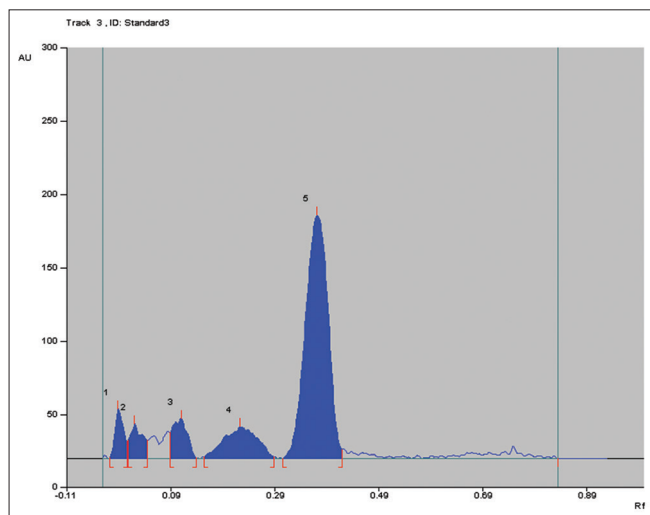


Fig. 8: Densitogram of the sample exposed to UV radiations

Stress degradation study

Significant degradation of ciprofloxacin was observed under neutral hydrolysis conditions. While lesser degradation was observed under alkaline hydrolysis and oxidative condition. 6.45% degradation was observed for ciprofloxacin in alkaline hydrolysis (3 ml in 0.1N NaOH) after keeping it at room temperature for ½ hrs. Ciprofloxacin showed oxidative degradation at room temperature for ½ h of about 6.96%. Ciprofloxacin showed degraded product peaks at Rf 0.41 and 0.89 under dry heat (oven 40°C 30 min). Ciprofloxacin was found to be degraded up to 7.28% with a decrease in an area only. Photolytic studies were also carried out by exposure of the drug to UV light for 24 h on 254 (Table 6 and Figs. 6-11).

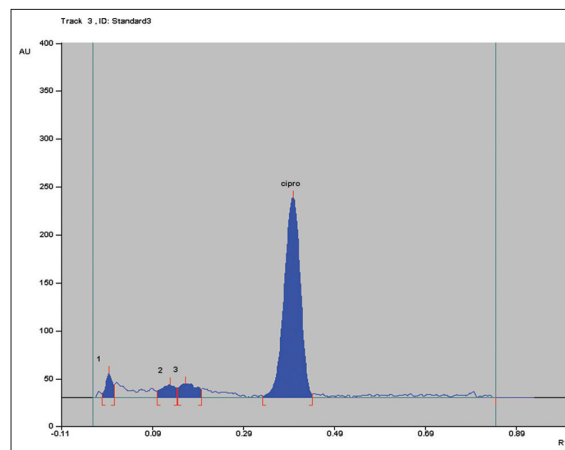


Fig. 9: Densitogram of acid (0.1 N HCL) treated sample

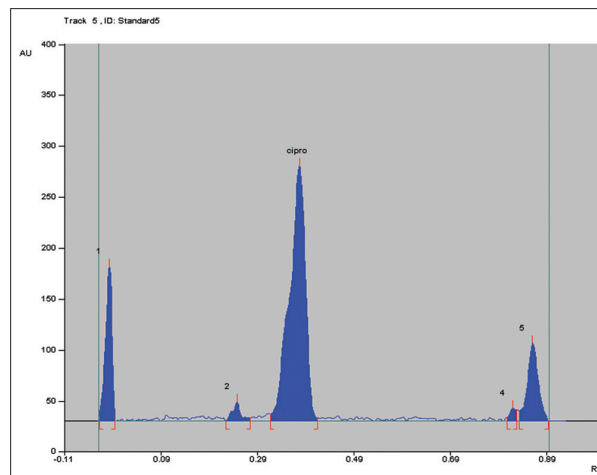


Fig. 10: Densitogram of the sample exposed to neutral hydrolysis

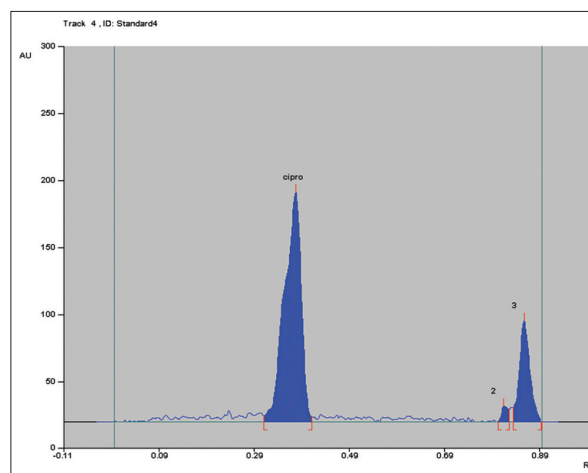


Fig. 11: Densitogram of oxide (3% H₂O₂) treated sample

CONCLUSION

The box-Behnken design and response surface methodology are useful tools for determining the sensitivity of ciprofloxacin Rf values to various chromatographic variables. Using a useful experimental design method, the saturation period, band duration, and migration distance were all optimized at the same time. It is a cost-effective method for generating a large volume of data in a short amount of time with a limited number of experiments. The proposed method for ciprofloxacin determination was found to be straightforward, precise, reliable, stable, and sensitive. The QbD method produced a more robust method that can generate accurate, high-quality, and reliable data during the process.

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AUTHOR CONTRIBUTION

RP studied and carry out the research work. RP and AG wrote the manuscript and ST and AK checked and finalized the manuscript.

CONFLICT OF INTREST

The authors declared that they have no conflict of interest.

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