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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF NAFTOPIDIL IN BULK AND DOSAGE FORM

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ABSTRACT

A new simple, precise, sensitive and validated RP-HPLC was developed for the estimation of Naftopidil in pharmaceutical dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C8 (4.6x150mm,5µ) and mobile phase comprised of Methanol : Water (90 :10 v/v). The flow rate was 0.8 ml/min with detection at 232 nm. The retention time was found to be 2.85 min. The linearity was found to be in the range of 1 - 5 µg/ml with correlation coefficient of 0.9998. The proposed method is accurate with 99.26% - 100.66 % recovery for naftopidil and precise (%RSD of repeatability, intraday and inter day variations were 0.77, 0.59-1.32, 0.63-1.72). The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.07552 and 0.2288 µg/ml respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from tablet excipients were found.

Keywords: Naftopidil, RP-HPLC, Validation, Methanol.

INTRODUCTION

Naftopidil, a phenopermine derivative (Fig.1), a selective α_1-adrenoreceptor antagonist, a calcium antagonist and a 5-HT_1A agonist[1].It is a renal urological drug that is utilized extensively for the treatment of arterial hypertension and benign prostatic hypertrophy(BPH)[2].

Literature survey reveals that few Chiral HPLC[3,4], RP-HPLC in bio samples[5-8] and phosphorimetric methods[9,10] reported for the estimation of naftopidil. No simple RP-HPLC method has been reported for the estimation in dosage forms. In the present study to develop a simple, accurate and precise RP-HPLC method for estimation of naftopidil in bulk and dosage form. The validation has been carried out as per ICH guidelines.

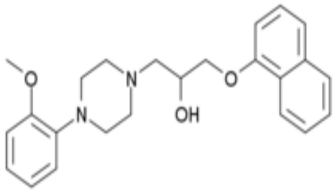

Fig. 1: Structure of Naftopidil

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Shimadzu LC 10 AT VP HPLC instrument equipped with UV detector and spinchrom software, Phenomenex Luna C8 (4.6 x 150 mm, 5 µ particle size) was used as stationary phase. Shimadzu Ax200 analytical balance and Sonicator Pci Ultrasonic 3.5L100H were used in the study.

Reagents and materials

The reference sample of naftopidil was supplied by Sun Pharma, Baroda. The formulation was procured from the local market. HPLC grade Methanol was purchased from Merck specialities private ltd, Mumbai .Triple distilled water was used throughout the experiment.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various

mobile phases tried, mobile phase containing Methanol: Water [90:10 % v/v] proportion with detection wavelength 232nm was selected, since it gave sharp peak with good symmetry within limits for the drug.

Chromatographic conditions
The optimized parameters which were used as a final method for the estimation of naftopidil represented in the Table 1.

Preparation of standard stock solutions
An accurately weighed quantity of naftopidil (10 mg) was transferred to 100 ml volumetric flask, dissolved and diluted to the mark with mobile phase to obtain standard stock solution having concentration of 100µg/ml.

Preparation of calibration curve
Aliquots of 0.1,0.2,0.3,0.4,0.5 ml standard stock solution (100 µg/ml) was transferred to the 10 ml of volumetric flasks and made up to the mark with mobile phase to get concentration of 1,2,3,4,5 µg/ml .An aliquot (20 µl) of each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curve was constructed by plotting the peak areas versus the concentration and the regression equation was calculated.
The fixed standard solution was prepared by transferring 0.3 ml of naftopidil (100 µg/ml) to 10 ml of volumetric flask and made up to the mark with mobile phase to get 3 µg/ml of naftopidil.

Sample preparation
Tablet powder equivalent to 10mg of naftopidil is weighed and transferred to 100ml standard flask. Small amount of mobile phase is added to dissolve and the volume is made up to the mark. Then it is filtered with 0.45 micron filter and sonicated. From this stock solution, 0.3ml is transferred to the 10 ml volumetric flask and volume is made up to 10ml to prepare 3µg/ml concentration. Prepared sample solution was analysed. (Table 7)

Method validation
The optimized Chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH, 2005) [(11).]

System suitability test
20 µL of the standard solution (3µg/ml) was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Specificity
Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, degradants or excipients. A volume of 20µl of working placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 2.85 min. Hence, the proposed method was specific for naftopidil.

Linearity
The linearity of calibration curve in pure solution was carried over the concentration range of 1-5 µg/ml through proposed HPLC method. The data was represented in Table 3.

Precision
The precision of the method was determined by repeatability and intermediate precision (intra-day and inter-day).

Repeatability
The Repeatability of the proposed method was ascertained by injecting six replicates of fixed concentration within the Beer's range and finding out the peak area by the proposed method. From this peak area %RSD was calculated. (Table 4)

Intra-day precision
Intra–day precision was determined by injecting three different concentrations (90 %, 100% and 110%) for three times in the same day. Peak area was measured and %RSD was calculated. (Table 4)

Inter-day precision
Inter-day precision was determined by injecting three different concentrations (90 %, 100% and 110%) for three days in a week. Peak area was measured and %RSD was calculated. (Table 4)

Accuracy
For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (50%, 100% and 150% of final concentration).A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table 5.

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the Flow rate (±0.2), organic solvent content (± 5%),wave length(±5nm). None of these alterations caused a significant change in peak area RSD, tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible. The results were tabulated in Table 6.

Limit of detection and Limit of quantification

Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope.LOD and LOQ were calculated by using equations, LOD=3.3 xσ/s and LOQ=10 x σ/s, where σ = standard deviation, S= slope of the calibration curve.(Table 4)

RESULTS AND DISCUSSIONS

To develop simple and economical RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with Phenomenex Luna C8 (4.6 x 150 mm,5µ)column and mobile phase comprising of Methanol : Water (90:10 v/v) at a flow rate of 0.8 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 232 nm based on peak area. The retention time was found to be 2.85 min. The optimised method was validated as per ICH guidelines. The system suitability parameters observed by using this optimised conditions were reported in Table2. A linearity range of 1-5 µg/ml with correlation coefficient 0.9998 was established. The result of recovery study by standard addition method ranging from 99.26 % to 100.66 % suggested good accuracy. The precision of the proposed method was checked in terms of the repeatability, inter-day and intra-day time periods. The low % RSD values of repeatability (0.77%), inter-day (0.63% - 1.72%) and intra-day (0.59 % – 1.32 %) variations reveal that the proposed method is precise. The LOD,LOQ values were found to be 0.07552µg/ml and 0.2288µg/ml respectively. The absence of interference peak, indicates that method can be used for routine analysis of naftopidil in pharmaceutical dosage form.

CONCLUSION

A rapid and reliable isocratic RP-HPLC-UV method for the determination of naftopidil has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method .It is highly specific,precise accurate and robust analytical procedure and its retention time 2.85 min allows the analysis of large number of samples in a short period of time. So this can be used for routine analysis.

Table 1: Optimised chromatographic conditions

Mobile phase	Methanol: water [90 :10% v/v]
Stationary phase	Phenomenex Luna C8 (4.6 x 150 mm ,5µ particle size)
Wavelength	232nm
Run time	10 min
Flow rate	0.8 ml/min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Table 2: System Suitability Test Parameters

System suitability parameters	Result
Retention time	2.85
Area	3996.152
Theoretical plate number	5861
Tailing factor	0.73

Table 3: Linearity data

Concentration (µg/ml)	Peak area
1	1329.636
2	2582.507
3	4006.359
4	5245.276
5	6645.769

Table 4: Validation parameters of the proposed method

Parameter	Results
Linearity (µg/ml)	1-5
Slope(b)	1329.5035
Intercept(a)	-26.6011
Correlation co efficient (r)	0.9998
Regression equation(y=mx+c)	Y=1329.5035x-26.6011
Precision	
Repeatability(%RSD, n=6)	0.77
Intraday precision(%RSD n=6)	0.59 -1.32
Interday precision(%RSD ,n=6)	0.63-1.72
% Recovery	99.26 – 100.66
Robustness	Robustted
LOD(µg/ml)	0.07552
LOQ(µg/ml)	0.2288

Table 5: Recovery data

Drug name	Levels	Amount added(µg/ml)	Amount recovered(µg/ml)	%Recovery	
Naftopidil	50 %	1.5	1.489	99.26	Mean %Recovery 99.75 SD 0.7889
	100%	3	2.98	99.33	
	150%	4.5	4.53	100.66	

Table 6: Results for robustness test

Parameters	Changes	% Recovery of Naftopidil
Organic phase variation	95%	99.4
	85%	98.7
Flow rate variation	1.0	98.6
	0.6	98.4
Wavelength variation	237	98.7
	227	98.2

Table 7: Analysis of formulation

Brand Name	Drug	Amount labelled (mg)	Amount found (mg)	%recovery	%RSD
Nafodil 75	Naftopidil	75	74.96	99.94	0.37

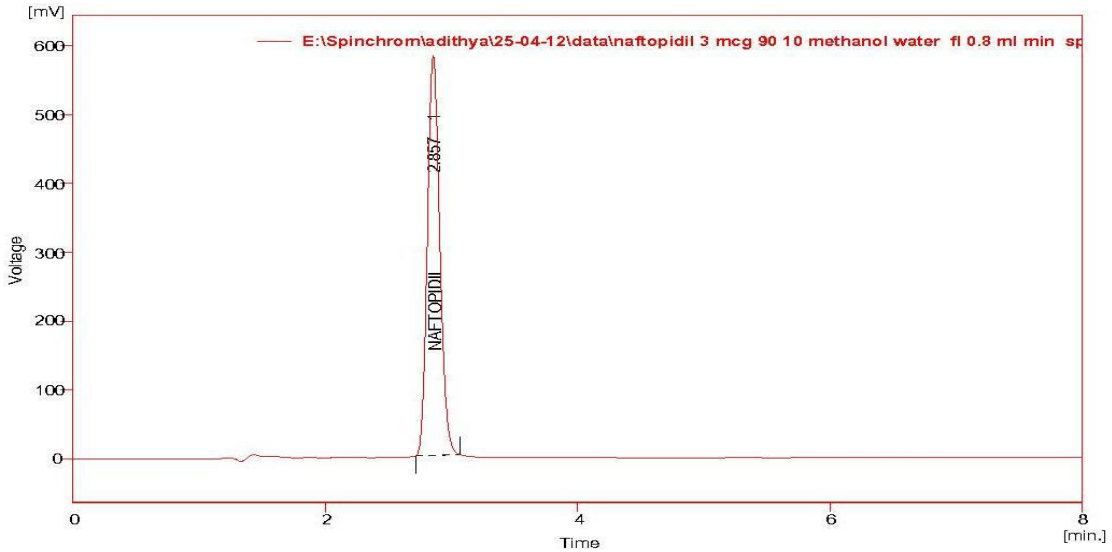

Fig. 2: Naftopidil API peak.

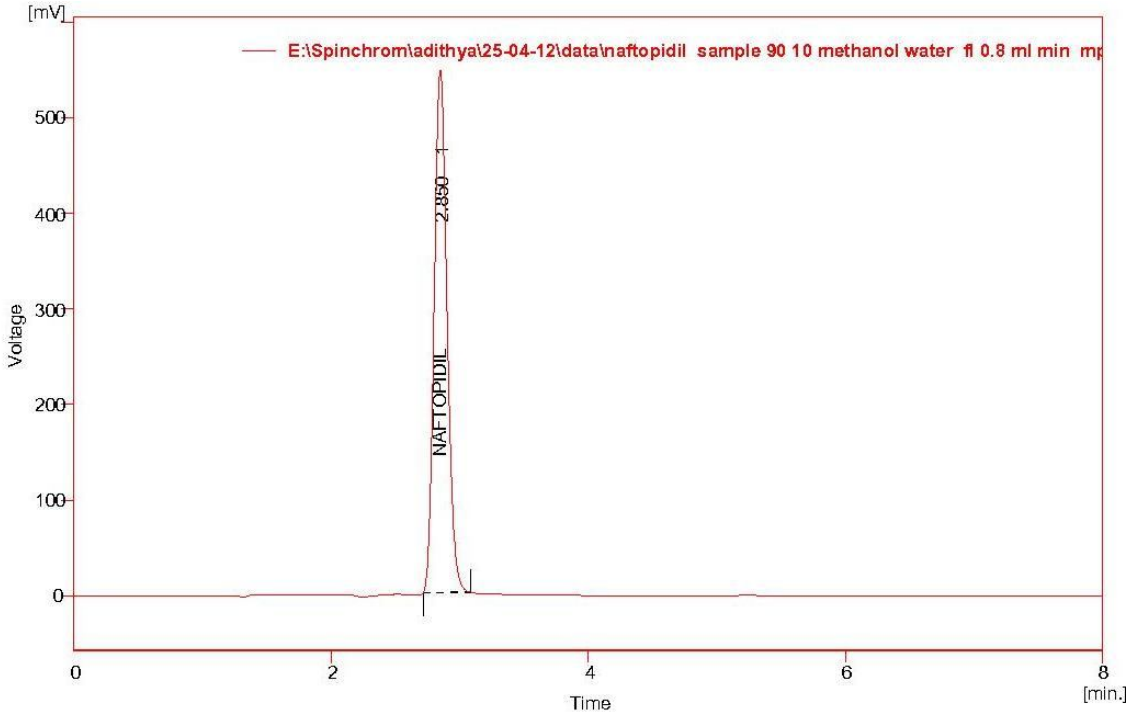

Fig. 3: Naftopidil formulation peak

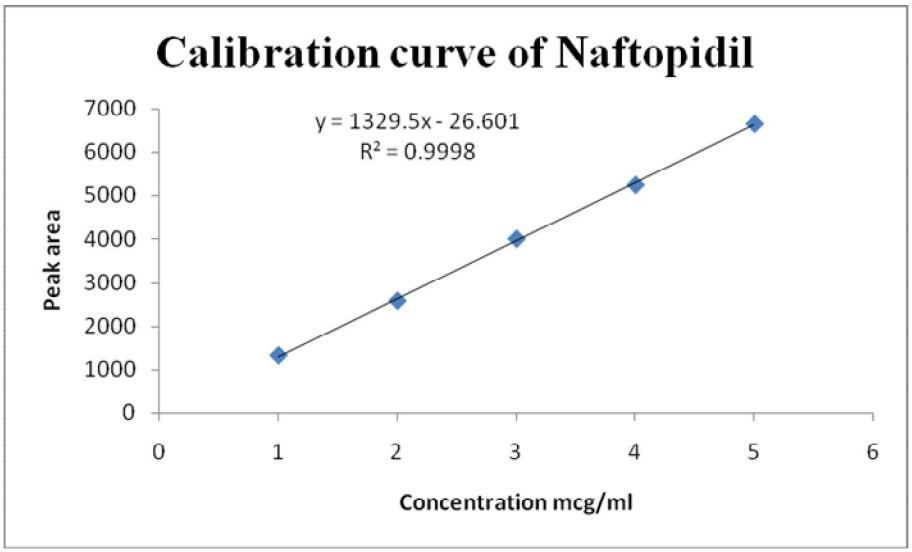

Fig. 4: Calibration curve of Naftopidil

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