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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF NORFLOXACIN IN BULK FORM

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ABSTRACT

A simple, selective, accurate and precise high-performance liquid chromatographic (HPLC) method for estimation of Norfloxacin in bulk form was developed & validated. The estimation was carried out on Phenomenax luna C-18 (250x4.6 mm, 5µ) column using a mobile phase consisting of 0.03 M potassium dihydrogen phosphate, triethyamine: Acetonitrile (88:12 v/v) of pH 3.8 adjusted with orthophosphoric acid, at a flow rate 1 ml/min. The UV detection was carried out at 280 nm. Method validation was performed as per the ICH guidelines. The method was validated for the precision, accuracy, linearity and robustness.

KEY WORDS: RP-HPLC estimation, Validation and Norfloxacin.

1. INTRODUCTION

Norfloxacin[1], chemically known as 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid, is a fluorized quinolone, inhibits, like the other members of this group, the gyrase of the bacterial DNA. This effect is responsible for the bactericidal action of norfloxacin. Follows a selection of sensitive bacili: most enterobacteriaceae (E.coli, klebsiellas, etc.), Pseudomonas aeruginosa, and many pathogenic enteric bacteria (Salmonella, Shigella, etc.), but also Neisseria (especially gonococci). Streptococci are partially resistant whereas anaerobic bacteria are completely resistant.[2] Norfloxacin is official in Indian Pharmacopoeia 2010[3], British Pharmacopoeia 2009[4] and United State Pharmacopoeia.[5] RP-HPLC, HPTLC and other spectrophotometric methods were reported for the estimation of Norfloxacin.[6-21]

As per literature survey, no analytical method on this mobile phase condition has been reported for estimation of Norfloxacin. In presented research work, we had developed a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Norfloxacin in bulk form for routine analysis. Chemical structure of norfloxacin is shown in Fig. 1

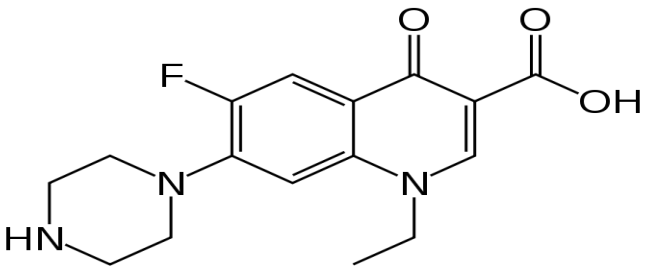

Figure 1: Chemical Structure of Norfloxacin

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

HPLC grade acetonitrile, potassium dihydrogen phosphate and triethylamine were used. Triple distilled water for HPLC was prepared in our laboratory.

2.2 Chromatographic conditions

Shimadzu prominence UFLC 2000 equipped with SPD-20A UV detector and Phenomenax Luna C_{18} (250x4.6 mm, 5µ) column. The mobile phase consisting of 0.03M potassium dihydrogen phosphate, 0.8% triethylamine : Acetonitrile (88:12 v/v) of pH 3.8 adjusted with orthophosphoric acid.

2.3 Preparation of standard solution

2.3.1 Preparation of stock solution of NF

Weigh accurately 10 mg of NF and transferred into 10 ml volumetric flask add 5 ml of acetonitrile and sonicated for 10 min and diluted up to mark with acetonitrile to get a stock solution having strength 1000 µg/ml.

2.3.2 Preparation of working Standard solution of NF

100 µg/ml solution of NF was prepared by diluting 1 ml stock solution to 10 ml with acetonitrile and further diluted with acetonitrile to get the concentration range of 1, 2, 3, 4, 5 µg/ml of NF.

2.3.3 Selection of Wavelength

Wavelength was selected with the help of UV-spectrophotometer. It was found 280 nm and shown in fig 2.

2.4 System suitability

System suitability testing is an integral part of analytical method. The tests are based on the concept that the equipment, electronics, analytical operation and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability parameters were calculated before the starting experimentation. It was determined by taking %RSD of retention time, peak area and theoretical plate of the five standards injections and shown in table 2.

2.5 Method validation[22-24]

2.5.1 Linearity and range

A linear relationship should be evaluated across the range of the analytical procedure. It should be established initially by visual inspection of a plot of signals as a function of analyte concentration of the content. For the establishment of linearity, a minimum of 5 concentrations are recommended. A standard linearity solution was prepared to attain concentration of 1, 2, 3, 4 and 5 µg/ml of the test concentration and the calibration curve was linear and regression equation found to be y = 48994x + 22324. Linearity was calculated and shown in fig 3 and table 3.

Acceptance criteria

Regression coefficient (r^2) should be 0.995 to 1.

2.5.2 Precision

The precision of an analytical method was determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation, relative standard deviation and standard error of mean. The inter-day precision study was performed on three different days i.e. day1, day2 and day3 at three different concentration and %RSD was calculated and shown in table 4.

Acceptance criteria

The %RSD of 6 determinations was NMT 2%.

2.5.3 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy was calculated as the percentage of recovery by the assay of the known added amount of the analyte in the sample. Accuracy assayed by using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). The accuracy of the method was evaluated triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated and shown in table 5.

Acceptance criteria

The recovery should be 98% to 102%.

2.5.4 Robustness

The robustness of the method was established by making the deliberate minor variation in the detection wavelength and flow rate. Method was performed by change the flow rate and wavelength. Robustness was studied using three replicates of concentration level at 100%. The %RSD in robustness study was less than 1% and this indicates that the method is precise, accurate and robust and recorded in table 6.

Acceptance criteria

Original method and changed method result should not vary ±1.0%

2.5.5 Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD was based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1). The value of LOD was found to be 0.033 µg/ml. LOD = (3.3 x SD) / Slope

2.5.6 Limit of quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOQ was based on the standard deviation of the response and the slope of the constructed

calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1). The value of LOQ was found to be 0.102 µg/ml.

LOQ = (10 x SD) / Slope

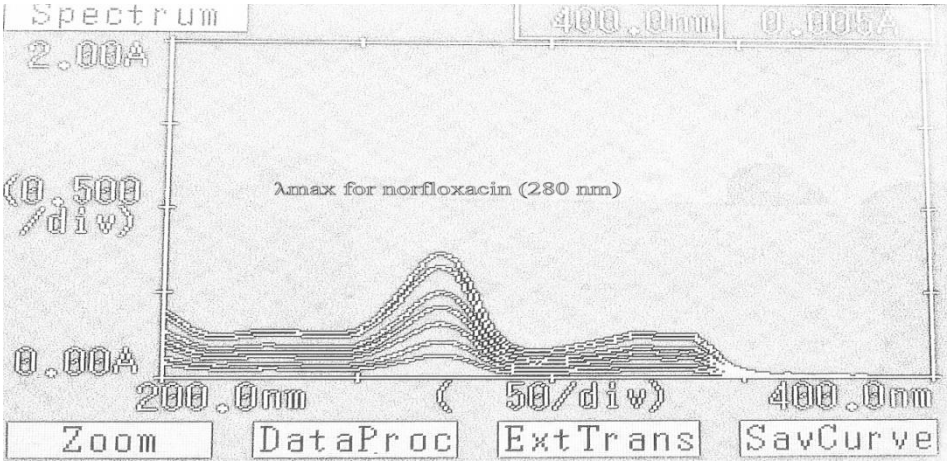

Figure 2: Spectra of norfloxacin

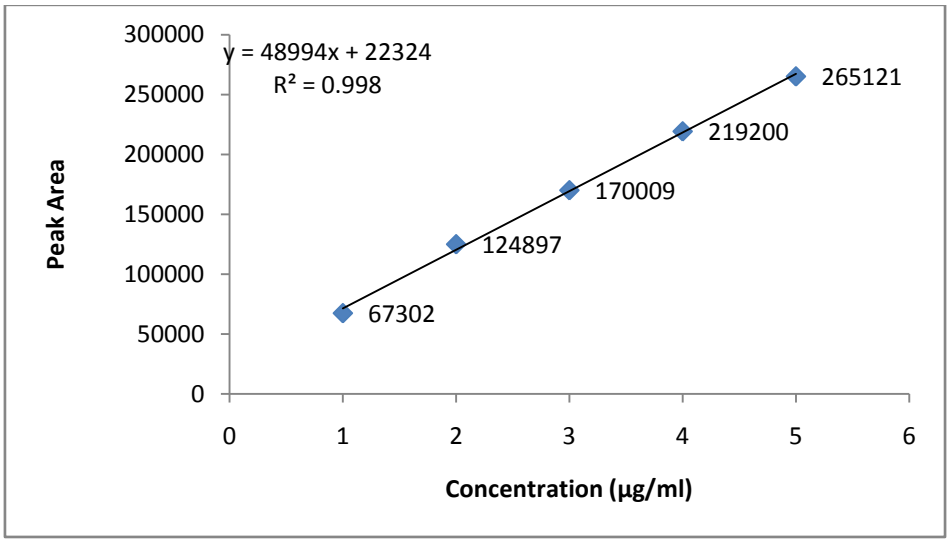

Figure 3: Calibration curve of NF

Table 1: Chromatographic system and conditions

Parameters	Conditions
Column	C_{18} (250mm × 4.6mm i.d) 5µm
Mobile phase	Acetonitrile: KH_2PO_4, triethyl amine (12:88)
Flow rate	1.0 ml/min
Detector	SPD-20A prominence UV/VIS detector
Detection wavelength	280 nm
Injector	77251 Rheodyne
Injection volume	20 µl
Software	LC solution
Pump	Binary prominence LC-20AD liquid pump
Nylon filter	0.45 µm Millipore

Table 2: System suitability parameters of NF

Sr. No.	Retention Time (NF)	Area	Theoretical Plates	Tailing Factor
1.	5.585	170009	1.324	2051.81
2.	5.584	169912	1.329	2069.25
3.	5.582	169900	1.326	2067.31
4.	5.584	170019	1.320	2039.29
5.	5.586	170085	1.319	2025.17
Avg.	5.5842	169985	1.323	2016.56
SD	0.00148	77.91	0.004159	32.164
%RSD	0.026	0.045	0.314	1.59

Table 3: Linearity data of NF

Sample No.	Concentration (µg/ml)	Peak Area (NF)
1.	1	67302
2.	2	124897
3.	3	170009
4.	4	219200
5.	5	265121

Table 4: Interday and Intraday Precision of NF

	Intraday Precision*			Interday Precision		
Concentration level	**Area***	**SD**	**%RSD**	**Area***	**SD**	**%RSD**
80%	124863.0	131.34	0.105	125115.3	108.87	0.087
100%	169773.8	425.00	0.25	169909.8	500.09	0.29
120%	218540.0	395.74	0.180	218851.7	348.62	0.159

*Mean of three replicates (n=3)

Table 5: Accuracy (%recovery) study of NF

Sr. No.	Amt. of Sample (µg/ml)	Spiked Concentration (µg/ml)	Area*	SD	%RSD	Recovery (%)
1.	3	80%	147218	970.51	0.659	99.8
2.	3	100%	172370	1081.50	0.627	101.38
3.	3	120%	195617	851.45	0.435	100.5

*Mean of three replicates (n=3)

Table 6: Percentage deviation of changed method for NF

Parameters		Area* (mean)	Active drug in %	% RSD
Flow rate (ml/min)	0.9	168253.4	98.96	0.96
	1.0	170196.0	100.1	0.10
	1.1	172921.3	101.7	0.85
Wavelength (nm)	279	172881.3	101.68	0.56
	280	170374.3	101.21	0.14
	281	169035.3	99.42	0.39

*Mean of three replicates (n=3)

Table 7: LOD and LOQ

Parameters	Readings
Wavlength maxima (nm)	280
LOD	0.033
LOQ	0.102

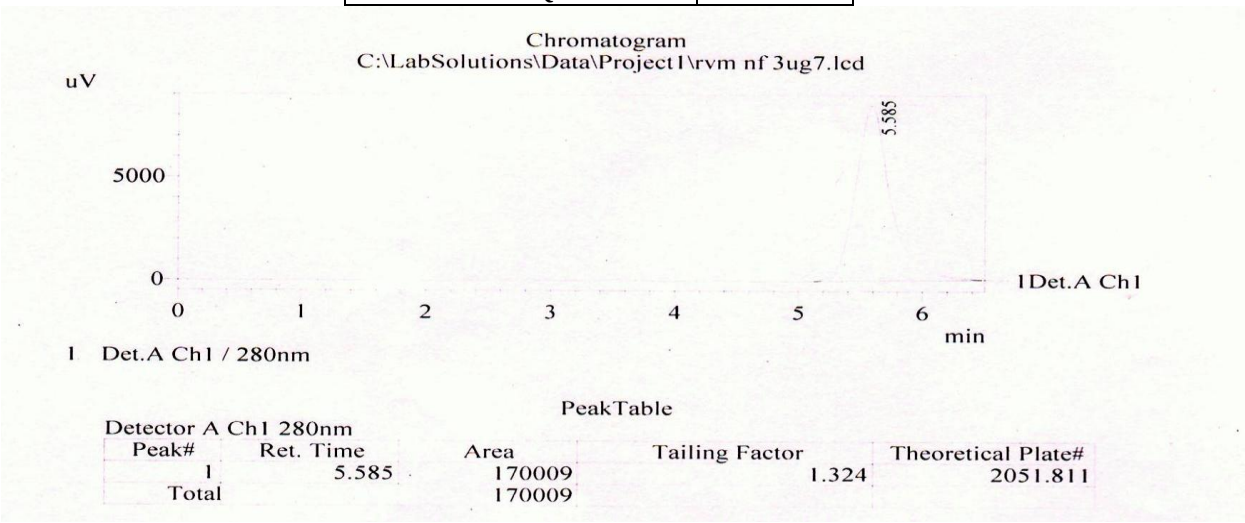

Figure 4: Chromatogram of Norfloxacin

3. RESULT AND DISCUSSION

High Performance Liquid Chromatographic method was developed and validated for estimation of norfloxacin. The proposed method was based on gradient elution. The estimation was carried out on Phenomenax luna C-18 (250x4.6 mm, 5µ) column using a mobile phase consisting of 0.03 M potassium dihydrogen phosphate, 0.8% triethylamine (TEA): Acetonitrile (88:12 v/v) of pH 3.8 adjusted with orthophosphoric acid, at a flow rate 1 ml/min. The UV detection was carried out at 280 nm. The calibration curve was linear over the concentration range 1-7 µg/ml and correlation coefficient (r^2) was found to be 0.998. The recoveries in accuracy study were found to be between 98-102% and %RSD was less than 2%. The theoretical plates were found to be more than 2000. The developed method can be successfully applied for the estimation of norfloxacin in bulk form and may also be useful for the simultaneous estimation of norfloxacin with other drugs like loperamide. The system suitability results are shown in table 2 All validation parameters were found within limits and are shown in table 3-7.

The validation results show that the developed methods are highly précised and accurate. The robustness study indicates that the developed method is robust and unaffected by minor change in the wavelength and flow rate.

4. CONCLUSION

A HPLC method was developed for the estimation of norfloxacin by reverse phase liquid chromatography in bulk form. The developed method was novel, simple, accurate, precise and reproducible, which can be used to estimate norfloxacin in bulk form for routine analysis and also in combination with other drug like Loperamide hydrochloride. The method was developed according to ICH guidelines.

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