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Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NADIFLOXACIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: In the present work, a rapid, precise and sensitive HPLC Method with UV detection (237 nm) for analysis of Nadifloxacin in Bulk was developed.

Methods: Chromatography was performed with a mobile phase containing a mixture of 0.05 %v/v trifluoro acetic acid and acetonitrile (65:35 v/v) with flow rate 1.2 ml min^{-1}. The proposed method was validated as per the standard guidelines.

Result: The retention time was found to be 12.3 min. In the range of 0.03-5 ppm, the linearity of Nadifloxacin shows a correlation co-efficient of 0.9997. Percentage recovery of the drug was found to be good (98-102%). Validation of the developed method was successful for precision, robustness, specificity and selectivity and ruggedness.

Conclusion: The developed HPLC method was found to be simple, sensitive, precise, accurate and reproducible and can be successfully used for the quantitative estimation of Nadifloxacin in bulk.

Keywords: Nadifloxacin, Bulk, Trifluoro acetic acid, Acetonitrile, HPLC.

INTRODUCTION

Nadifloxacin (Fig. 1) is a potent, broad-spectrum, quinolone agent approved for topical use in acne vulgaris and skin infections. As exposure of pathogenic and colonizing bacteria to antibiotics results in drug resistance, it is not desirable to use an important, broad-spectrum antibiotic, which belongs to a class of agents widely used systemically to treat a wide variety of infections, as a topically applied preparation. On this basis, Nadifloxacin is not a good option for topical treatment of acne when other effective non-antibiotic treatments are available. Nadifloxacin has potential as a topical agent for short-term treatment of skin infections. The arginine salt of its (-)-(S)-isomer is being developed as a parenteral agent based on its potency against methicillin and quinolone-resistant *Staphylococcus aureus*. Several methods for determination of Nadifloxacin have been developed including UV-VIS spectrophotometric method [1] HPTLC [2-3] and HPLC [4-5]. For routine analysis, a simple and cost effective analytical method is preferred. A simple and rapid assay procedure was developed using the sample preparation and mobile phase preparation as described in Ref. 5. The objective of present study was to develop a simple, precise, accurate and economic HPLC analytical method with better detection range for the estimation of S-Nadifloxacin in bulk drugs. The developed method was validated as per ICH guidelines. And suitable statistical tests were performed on validation data.

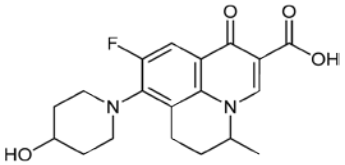

Fig. 1: Structure of Nadifloxacin

MATERIALS AND METHODS

Instrument

Specifications of HPLC instrument used are as follows. A gradient high pressure liquid chromatograph (Shimadzu, class lc-series), system controller SCL 10AVP, with LC-10 AT pump and a Hypersil C-18 column (150 mm x 4.6 mm i. d., particle size 5 µm) was used. The HPLC system was equipped with the Shimadzu class VP-5.0 software (system-1). Another HPLC system was used with following configuration: Agilent 1200 series, instant pilot software Chemstation plus, with micro-vacuum degasser G1379B, binary pump G1312B, diode array detector SL G1315C, thermostated column compartment G1367C (system-2). UV/Vis spectrophotometer (Shimadzu), sonicator (Lab companion), balance (Mettler Toledo) and pH meter (Lab India) were used in the research.

Chemicals and reagents

All the chemicals used were either of A. R. Grade or HPLC grade. Acetonitrile (RANKEM, HPLC Grade), Tri-Fluro Acetic acid (MERCK, AR Grade), Ammonia Solution (RANKEM, AR Grade) and Water (Milli Q) were used in the research work. Nadifloxacin was obtained as a gift sample from Apollo Pharmaceuticals, Mumbai, India.

Determination of wavelength

Wavelength determination of Nadifloxacin was carried out by the procedure as suggested by Devhadrao et al [6]. Solution of Nadifloxacin (1 ml) was prepared in 10 ml methanol (100 ppm). 1 ml was pipette out from 100 ppm solution and volume was made up with 10 ml methanol (10 ppm). The λ max was determined on UV – visible spectrophotometer in the range 200 – 400 nm. The λ max value for Nadifloxacin was found to be 237 nm.

Selection of Mobile Phase

The standard solution of Nadifloxacin was run and different combination of solvents was tried for isocratic method. The peaks found were well resolved and symmetric. From the various conditions tried, isocratic elution method using Buffer: Acetonitrile (70:30) was selected, since it gave sharp peaks with symmetry within limits and significant retention time.

Preparation of Solutions

Blank solutions

Diluent was prepared using water: Acetonitrile (50:50) and pH was adjusted to 8.5 by using liquid ammonia.

Standard solutions

10 mg Nadifloxacin was weighed accurately. Transferred into 10 ml volumetric flask and sufficient amount of diluent was added. Then it was sonicated to dissolve the drug and volume, was made up with diluent (1000 ppm). 1 ml of this solution was transferred to 10 ml volumetric flask and made up the volume with diluent (100 ppm). 0.1 ml from 100 ppm solutions was transferred to 10 ml volumetric flask and made up the volume with diluent to get 1 ppm Nadifloxacin standard solution.

Sample solution

50 mg Test sample of Nadifloxacin was transferred in to 50 ml volumetric flask. Sufficient amount of diluent was added and sonicated for 2 min. Then volume was made up with diluent.

Preparation of Mobile Phase

Buffer Preparation: 1 ml of tri-fluro acetic acid (0.05%v/v) was added in 2000 ml water & sonicated. The mobile phase was prepared by mixing buffer & acetonitrile.

Chromatographic Conditions

The contents of the mobile phase were phosphate buffer solution (pH: 4.5) and acetonitrile in the ratio of 65:35 percent v/v. The mobile phase was filtered through 0.45-µm-membrane filter and sonicated for 15 min. The flow rate of the mobile phase was maintained at 1.2 ml/min. The column temperature was set at 40 □□1° C and the detection was carried out by UV-Detector wavelength at 237 nm. The run time was set at 25 min and the volume of the injection loop was 5 µL. Prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The data were acquired, stored and analyzed with the software class N-2000 CHROMTECK (SHIMADZU). The chromatogram obtained through the injection of the placebo solution did not contain any other peak at the retention time of Nadifloxacin. The chromatogram peak purity tools show that the peak was 100%. Thus, it was shown that the peak at 12.3 min was not due to any interference from the excipients in the formulation.

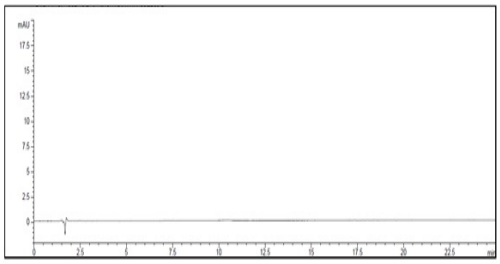

Fig. 2: Chromatogram of placebo solution of Nadifloxacin

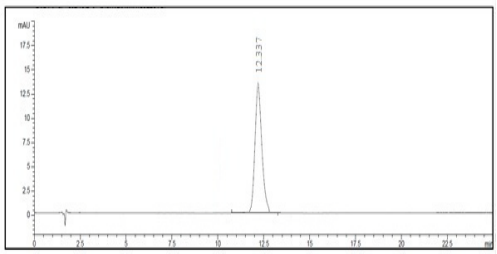

Fig. 3: Chromatogram of Nadifloxacin

The Liquid Chromatographic method was validated for the following parameters:

Calibration procedure

The calibration curve was plotted with five concentrations of the standard drug solution 0.03-10.0 ppm and chromatography was repeated five times for each dilution. The linearity was evaluated by linear regression analysis. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Five determinations were carried out for each solution, peak areas were recorded for all the solutions. The correlation graph was constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the injected amounts of the respective concentrations.

Linearity and range

By using the working standard, aliquots of 0.1, 0.2, 0.3, 0.6, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0 ppm were prepared with blank solution. Five dilutions of each of the above mentioned concentrations were prepared separately and from these five dilutions, 5 µl of each concentration of the drug were injected into the HPLC system and their chromatograms were recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

Precision

The precision of the assay was determined in terms of intra and inter-day variation in the peak area for a set of drug solution of known concentration of 80%, 100% and 120% (8 ppm, 10 ppm and 12 ppm respectively) assayed five times on the same day and on three different days. The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of standard deviation to the mean with 100(CV=SD/MEAN X 100).

Robustness

As defined by the ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 0.1 ml/min in flow rate of the mobile phase.

Specificity and Selectivity

The specificity of the RP-HPLC method was determined by using the parameters like retention time (t_R) and tailing factor (T_f). Tailing factor for peaks of Nadifloxacin should be less than 2% and should have identical retention time.

Ruggedness of the developed method

Ruggedness of the method was determined by running the developed method for Nadifloxacin analysis in two different HPLC systems as mentioned in the instrumentation section above. The results of the two different HPLC systems are compared and discussed in the result and discussion section below.

RESULTS AND DISCUSSION

The run time was set at 25 min and the retention time for Nadifloxacin was found 12.3 min as shown in Figure 2. The sample solution was injected 10 times and the retention times were found to be approximately same. Averages of 10 such determinations of peak areas are shown in Table No. 1.

When the concentrations of Nadifloxacin and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2 = 0.9997) was observed between the concentration of Nadifloxacin and the respective peak areas in the range 0.1 – 10.0 µg/ml. Regression of Nadifloxacin was found to be Y= 9.9859x -0.2301, where 'Y' is the peak area and 'X' is the concentration of Nadifloxacin (Fig. 4, Table No. 2).

The proposed HPLC method was validated for intra and inter-day variation. When the solution containing 5, 10 and 20 µg/ml of Nadifloxacin was repeatedly injected on the same day, the coefficient of variance (CV) in the peak area for five replicate injections was found to be less than 0.4 %.

Also the inter day variation (3 days and five injections) was found to be less than 0.5% (Table No. 3).

Table 1: Calibration of RP- HPLC Method for Determination of Nadifloxacin

Concentration (µg/ml)	Peak Area (mAU)*
0.1	1.01513
0.2	2.03207
0.3	3.05863
0.6	6.09757
1.0	9.87983
2.0	21.35481
3.0	30.87639
4.0	39.47862
5.0	50.15476
10.0	99.98535

*Mean of five determinations

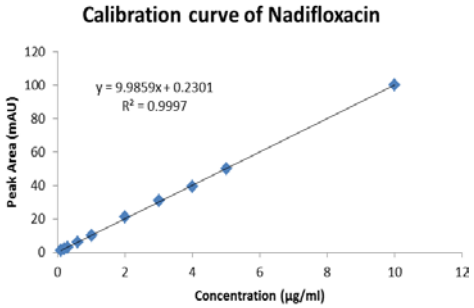

Fig. 4: Calibration curve of Nadifloxacin

Table 2: Results of the data analysis for the quantitative determination of Nadifloxacin

Statistical parameters	RP-HPLC
Concentration range, µg/ml	0.1-10.0
Regression equation	Y= 9.9859x -0.2301
Correlation co-efficient (r^2)	0.9997
Limit of detection (LOQ), µg/ml	0.020
Limit of quantification (LOD), µg/ml	0.006

Table 3: Intra and Inter-Day Precision for Nadifloxacin

Concentration of Nadifloxacin (µg/ml)	Observed concentration (µg/ml) of Nadifloxacin Found			
	Intra-Day		Inter-Day	
	Mean (n=5)	C. V. (%)	Mean (n=5)	C. V. (%)
5	5.02	0.26	5.04	0.24
10	10.1	0.25	10.3	0.28
20	20.1	0.31	20.1	0.43

Known amounts of the drug solution (8, 10 and 12 µg/ml) were subjected to the estimation of the drug for the recovery studies. There was a high recovery of Nadifloxacin (100.5%, 100.2%, and 100.1%) indicating that the proposed procedure for the determination of Nadifloxacin is highly accurate (Table No. 4).

The method was found to be robust as the result shows that there is no significance change in the result observed when there is a little change in the flow rate of the media. We can see that the retention time is almost similar when the flow rate is decreased a little bit by 0.2 ml/min (1.0 ml/min) and when increased a little bit by 0.2 ml/min (1.4 ml/min) (Table No. 5).

Number of theoretical plates was found to be more than 2000 and the tailing factor was found to be less than 2.0 (Table No. 6). Hence the developed method meets the criteria of system suitability.

When performed in two different systems (agilant and Shimadzu) under similar conditions, both the systems gave similar results (Table No. 7). Hence the ruggedness of the system is confirmed.

Table 4: Accuracy of Lamotrigine

Sample	Percentage (%) recovery	Mean
S1-80%	99.7	100.5
S2-80%	101.3	
S3-80%	100.4	
S1-100%	100.5	100.2
S2-100%	101.3	
S3-100%	98.7	
S1-120%	101.0	100.1
S2-120%	98.9	
S3-120%	100.4	

Table 5: Robustness Results for variations in Flow Rate (ml/min)

Method parameter → / Flow rate (ml/min) ↓	Retention time (min)	Tailing factor
1.0	10.6	0.849
1.2	10.4	0.897
1.4	10.4	0.982

Table 6: System Suitability Parameters

S. No.	Parameters	Obtained Values
1	Theoretical plates (N)	54419
2	Tailing factor (T)	0.897

Table 7: Ruggedness of method

	Retention time	Peak Area
Agilant	12.337 min	15.0697
Shimadzu	12.430 min	18632

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise and accurate HPLC method for the quantitative estimation of Nadifloxacin. The results showed that the proposed method is highly reproducible. The HPLC method developed in the present study has been used to quantify Nadifloxacin in bulk.

CONFLICT OF INTERESTS

Declared None

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