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METHOD DEVELOPMENT AND VALIDATION OF NEWLY SYNTHESIZED CIPROFLOXACIN HYDRAZIDE BY RP HPLC

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A new RP-HPLC method was developed for the estimation of hydrazide derivative of ciprofloxacin which is synthesised and evaluated for its antibacterial activity. Ciprofloxacin hydrazide was well eluted on an isocratic c18 column (Kromosil 100-5 C_{18} column 250 mm × 4.6 mm) utilizing a mobile phase composition of Acetonitrile: phosphate Buffer pH 3 (40 : 60 v/v) at a flow rate of 0.8 ml/min with UV detection at 266 nm. The retention time was 2.535 ± 0.04 mins. The developed method was validated for specificity, linearity, precision, accuracy, LOD, LOQ and robustness. The developed reverse phase liquid chromatography method can be applied in routine analysis for drug discovery studies on this newly synthesised compound. The determined validation parameters were within the specified ICH guideline limits.

KEYWORDS : Ciprofloxacin hydrazide, RP-HPLC, validation.

INTRODUCTION

Ciprofloxacin is an antibacterial drug used to treat various bacterial infections. It is a Fluoroquinoline derivative belongs to DNA gyrase inhibitors for the treatment of broad spectrum bacterial infections in adults and children. Chemically, ciprofloxacin is cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid [1, 2]. A new hydrazide derivative was synthesised to explore the nucleus and its antibacterial potency. The biological activity was good for the synthesised moiety. Thus to fasten the drug discovery process, a new RPHPLC method was developed and validated.

The purpose of the present work is to develop simple and economic RP-HPLC method for the estimation of newly synthesised ciprofloxacin derivative and perform validation of the method as per International Conference of Harmonisation (ICH) guidelines [1]. The developed method has been validated by evaluation of system suitability, linearity, limit of detection and limit of quantification, precision and accuracy.

Literature survey revealed methods for the estimation of ciprofloxacin alone in tablets by RP-HPLC [3-8]. Hence an attempt has been made to develop RP-HPLC method for the estimation of new derivative of ciprofloxacin in the current research.

MATERIALS AND METHODS

Materials : Ciprofloxacin was obtained as a gift sample from MYLAN Labs Pvt. Ltd. HPLC grade acetonitrile, reagents and HPLC water were procured from Merck India.

Instrumentation and analytical conditions

HPLC: Agilent 1120 compact LC chromatographic system, with variable wavelength UV detector and Rheodyne injector with 20µl fixed loop was used for the chromatographic separation. Ezchrome software was used for data analysis. Chromatographic separation was carried out on a c18 column (Kromosil 250nm × 4.5mm).

AXIS AGN204- PO electronic balance was used for weighing purpose. Ultrasonic bath sonicator was used for degassing purpose.

Chromatographic conditions

Column : Kromosil 100-5 C_{18} Column [250 mm × 4.6 mm]

Detector Wave length : 266 nm

Mobile Phase : Acetonitrile: Phosphate Buffer pH 3 (40 : 60 v/v)

Flow : 0.8 ml/min

Injection Volume : 20 µL

Diluent : Mobile phase

Run Time : 10minutes

Temperature : Ambient

Method development

Spectroscopic determination of Ciprofloxacin hydrazide indicated that the drug absorbs maximum at 266nm, hence 266 nm was selected as the detection wavelength. Several different mobile phases were used for the initial trials in the estimation, but optimum results were attained with Acetonitrile: Phosphate Buffer pH 3 in the ratio of 40:60 v/v. The peak was shown in figure 1.

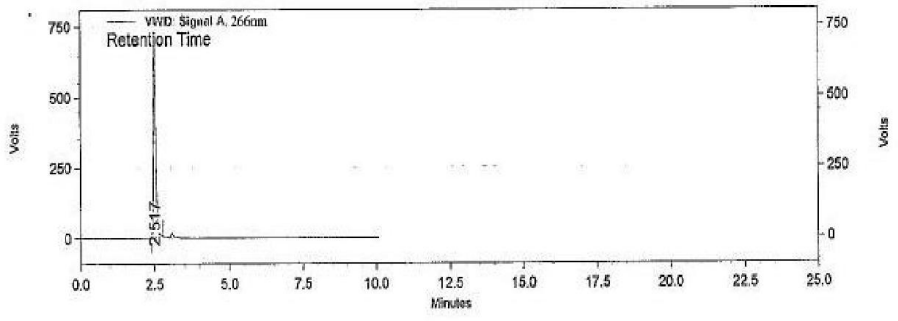

Fig. 1. Optimised Chromatogram of Ciprofloxacin hydrazide

Mobile phase composition

Acetonitrile and Phosphate Buffer pH adjusted to 3 were mixed in the ratio of 40 : 60 v/v and filtered through a 0.45µ membrane filter and sonicated for 40min.

Preparation of standard stock solution

25 mg of Ciprofloxacin hydrazide was weighed accurately and transferred in to 25 ml volumetric flask and dissolved in 10ml of mobile phase and sonicated it for 10min and made up to the mark with the mobile phase to obtain a final concentration of 1000µg/ml.

Selection of Analytical Concentration Range and Construction of Calibration Curve for Ciprofloxacin hydrazide

Appropriate aliquots ranging from 0.5 ml to 2.5 ml were pipetted out from the working stock solution (1000 µg/ml of Ciprofloxacin hydrazide) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range 50-250 µg/ml of Ciprofloxacin hydrazide. Triplicate dilutions of each of the above mentioned concentrations were prepared, 20 µL of each concentration of the drug was injected into the HPLC system.

Method Validation

System suitability

System suitability was carried out by injecting 6 replicate injections of 250 µg/ml concentration. It is checked on each day of validation to evaluate the performance of the system. The parameters like theoretical plates, retention time, tailing factor and its % RSD were determined. The results were given in the table 1.

Table 1. System suitability parameters of Ciprofloxacin hydrazide (*n* = 6)

S.no	Parameters	Results
1	Theoretical plates	11467
2	Tailing factor	0.7
3	Retention time	2.535±0.04
4	% RSD	0.082

Linearity and range

The peak areas corresponding to the concentration range of Ciprofloxacin hydrazide 50-250 µg/ml prepared in triplicate and were plotted against the respective concentrations. The calibrated curves were linear in the range studied with mean correlation coefficient of 0.997 and the representative calibration curve was shown in figure 3. Regression analysis was given in the table 2.

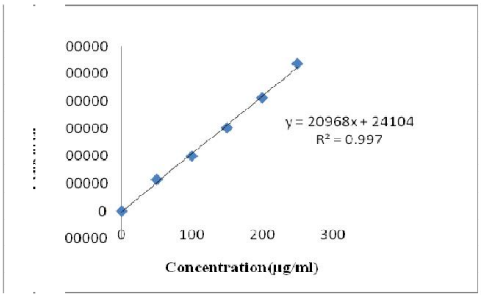

Fig. 2. Calibration curve of Ciprofloxacin hydrazide

Table 2. Linearity of Ciprofloxacin hydrazide (*n* = 3)

Parameters	Valves
Linearity range	50-250 µg/ml
Slope	20968
Intercept	24104
Correlation coefficient	0.997
Regression equation	$y = 20968\,x + 24104$

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product. The recovery was calculated at three spike levels *i.e*., 80%, 100% and 120%. The results of recovery were given in the table 3.

Table 3. Results for Accuracy (*n* = 3)

Recovery level	Amount Added (mg)		Amount Found (mg)	% Recovery (%w/w)
	Std.	Test		
80%	60	20	76.64	95.25
100%	80	20	98.56	98.66
120%	100	20	115.42	98.82
Mean %	97.576			

Precision

The precision of the method was determined by the injection of six replicates of 250 µg/mL concentration. Precision was performed intraday (same day) with three intervals as well as inter day (three successive days). The results of intraday were given in the table 4.

Table 4: Precision of Ciprofloxacin hydrazide

Precision		% RSD
Intraday		1.06
Interday	Day 1	1.06
	Day 2	1.02
	Day 3	1.03

Limit of Detection and Limit of Quantitation

The LOD and LOQ were calculated from the slope (s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10σ/s. The values were tabulated in table 5.

Table 5: Results for LOD and LOQ

Parameters	Values (µg/mL)
LOD	1.57
LOQ	1.87

Robustness

Robustness was carried out by change in the flow rate (± 0.2 ml/min) and variation wavelength (± 2 nm). Solution of 100 µg/ml concentration was prepared and injected in

triplicate for each varied operational condition and %RSD was calculated. The results were given in the table 6.

Table 6. Results for Robustness (*n* = 3)

Parameters		%RSD
Wavelength	264	1.19
± 2nm	268	0.08
Flow rate	0.7	0.68
±0.2ml/min	0.9	0.91

Results and discussion

The ciprofloxacin was derivatized to get hydrazide derivative. This compound was screened for antibacterial activity against bacterial strains and found to be good at biological activity. The absorption maximum for the Ciprofloxacin hydrazide drug was found to be at 266 nm in the diluent used. Hence 266 nm was selected as the detection wavelength. Changes in chromatographic conditions were performed for the method development like variations in mobile phases and compositions and variations in RP HPLC columns. The optimum results were attained with Acetonitrile: Phosphate Buffer pH 3 (40 : 60 v/v) with Kromosil 100-5C_{18} Column [250 mm × 4.6 mm]. The system suitability was carried using 6 injections and the number of theoretical plates was more than 2000. The retention time for the drug is at 2.535 ± 0.04. Tailing factor was less than 1.5. The peaks obtained were symmetric. The linearity for the Ciprofloxacin hydrazide was observed in the range of 50-250µg/mL. The accuracy of the method was studied by doing recovery studies by standard addition method for the drug. The recovery was calculated and found to be 97.57 % w/w. The % RSD was less than 2 which indicates a good accuracy of the method. The method developed was precise from the data of interday and intraday precision. The % RSD was less than 2 for precision. LOD and LOQ of emtricitabine were 1.57 µg/mL and 1.87 µg/mL respectively. The robustness of the method was determined by changing the wavelength (± 2 nm) and flow rate (± 0.1 mL/min) of the optimised method. The results were found to be within the limits of ICH guidelines.

Conclusion

The proposed RP-HPLC method was developed and validated as per the International Conference of Harmonisation (ICH) guidelines, and was found to be to be economic and simple due to use of readily available mobile phase and lack of extraction procedures. The results of linearity, precision, accuracy were within the limits. The proposed method was reliable, rapid and robust. Therefore, this method can be employed in drug discovery and quality control to estimate the amount of ciprofloxacin hydrazide.

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