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[Research article]

Method Development and Validation of Naftopidil by Reverse Phase-HPLC in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A simple, sensitive accurate precise and rapid RP-HPLC method was developed for the estimation of Naftopidil in bulk and pharmaceutical formulation. The chromatographic conditions used for separation was Kromosil C18 (150×4.6mm, 3.5µ) and the mobile phase comprised of acetonitrile and water in the ratio of 50:50 %(*v/v %)* The flow rate was 1ml/min with the detection at 232nm.The retention time was found to be 3.245min.The proposed method is accurate .The linearity was found to be in the range of 50-150 µg/ml with correlation coefficient of 1. The repeatability and intermediate precision was found to be less than 2%. The Limit of Quantitation (LOQ) were found to be 1.13 µg/ml and 3.43 µg/ml respectively. The method was successfully applied to Pharmaceutical formulation.

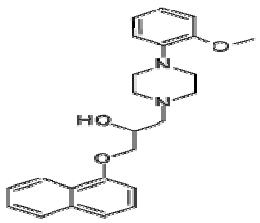
Keywords: Naftopidil, RP-HPLC, Validation, Methanol, ACN.

INTRODUCTION

Naftopidil is a novel phenylpiperazine vasodilator drug with selective alpha-1 adrenoceptor-blocking activity, which is undergoing clinical evaluation in patients with essential hypertension[1]. Naftopidil is an α1-adrenergic receptor antagonist (α1-blocker) used to treat lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH). Naftopidil has distinct characteristics because it has three times greater affinity for the α1D-adrenergic receptor subtype than for the α1A subtype [2].

Naftopidil is chemically, 1-[4-(2-Methoxyphenyl) piperazin-1-yl]-3-naphthalen-1- yloxy- propan-2-ol, represented in figure 1.

Fig 1: 1-[4-(2-Methoxyphenyl) piperazin-1-yl]-3-naphthalen-1- yloxy- propan-2-ol



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Litreature survey reveals that a chiral HPLC method was developed for separation and analysis of Naftopidil enantiomers(3).Further a fluorescence method(4),chemiluminescence method (5) and direct determination of naftopidil in phosphor - rescence were reported(6). A HPLC method was developed for determination of naftopidil in biological samples [7].

MATERIALS AND METHODS

Quantitative HPLC was performed on an isocratic high pressure liquid chromatography (Waters model 2695) Equipped with a photodiode array detector capable of operating in the range of 190 nm to 800 nm with Kromosil C18 (150×4.6mm, 3.5µ).

REAGENTS AND CHEMICALS

Orthophosphoric acid of AR grade, methanol of HPLC grade, acetonitrile of HPLC grade and water HPLC grade were obtained from Rankem Chemicals Ltd., Mumbai. Naftopidil was obtained as a gift sample from Aurobindo pharma, India. The commercially available Naftopidil tablets were procured from the local Pharmacy.

CHROMATOGRAPHIC CONDITIONS

The mobile phase consisting of acetonitrile and water (pH adjusted 2.4 by orthophosphoric acid) in the ratio of 50:50 %(*v/v %)* was filtered through 0.45µ membrane filter before use, degassed and pumped from the solvent reservoir into the column at a flow rate of 1 ml/min. The detection was monitored at 232 nm and the run time was 5 minutes. The volume of injection loop was 4 µl and prior to the injection of the drug solution; the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The column and the HPLC system were maintained at 45°c temperature.

Preparation of standard stock solution

50 mg of Naftopidil was accurately weighed and transferred into a 50 ml clean dry volumetric flask, and 30 ml of methanol and 20 ml of water was added, sonicated for 10 minutes to dissolve the contents and diluted to volume with water to get a concentration of 1mg/ml (stock 1). 5 ml of the above stock solution was transferred to a 25 ml volumetric flask and made up to the mark to get a concentration of 200 µg/ml (stock 2).

Preparation of sample solution

10 tablets of Naftopidil were accurately weighed and powdered. Transfer the powder equivalent to 50 mg of Naftopidil into 50 ml of clean, dry, volumetric flask. To this 30 ml of Methanol and was added and sonicated for about 10 minutes, further the volume was made up with diluent and then filtered through 0.45 micron filter. Further 1 ml of the filtrate was diluted to 10 ml with water.

METHOD DEVELOPMENT

A C18 column (150×4.6mm, 3.5µ) as a stationary phase with a mobile phase of acetonitrile and water at a flow rate of 1.0 mL/min and a detection wavelength of 232 nm afforded the best separation of Naftopidil. The standard solutions prepared as above were injected into the 10 µl loop and the chromatogram was recorded as shown in fig 2. The retention time of Naftopidil was found to be 3.245 min. The calibration curve was constructed by plotting concentration versus peak area ratio. The amount of Naftopidil present in sample was calculated through the standard calibration curve.

ASSAY

Ten tablets each containing 50 mg were weighed accurately and powdered. A quantity equivalent to 50 mg of Naftopidil was weighed accurately and transferred to 50 ml volumetric flask containing 30 ml of methanol. The contents were sonicated for 20 min. and made up to the mark with the water. The resulting solution is filtered through 13 mm × 0.45µm PVDF. 1mL of the above solution was pipette into 10mL volumetric flask and made up with water. The solution obtained was diluted with the water so as to obtain a concentration in the range of linearity previously determined for the pure drug. The 20µl sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of Naftopidil present in tablet formulation was determined by comparing the peak area from the standard. The results were furnished in Table 1.

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)[8]

LINEARITY AND RANGE

The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The standard curve was obtained in the concentration range of 50-150 µg /ml. The peak area ratios of the drug versus concentration were found to be linear and the results are furnished in Table 2**.**The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits. The linearity graph was shown in fig 3.

ACCURACY

Accuracy of the method was performed by preparing the placebo of the drug formulation according to the formulation procedure. To the required quantity of placebo, a known quantity of Naftopidil with the same proportion as in the drug formulation was added to get three concentrations (50, 100, 150 µg/mL of Naftopidil). Results have shown that the recovery of Naftopidil is within 98.0–102%, and the RSD is lower than 2.0%. The results are shown in Table 3.

PRECISION

Repeatability

Repeatability of the method was evaluated by calculating the RSD of the peak areas of six replicate injections for the standard concentration (100%) of Naftopidil, which was found to be 0.6%.The results are furnished in Table 4.

Intermediate precision (ruggedness)

The Intermediate precision method was also evaluated by analyzing six samples of Naftopidil by two analysts in the same laboratory using different HPLC systems. Results of this study showed that the percentage RSD of Naftopidil was 0.8% indicating a good intermediate precision of the method. The results are tabulated in Table 5.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ*)*

The LOD and LOQ for Naftopidil were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Naftopidil. The results were shown in Table 6.

ROBUSTNESS

The robustness was checked by changing the flow rate to 0.8 and 1.2 ml/ min, and column oven temperature 40°c to 50°c, did not affect peak response and retention time so the method suits best and the results are shown in Table 7.

Fig 2.Chromatogram of Naftopidil standard solution

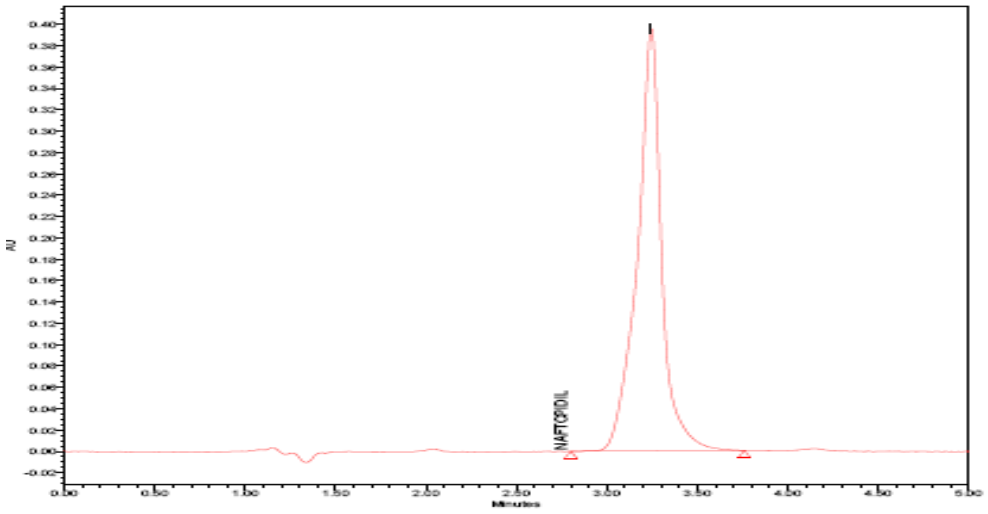

Table1 Quantitative estimation of Naftopidil in tablet dosage form

S. NO.	Tablet Sample	Label Claim in mg/tablet	Peak Area		Amount found mg/tablet	Percentage content of Drug
			Test	Standard		
1	Naftopidil	50	3728129	3772820	49.4	98.81

Fig 3: Linearity graph for Naftopidil

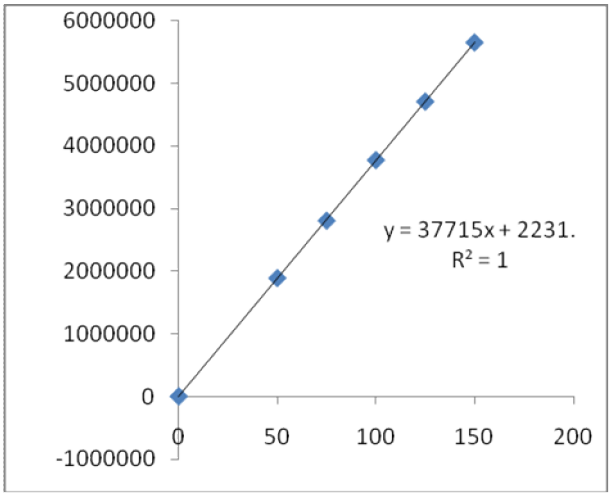

Table 2: Linearity data of Naftopidil

S.no.	Concentration (µg/ml)	Area of the peak(mv)
1	50	1889821
2	75	2808562
3	100	3776483
4	125	4712756
5	150	5656587

Table 3: Accuracy data of Naftopidil

Sample	Area	Amt added (µg/ml)	Amt recovered (µg/ml)	%Recovery	Mean
50%-Rec-1	1801854	50	49.9	99.9	99.86
50%-Rec-2	1818914	50	50.1	100.2	
50%-Rec-3	1802814	50	49.8	99.6	
100%-Rec-1	3728129	100	99.9	99.9	99.93
100%-Rec-2	3719305	100	99.8	99.8	
100%-Rec-3	3718658	100	100.1	100.1	
150%-Rec-1	5640826	150	149.8	99.8	99.4
150%-Rec-2	5606940	150	148.6	99.0	
150%-Rec-3	5694400	150	149.2	99.4	

Table 4: Repeatability data of Naftopidil

S.No	Standard	Chromatographic peak area(mv)
1	Injection 1	3731063
2	Injection 2	3758291
3	Injection 3	3706078
4	Injection 4	3741252
5	Injection 5	3700483
6	Injection 6	3722900
7	Mean	3726678
8	SD	21706
9	%RSD	0.6

Table 5 intermediate precision of Naftopidil

S.No	Sample solution	Chromatographic peak area(mv)
1	Injection 1	3745879
2	Injection 2	3721458
3	Injection 3	3768542
4	Injection 4	3702368
5	Injection 5	3710245
6	Injection 6	3700365
	MEAN	3736542
	STANDARD DEVIATION	22546
	%R.S.D	0.8

Table 6: LOD and LOQ data of Naftopidil

S.NO	Name	LOD Value (µg/ml)	LOQ Value (µg/ml)
1.	Naftopidil	1.13	3.43

Table 7: Robustness data of Naftopidil

Effect of variation in Column Oven Temperature

System suitability parameters	Variation in column oven temperature(°C)			Acceptance criteria
	40	45	50	
Tailing factor	0.931	0.937	0.921	NMT 2.0
Theoretical plates	3092	3460	3103	NLT 2500

Effect of variation in flow rate

System suitability parameters	Variation in flow(ml/min)			Acceptance criteria
	0.8	1.0	1.2	
Tailing factor	0.921	0.937	0.916	NMT 2.0
Theoritical plates	3361	3460	2945	NLT 2500

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of Naftopidil in bulk and tablet dosage form. The developed method is cheap, easy, and it gives sharp peak with high resolution. The assay provides a linear response across a wide range of concentrations. Low intra-day % RSD coupled with excellent recoveries. The proposed method is highly precise accurate and robust analytical procedure and its RT is 3.245 min allows the analysis of larger number of samples in short period of time. So this developed method can be used for the routine analysis of Naftopidil in tablet dosage form.

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