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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NAFTOPIDIL IN BULK AND DOSAGE FORM USING RP HPLC

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ABSTRACT

A reversed phase High Performance Liquid Chromatographic method for the estimation of Naftopidil has been developed. The drug was separated on C 18 using Pot. Dihydrogen o-phosphate: ACN in ratio of 35:65 at flow rate of 1.0 ml/min. Components are detected imultaneously at 232 nm using UV detector. The detection limits for Naftopidil was 0.094 µg/ml where as the quantitation limits was 0.28 µg/ml. Linearity range was established in range of 05–10 µg/ml for Naftopidil. Recovery of the added Naftopidil standard mixture in tablet solution was found 100.8 % with Relative standard deviation (*n*=3) of 0.384 %. The proposed method has been applied to the determination of Naftopidil in commercial products. The results obtained by methods were in good agreement of true values. The proposed method is simple, accurate, reproducible and suitable for routine analysis.

KEYWORDS: Naftopidil, RP HPLC, ICH.

INTRODUCTION

Introduction to High Performance Liquid Chromatography

HPLC is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. [1-4]

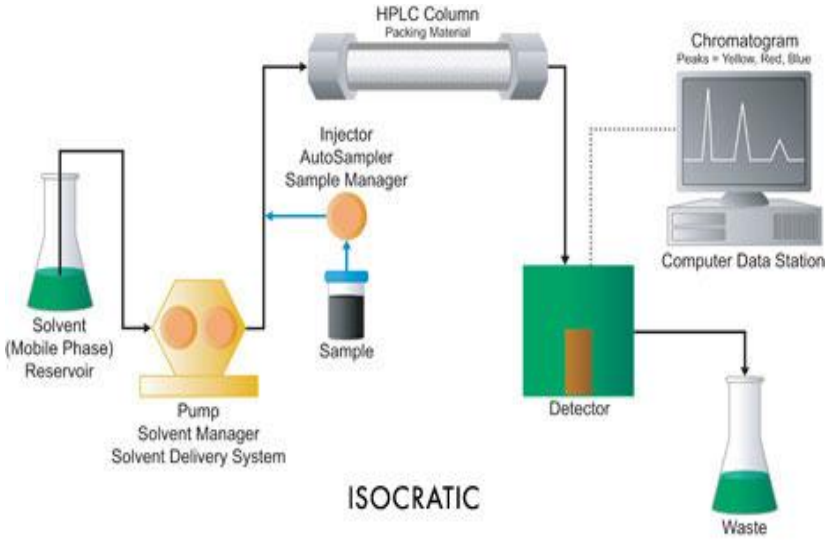

Fig. 1: A schematic diagram of HPLC equipment.

Various components of HPLC

1. A solvent delivery system, including pump,
2. Sample injection system,
3. A chromatographic column,
4. A detector,
5. A strip chart recorder,
6. Data handling device and microprocessor control.

Selection of buffer as a mobile phase in HPLC

In RP-HPLC the retention of analyte are related to their hydrophobicity. The more hydrophobic the analyte the longer it is retained. When an analyte is ionized it become less hydrophobic and retention decreased .When pH increases acid loose a proton and base gain a proton when pH decreases and become ionized.[5-6]

For the most robust method it is recommended that separation be develop a mobile phase pH, where a retention of ananlyte is little affected by changes in pH when separating bases. For example - acid mobile phase usually show better reproducibility then neutral mobile phases.[7-8]

Analytical method validation

Validation is an act of proving that when any procedure, process, equipment, material, activity or system is performed as expected under given set of conditions then it should give the required accuracy, precision, sensitivity, ruggedness to the method /system etc. When

extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.[9]

The various validation parameters used as per ICH guideline are:[10]

1. Accuracy.
2. Precision (intraday and inter day precision, Repeatability and Reproducibility).
3. Linearity.
4. Limit of Detection (LOD) and Limit of Quantitation (LOQ).
5. Ruggedness.
6. Selectivity/ Specificity.
7. Robustness/ Ruggedness.

Introduction of Naftopidil

Naftopidil is an α1-adrenergic receptor antagonist (α1-blocker) used to treat lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH). Different from tamsulosin hydrochloride, in that it has higher and extremely higher affinity respectively, for the α1A-adrenergic receptor subtype than for the α1D type, naftopidil has distinct characteristics because it has a three times greater affinity for the α1D-adrenergic receptor subtype than for the α1A subtype.[11]

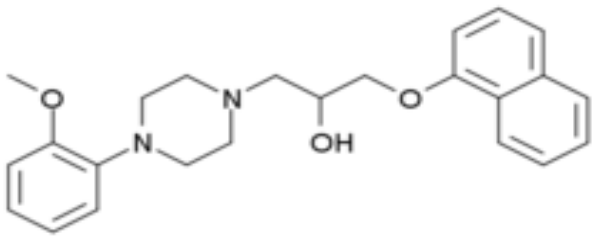

Fig. 2: Structure of Naftopidil.

IUPAC Name

4-(2-Methoxyphenyl)-alpha-((1-naphthalenyloxy)methyl)-1-pioerazineethanol dichloride;(+-)-1-(4-(2Methoxyphenyl)piperazinyl) -3- (1-naphthyloxy)propan-2-ol dichloride.

Molecular formula $C_{24}H_{28}N_2O_3$.

Molecular Weight 392.5g/mol.

Pharmacodynamics

Naftopidil is a novel alpha-1 adrenoreceptor blocker. The phenylpiperazine derivative competitively inhibits prazosin-binding prostatic membrane receptors.[12] The selective action against adrenoreceptors leads to reduced blood pressure and prostate pressure. Naftopidil reduces the bladder outlet obstruction in benign prostate hyperplasia patients.[13]

Mechanism of Action

Benign prostatic hyperplasia (BPH) is common in men above a certain age throughout the world. Alpha1-adrenoceptor antagonists is widely used as a conservative treatment to relieve bladder outlet obstruction due to benign prostatic enlargement.[14] Naftopidil is a newly synthesized alpha1-blocker that has been found to be effective in the treatment of BPH. This drug is highly selective for the Alpha1A-, and Alpha1D-adrenoceptor subtypes, with an affinity for the Alpha1D-adrenoceptor that is 3- and 17-fold higher than that for the Alpha1A- and Alpha1B-adrenoceptors. Pharmacokinetics In healthy adult volunteers, after once oral administration of Naftopidil alone with 25mg, 50mg and 100mg.[15]

MATERIALS AND METHODS

Chemicals and reagents

Naftopidil bulk drug was made available from Cadila Healthcare Pvt.Ltd Ahmedabad, Gujrat. Orthophosphoric acid, methanol, Perchlorate buffer, Acetonitrile & Pot. Dihydrogen o-phosphate were obtained from Loba Chem. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment. Equipments: The Waters HPLC system with a UV or photo diode array detector was used for method development and validation. The output signal was monitored and processed by using Empower software. Chromatographic condition: The mobile phase used Pot. Dihydrogen o-phosphate: CAN in ratio of 35:65 at flow rate of 1.0 ml/min. The analytical column used Inertsil ODS C18 (4.0 x 250mm, 6 detection was carried out at a wavelength of 232 nm for a run time of 6 min. Diluent used as Methanol.

Preparation of standard stock solution of Naftopidil

10 mg of Naftopidil was weighed accurately and transferred into 10 ml volumetric flask. About 10 ml of HPLC grade Methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent to form 1000 µg/ml solution. 1.0 ml of the stock solution was further diluted in a 10 ml volumetric flask with same solvent to form 100 µg/ml solutions and 1.0 ml of 100µg/ml was diluted further up to 10 ml with same solvent. The final

solution contained about 10 µg/ml of Naftopidil. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use.

Preparation of Sample Solution of Naftopidil

One tablet was weighed, powdered and then the weight was transferred into a 100mL volumetric flask, 50 mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

RESULT AND DISCUSSION

Chromatograhic prameters were preliminary optimized to develop RP HPLC method for estimation of Naftopidil with short analysis time 6 min, and accepted resolution (>2). The isoabsoptive point of Naftopidil was 232 nm. In order to identify a suitable organic modifier, various compositions of methanol, Pot. Dihydrogen o-phosphate and acetonitrile were tested along with different buffer. Different columns like Inertsil and Inspire columns were tried. Resolution and peak tailing were the measure problem while we are during developemnet of method. Resolution and peak separation were very less when we are using one mobile phase, to increase the resolution and better peak separation of methanol and water were used in isocratic mode. Finally, separation for the determination Naftopidil was carried out by isocratic elution with a flow rate of 1.0 ml/min at 232 nm using inertsil (ODS 250 x 4.6 mm) the standard chrometogram shown in fig 3.

Optimized Method: Drug was eluted with good resolution, retention time all the parameters like Plate count and Tailing factor were within the limits.

Mobile phase

Pot. Dihydrogen o-phosphate: ACN 35: 65.

Chromatographic conditions

Flow Rate. 1.0 ml/min.

Column. ODS (215x4.6mm).

Detector Wavelength. 232 nm.

Column Temperature. 30^{o}C.

Injection Volume. 10uL

Run Time. 6 min.

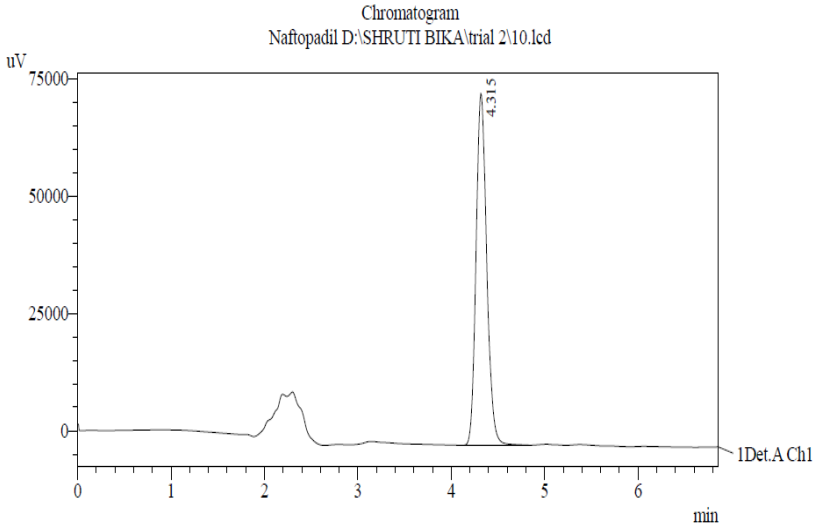

Fig. 3: Optimized chromatogram of Naftopidil.

Table1: Final trial's observations.

RET.TIME	AREA	HEIGHT	THEORETICAL PLATES	TAILING FACTOR
4.315	595450	75027	42010.484	1.218

Results and discussion

Here resolution was good and theoretical plate count for Naftopidil was **42010.484** which is greater than 2000. The selected and optimized mobile phase was Pot. Dihydrogen o-phosphatel: ACN in ratio of 35:65 and conditions of run were flow rate of 1 ml/min at detection wavelength of 232 nm for 6 minutes where resolution, shape of peak was found good with high number of theoretical plate counts and better symmetry. Hence chromatographic condition was suitable for method development.

METHOD OF VALIDATION

The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. [16-17]

Specificity

Preparation and running of Naftopidil

The solution of Naftopidil was prepared. Accurately weighed 10 mg of Naftopidil was transferred to 100 ml of volumetric flask and 100 ml of methanol was added to it. Now the conc. Is 100µg/ml, from this solution 1 ml of was taken in 100 ml flask and 50 ml of methanol was added common excipients used in tablet formulation such as 8% starch, 7% magnesium stearate, 1% talc and remaining lactose (for 100 µg/ml) were added in this

solution and were sonicated for 20 minutes. Then solution was filtered through membrane filter and volume was made up to the 100 ml with solvent system and degassed under ultrasonic bath prior to use. The solution was then injected into the HPLC system. The chromatogram obtained of synthetic mixture is shown in figure 4.

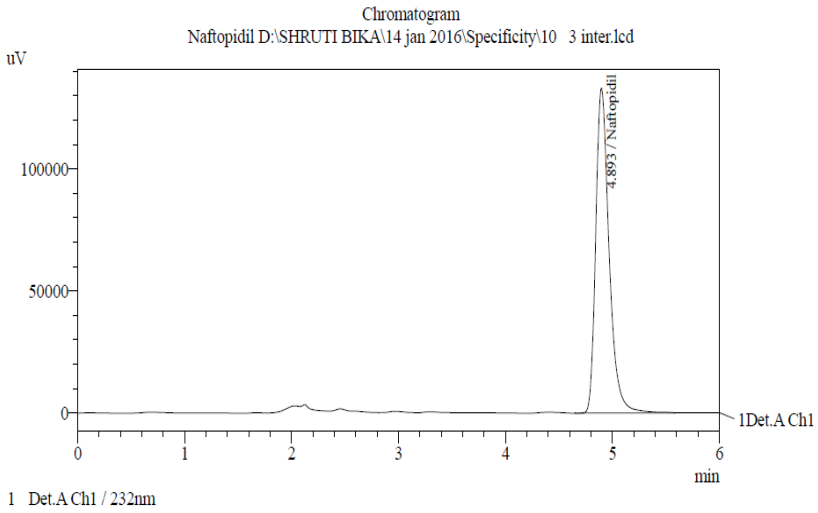

Fig.4: Chromatogram for formulated synthetic tablet mixture.

Result and discussion

No excepients peaks were found at the retention time of Naftopidil hence Specificity studies indicated that the excipients did not interfere with the analysis.

Linearity

Linearity range was found for Naftopidil from 0.5µg/ml to 10 µg/ml concentration. The correlation coefficient was found to be 0.999 which showed good linearity between ranges. The slope was found to be 5935.8 and intercept was found to be 104983

Preparation of calibration curve of standard API

The standard solutions of Naftopidil in the concentration range of 0.05 µg/ml to 10 µg/ml were obtained by transferring 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml from stock solution 10 µg/ml to the series of six volumetric flasks of 10 ml. The volumes in each volumetric flask were made up to the mark. The solutions were filtered, degassed and were injected into column. The run time was 6 min and the peak areas were measured. The calibration data are shown in **table no. 2** and calibration curve is shown in **figure 5.**

Table 2: Calibration curve data for Naftopidil.

S. no	Conc (µg/ml)	Area 1	Area 2	Area 3	Mean	SD	%RSD
1.	0.5	121114	119935	117696	119581.7	1736.178	1.451876
2.	1	197197	195548	193417	195387.3	1895.115	0.969927
3.	2	312643	310012	309112	310589	1834.853	0.590766
4.	3	404326	395905	390170	396800.3	7120.344	1.79444
5.	4	530409	528933	527751	529031	1331.707	0.251726
6.	5	622250	618630	609693	616857.7	6463.392	1.047793
7	6	711094	711936	718094	713708	3821.647	0.535464
8	7	845603	838344	819620	834522.3	13406.45	1.606482
9	8	942765	945286	938975	942342	3176.693	0.337106
10	9	1049186	1054295	1051568	1051683	2556.441	0.243081
11	10	1157703	1148534	1153069	1153102	4584.589	0.397587

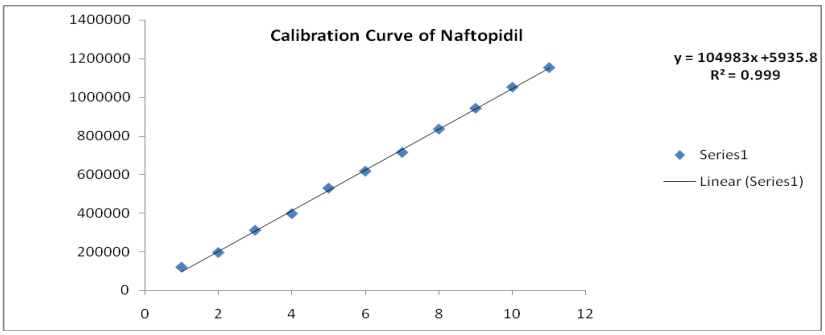

Fig.5: Calibration Curve of Naftopidil.

Accuracy

Accuracy of analytical method expresses the closeness of agreement between the value which is expected either as conventional true value or an accepted reference value and the value found. The results of analysis, obtained in three groups containing three replicate experiments with API and different tablet dosage forms, had good agreement with the labeled amount of the drug. The results are shown in **Table 3.**

Table 3: Accuracy.

Conc.	Area	Mean area	SD	%RSD
80%	434426			
	434112			
	431546			
		433361.33	5003.288	0.921368
100%	536348			
	533893			
	534592			
		534944.3	1264.856	0.236446
120%	631478			
	643323			
	629904			
		634901.7	7335.428	1.155364

Table 4 : Data showing Recovery Study.

Conc	C1 Conc µg/ml	Mean area y= 104983x + 5935.8	C2 Conc x = (y - 5935.8) / 104983	% Recovery (c2/c1*100)
80%	4	433361.3	4.071	101.784
100%	5	534944.3	5.038	100.779
120%	6	634901.7	5.991	99.852
			Mean % Recovery	100.805

RESULT AND DISCUSSION

The mean % recovery was found to be 100.8 % for Naftopidil. The values for % recovery for 80%, 100% and 120% were 101.784% , 100.779% and 99.852% respectively they fall within the limit, hence it can be said that the proposed method was accurate.

Precision

Repeatability

Repeatability precision was determined by using six time repetitions of the single target concentration that is equivalent 100% level of target range that is 7µg/ml for Naftopidil. The results are shown in **table 5.**

Table 5: Data showing repeatability analysis.

S.No.	Conc. µg/ml	Area
1.	10	1169882
2.	10	1149952
3.	10	1175767
4.	10	1162677
5.	10	1166160
6.	10	1166748
Mean		1165198
SD		8669.112
%RSD		0.744004

Result: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml Naftopidil, it showed RSD of 0.744004 %. Thus, it can be concluded that the analytical technique showed good repeatability.

Intermediate precision

A. Intra-day precision

For intra-day precision studies the drug having concentration value 80%, 100% & 120% of the target concentration (n = 3), were injected in triplicate at same day at different times periods into the HPLC system. The result are shown in **table 6.**

Table 6: Data for the intra-day precision analysis

Conc.	Time	Area	Mean area	SD	%RSD
	9:00 AM	933400			
80%	11:00 AM	933418			
	1:00 PM	933510			
			933442.6667	59.0028241	0.006320991
	9:00 AM	104855			
100%	11:00 AM	104726			
	1:00 PM	103492			
			104357.6667	752.4588582	0.721038408
	9:00 AM	129710			
120%	11:00 AM	129989			
	1:00 PM	129789			
			129829.3333	143.8065831	0.110765864
				MEAN %RSD	0.279375088

Result

Intraday study showed a RSD of 0.279375 % for Naftopidil. It showed that the mean % RSD was found to be within acceptance limit (≤2%). Thus, it can be concluded that the analytical technique showed a good intra-day precision.

Inter-day precision

For inter-day studies the drug having concentration value 80%, 100% & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system at three different days. The results are shown in **table 7.**

Table 7: Data for the inter-day precision analysis.

Conc.	Time	Area	Mean area	SD	%RSD
	DAY 1	933410			
80%	DAY 2	933415			
	DAY3	933520			
			933448.3333	62.11548319	0.00665441
	DAY 1	104595			
100%	DAY 2	104856			
	DAY3	103682			
			104377.6667	616.436804	0.590583047
	DAY 1	129930			
120%	DAY 2	129269			
	DAY3	129929			
			129709.3333	381.3401806	0.293995945
				MEAN %RSD	0.297077801

Result: Inter day study showed a RSD of 0.297077801% for Naftopidil. It showed that the mean % RSD was found to be within acceptance limit (≤2%). Thus, it can be concluded that the analytical technique showed a good inter day precision.

Limit of detection and Limit of quantification

Limit of detection is the lowest amount of analyte that can be detected but not quantitated as an exact value and Limit of quantitation is the lowest amount of analyte that can be quantitatively determined in a sample with suitable precision and accuracy.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

LOD = 3.3(SD/S) and

LOQ = 10(SD/S)

Where, SD = Standard deviation of the response, S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The results are shown in Table 8.

Table 8: Limit of detection and quantitation.

	Linearity 1	**Linearity 2**	**Linearity 3**	**Intercept SD**	**Slope mean**
Intercept	85338	82515	79176	3084.599	
Slope	107308	107382	107356		107348.7

LOD = 3.3 * Intercept/Slope

LOD = 3.3 * 3084.599/107348.7

LOD = 0.094823

LOQ = 10 * Intercept / Slope

LOQ = 10* 3084.599/107348.7

LOQ = 0.287344

Result

The LOD was found to be **0.094823** µg/ml and LOQ was found to be 0.**287344** µg/ml which showed that sensitivity of the method was high.

ROBUSTNESS

Flow rate variation by minus 0.1 ml/min (0.9 ml/min) was set keeping all other conditions same as before and triplicate observation of the single target concentration that is 10µg/ml for Naftopidil were determined.

Flow rate variation by plus 0.1 ml/min (1.1 ml/min) was set and triplicate observation of the single target concentration that is 10µg/ml for Naftopidil were determined

Table 9:

Flow rate at 0.9ml/min

Conc (µg/ml)	Area
10	1354683
10	1312504
10	1311747
Mean	1326311
SD	24573.5
%RSD	1.85277

At 1.1 ml/min

Conc µg/ml	Area
10	1079775
10	1080000
10	1084535
Mean	1081437
SD	2685.593
%RSD	0.248336

Change In Oven Temperature

Oven temperature variation by minus 3 degree (27 degree) was set for the analysis of variation in oven temperature by using triplicate observation of 10µg/ml of Naftopidil.

Table 10:

Oven temperature At 27 degree

Conc (µg/ml)	Area
10	1181042
10	1181376
10	1182883
Mean	1181767
SD	980.8063
%RSD	0.082995

At 33 degree

Conc (µg/ml)	Area
10	1292139
10	1290838
10	1290838
Mean	1291272
SD	751.1327
%RSD	0.05817

Table 11:

Change In Wavelength By minus 5nm (227 nm)

Conc (µg/ml)	Area
0	1284072
10	1289865
10	1289314
Mean	1287750
SD	3197.421
RSD	0.248295

By plus 5 nm (237 nm)

conc (µg/ml)	Area
10	1276197
10	1290807
10	1289314
Mean	1285439
SD	8038.831
%RSD	0.625376

Result: No significant differences in the results were obtained by making variations in the flow rate, wavelength and oven temperature within the established limits in the developed method. Thus the method was robust.

Table 12: Analyst 1

CONC(µg/ml)	AREA
10	1169882
10	1159335
10	1175767
MEAN	1168328
SD	8325.494
%RSD	0.712599

Analyst 2

CONC(µg/ml)	AREA
10	1189227
10	1186335
10	1190908
MEAN	1188823
SD	2313.07
%RSD	0.194568

ROGGEDNESS

Ruggedness was tested by keeping all the method conditions same but with different analysts i.e observations were taken by analyst 1 and then by analyst 2**.**

Table 12: Analyst 1

CONC(µg/ml)	AREA
10	1169882
10	1159335
10	1175767
MEAN	1168328
SD	8325.494
%RSD	0.712599

Analyst 2

CONC(µg/ml)	AREA
10	1189227
10	1186335
10	1190908
MEAN	1188823
SD	2313.07
%RSD	0.194568

Result: No significant differences in the results were obtained by taking observations by two different analysts. The % RSD value was within the limits (less than 2%), thus the method was rugged.

SUMMARY AND CONCLUSION

Table 13: Summary Table

S.No.	Parameters	Naftopidil
1.	Retention Time (min)	4.5
2.	Theoretical Plates	49039.312
3.	Asymmetry	1.238
4.	Wavelength	232nm
5.	Concentration range	0.5-10 µg/ml
6.	Regression equation y = mx + c	y = 104983x + 5935.8
7.	Correlation coefficient r^2	0.999
8.	Slope (m)	104983
9.	Intercept (c)	5935.8
10.	Oven temperature	30 degree
11.	Flow rate	1 ml/min

CONCLUSION

RP-HPLC methods were developed for Naftopidil as API. In RP-HPLC estimation methanol was used as solvent and measurement was done at 232 nm with mobile phase Pot.

Dihydrogen o-phosphate: ACN in ratio of 35:65 at flow rate of 1.0 ml/min. Linearity range was found to be 0.5-10 µg/ml.

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