

Development and validation of stability-indicating high-performance thin-layer chromatography method for estimation of Naftopidil in bulk and in pharmaceutical formulation

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ABSTRACT

A simple, selective, precise and Stability-indicating High-performance thin-layer chromatographic method for analysis of Naftopidil both in a bulk and in pharmaceutical formulation has been developed and validated. The method employed, HPTLC aluminium plates precoated with silica gel as the stationary phase. The solvent system consisted of hexane: ethyl acetate: glacial acetic acid (4: 4: 2 v/v). The system was found to give compact spot for Naftopidil (R_f value of 0.43 ± 0.02). Densitometric analysis of Naftopidil was carried out in the absorbance mode at 253 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.999 \pm 0.0001$ with respect to peak area in the concentration range 200 - 1200 ng per spot. The method was validated for precision, recovery and robustness. The limits of detection and quantification were 20.35 and 61.68 ng per spot, respectively. Naftopidil was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation and thermal conditions. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different R_f value. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of investigated drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of Naftopidil in bulk drug and pharmaceutical formulation. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Naftopidil;
HPTLC;
Validation;
Stability;
Degradation.

INTRODUCTION

Naftopidil is chemically known as chemically 1-[4-(2-methoxyphenyl)piperazin-1-yl]-3-(1-naphthyl)propan-2-ol. The molecular formula is $C_{24}H_{28}N_2O_3$. This corresponds to a molecular weight of 392.49 g/mol. It is used in the treatment of hyperten-

sion. Naftopidil exerts its antihypertensive action via alpha1 adrenoceptor blockage and Ca^{2+} antagonism in vascular smooth muscle. Naftopidil competitively inhibited specific [3H] prazosin binding in prostatic membranes of humans. Naftopidil was selective for the alpha 1d-adrenoceptor with approximately 3- and 17-fold higher affinity than for the alpha 1a- and alpha 1b-

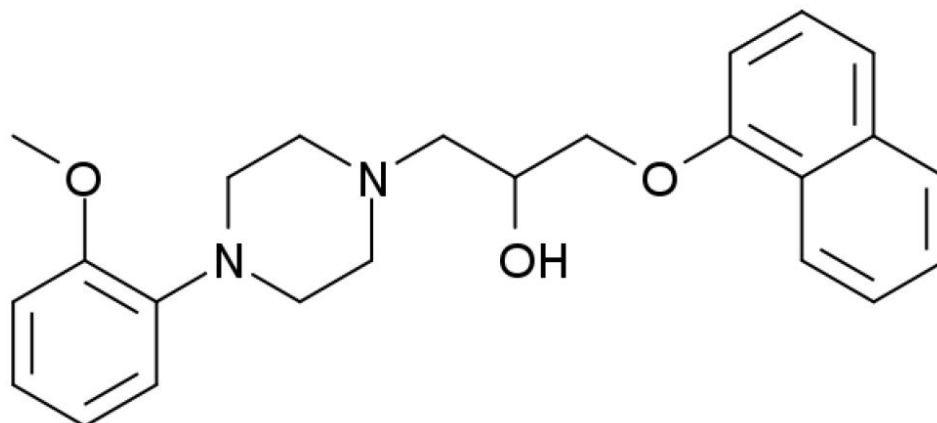


Figure 1 : Chemical structure of Naftopidil

adrenoceptor subtypes, respectively. In addition to the antagonistic action of this agent on the alpha 1 adrenergic receptors of prostatic smooth muscle naftopidil may also act on the lumbosacral cord and thus may improve collecting disorders in patients with benign prostatic hyperplasia^[1-2].

Literature survey revealed Several chromatographic methods including liquid chromatography–UV (LC–UV)^[3-6], LC – isotope dilution mass spectrometry^[7], and HPLC mass spectrometry (LCMS)^[8] have been developed to measure naftopidil in biological fluids. However to our knowledge no information related to the stability indicating high performance thin-layer chromatography (HPTLC) determination of Naftopidil in pharmaceutical dosage forms has ever been mentioned in literature. HPTLC is a widely used analytical technique due to its advantages of low operating cost, high sample throughput, and minimum sample preparation requirement. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus reducing the analysis time and cost per analysis^[9].

Hence, the objective of the present study was to develop a stability-indicating HPTLC method^[10] for estimation of Naftopidil as bulk drug and in formulations and to perform stress studies under a variety of ICH-recommended test conditions^[11]. The proposed method was validated for linearity, accuracy (recovery studies), specificity, precision, robustness, ruggedness, LOD (limit of detection), LOQ (limit of quantitation), and repeatability according to the ICH guidelines^[12,13] and its updated international convention^[14].

EXPERIMENTAL

Chemicals and reagents

Naftopidil was supplied as a gift sample from Cadila Health Care Ltd., Ahmedabad, India. All chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

HPTLC instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on pre-coated silica gel aluminium plates 60 RP-18 F₂₅₄ (10 × 10 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were prewashed with methanol and activated at 60 °C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of hexane: ethyl acetate: glacial acetic acid (4: 4: 2 v/v), and 10 ml of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2). The length of the chromatogram run was approximately 8 cm. Subsequent to development; the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WINCats software.

Preparation of standard solution and linearity study

An accurately weighed quantity of 10 mg Naftopidil was transferred to 10 ml volumetric flask, dissolved in methanol and volume was made up to mark with the same solvent to obtain concentration 1000 ng/ml. Aliquots of standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µl of Naftopidil was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtain the concentration of 200, 400, 600, 800, 1000 and 1200 ng per spot. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated six times.

Method validation

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng per spot of Naftopidil). The intra and inter-day variation for the determination of Naftopidil was carried out at three different concentration levels of 400, 600 and 800 ng per spot.

Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine detection and quantification limit, Naftopidil concentrations in the lower part of the linear range of the calibration curve were used. Naftopidil solutions of 200, 240, 280, 320, 360 and 400 ng/spot were prepared and applied in triplicate. The LOQ and LOD were calculated using equation $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$, where, N is standard deviation of the peak areas of the drugs ($n=3$), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for Naftopidil in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of Naftopidil was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Ruggedness

Ruggedness of the method was performed by spot-

ting 600 ng of Naftopidil by two different analyst keeping same experimental and environmental conditions.

Accuracy

The analysed samples were spiked with extra 80, 100 and 120% of the standard Naftopidil and the mixture were analysed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

Robustness

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of hexane-ethyl-acetate-glacial acetic acid (3:5:3.5:1.5 and 4.5:4.5:2.5, v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of $\pm 5\%$. The plates were prewashed by methanol and activated at $60 \pm 5^\circ\text{C}$ for 8, 10 and 12 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20 and 40 min.

Application of proposed method to tablet formulation

To determine the concentration of Naftopidil in tablets (labeled claim: 25 mg per tablet), the contents of ten tablets were weighed, their mean weight determined and they were finely powdered. The powder equivalent to 25mg of Naftopidil was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 20 min and the volume was made up to 100 ml. The resulting solution was filtered using 0.41 µm filter (Millifilter, Milford, MA). The above solution (600 ng per spot) was applied on TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate.

Forced degradation of Naftopidil

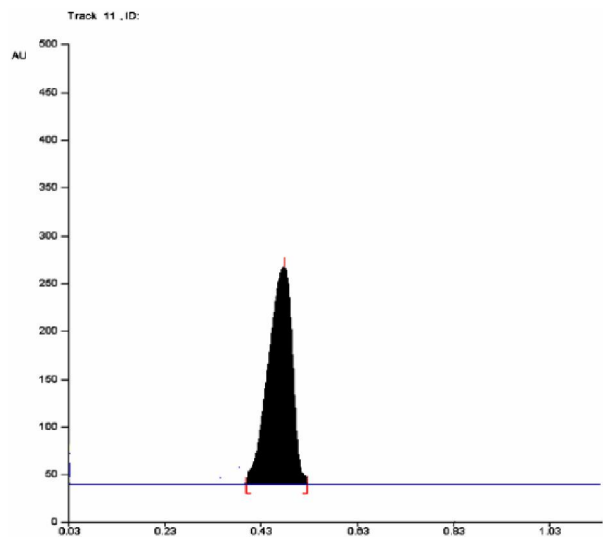
Acid and base induced degradation

The 10 mg of Naftopidil was separately dissolved in 10 ml of methanolic solution of 0.5M HCl and 0.5M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1ml of above solutions

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TABLE 1 : Linear regression data for the calibration curves^a

Linearity range (ng per spot)	200 -1200
R ² ± S.D.	0.9992 ± 0.197
Slope ± S.D	6.8177 ± 0.197
Intercept ± S.D	33.362 ± 0.197

^a n = 3.

Figure 2: A typical HPTLC chromatogram of Naftopidil ($R_f = 0.43$) in hexane: ethyl acetate: glacial acetic acid (4:4:2 v/v) at 253 nm

was taken and neutralized, then diluted up to 10 ml with methanol. The resultant solution were applied on TLC plate in triplicate (2 μ l each, i.e. 200 ng per spot). The chromatograms were run as described in Section 2.2.

Hydrogen peroxide-induced degradation

The 10 mg of Naftopidil was separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (3.0%, v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on TLC plate in triplicate (2 μ L, i.e. 200 ng per band). The chromatograms were run as described in Section 2.2.

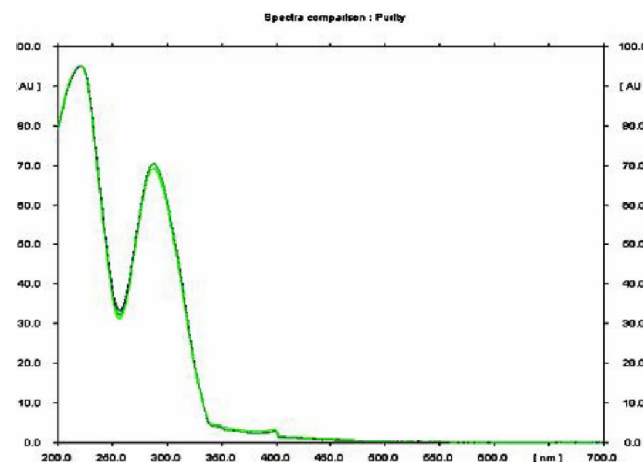
Photochemical degradation

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 8 h. The resultant solution (2 μ L, i.e. 200 ng per band) was applied on a TLC plate and chromatograms were run as described in section 2.2.

TABLE 2 : Intra-day and inter-day precision of HPTLC method

Drugs	Conc. ng/spot	Intra-day		Inter-day	
		S.D.	% R.S. D.	S.D.	% R.S.D
NAFTO	400	7.848	0.28	7.099	0.25
	600	12.020	0.30	5.179	0.13
	800	6.434	0.11	11.77	0.21

* mean of three estimations


Figure 3 : A typical overlain spectrum of standard drug and drug extracted from tablet.

Dry heat degradation product

The powdered drug was stored at 60°C for 8 h under dry heat condition showed significant degradation showed additional peak at R_f value of 0.26 & 0.79. In all degradation studies, the average peak areas of Naftopidil after application (2 μ L, i.e. 200 ng per band) of three replicates were obtained.

RESULTS AND DISCUSSION

Development of optimum mobile phase

TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, hexane: ethylacetate (2.5: 2.5v/v) gave good resolution with R_f value of 0.50 for Naftopidil but typical peak nature was missing. Finally, the mobile phase consisting of Hexane: ethylacetate: glacial acetic acid (4:4:2 v/v) gave a sharp and well defined peak at R_f value of 0.43 (Figure 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Calibration curve

The linear regression data for the calibration curves

TABLE 3 : Recovery study

Drug	Label claim (mg/tablet)	Amount of Standard drug added (%)	Drug Recovered	% R.S.D.
		0	100.02	0.52
		80	100.79	0.014
NAFTO	25	100	100.22	0.15
		120	99.70	0.19

* mean of three estimations at each level

TABLE 4 : Robustness of the method ^a

Parameter	S.D. of peak area	% R.S.D.
Mobile phase composition	33.8	0.82
Mobile phase volume	48.47	1.18
Development distance	39.02	0.97
Activation of TLC plate	12.17	0.29
Duration of saturation	22.14	0.54
Time from spotting to chromatography	46.09	1.12
Time from chromatography to scanning	47.47	1.15

^a n=6

TABLE 5 : Summary of validation parameter

Parameter Data	NAFTO
Linearity range (ng per spot)	200 – 1200
Correlation coefficient	0.9994
Limit of detection (ng per spot)	20.35
Limit of quantification (ng per spot)	61.68
Recovery (n = 6)	100.24
Ruggedness(% R.S.D)	
Analyst-I(n=6)	0.63
Analyst-II(n=6)	1.29
Precision (% R.S.D.)	
Repeatability of application (n = 6)	0.69 - 0.72
Inter-day (n = 6)	0.23 – 0.26
Intra-day (n = 6)	0.25 – 0.29
Robustness	Robust
Specificity	Specific

showed good linear relationship over the concentration range 200-1200 ng/spot.

Validation of method

Precision

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% R.S.D.). The results depicted revealed high precision of the method is presented in TABLE 2.

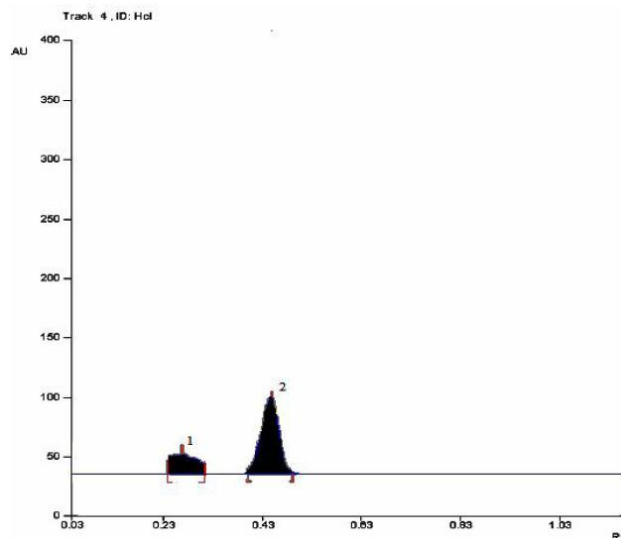


Figure 4 : HPTLC chromatogram of acid (0.5 N HCl, 8h, R.T.) treated Naftopidil; peak1 (impurity) (R_f : 0.25), peak2 (Naftopidil) (R_f : 0.43).

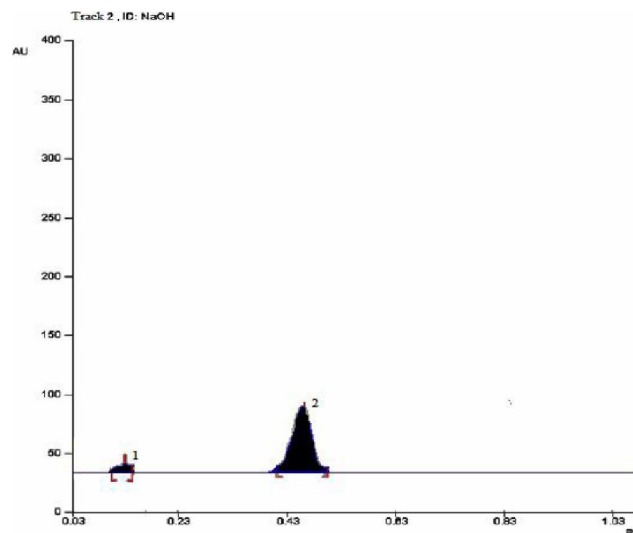


Figure 5 : HPTLC chromatogram of base (0.5N NaOH, 8h, R.T.) treated Naftopidil; peak1 (impurity) (R_f : 0.15), peak2 Naftopidil (R_f : 0.43)

LOD and LOQ

Detection limit and quantification limit was calculated by the method as described in section 2.4.2. The LOQ and LOD were found to be 20.35 and 61.68ng respectively. This indicates that adequate sensitivity of the method.

Recovery studies

The proposed method when used for extraction and subsequent estimation of Naftopidil from the pharmaceutical dosage form after over spotting with 80, 100

TABLE 6 : Degradation of Naftopidil

Sr no.	Sample exposure condition	No. of degradation product (R_f)	Naftopidil remained (ng/1500ng) (\pm SD, n=3)	Recovery (%)
1	1 M HCl, 8h, RT	1 (0.25)	191.3	95.65
2	1 M NaOH, 8h, RT	1(0.15)	188.86	94.43
3	3% H_2O_2 , 8 h, RT	2 (0.16,0.79)	162.02	81.01
4	Heat, 3h, RT	2(0.26,0.79)	185.02	96.46
5	Photo, 8h	1(0.81)	192.92	92.56

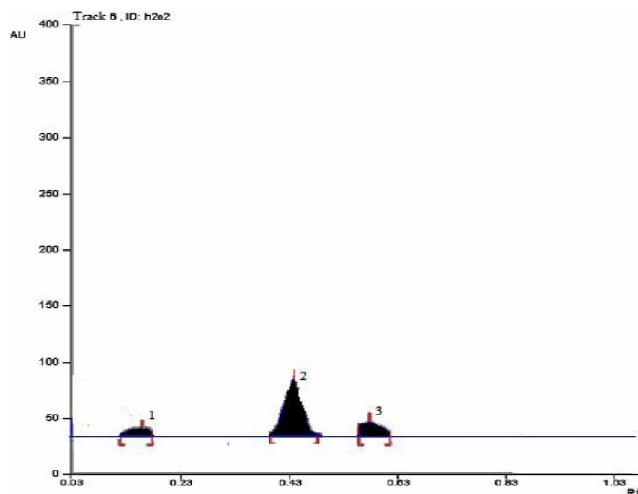


Figure 6 : HPTLC chromatogram of hydrogen peroxide (3% w/v, 8 h, R.T.) treated Naftopidil; peak1 (impurity) (R_f : 0.16), peak 3 (impurity) (R_f : 0.51), peak 2 (Naftopidil) (R_f : 0.43).

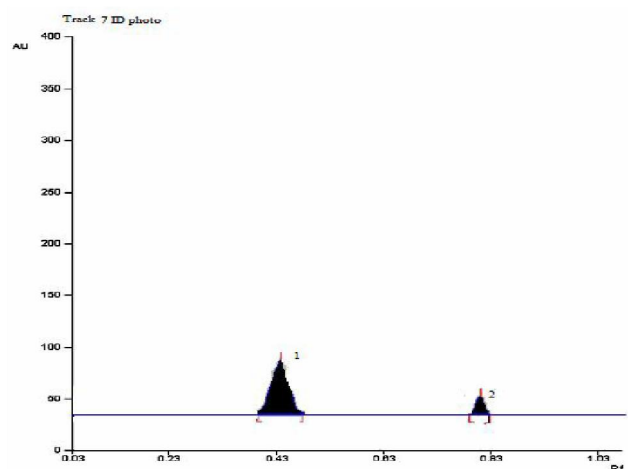


Figure 7 : HPTLC chromatogram of photo degraded (8 h) treated Naftopidil; peak 1 (Naftopidil) (R_f : 0.43), peak 2 (impurity) (R_f : 0.81)

and 120 % of additional drug; afforded good recovery of Naftopidil. The amounts of drug added and determined and the % recovery are listed in TABLE 4.

Specificity

The peak purity of Naftopidil was assessed by comparing the spectra at peak start, peak apex and peak

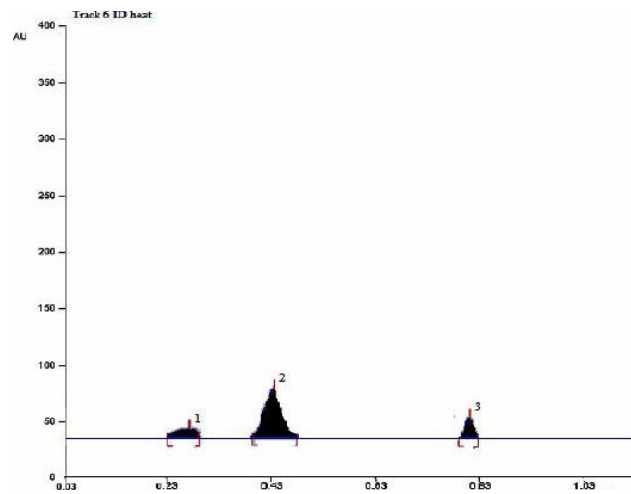


Figure 8 : HPTLC chromatogram of heat degraded (8 h) treated Naftopidil; peak 1 (impurity) (R_f : 0.26), peak 2 (Naftopidil) (R_f : 0.43), peak 3 (impurity) (R_f : 0.79)

end positions of the spot, i.e., $r_2(S, M) = 0.9998$ and $r_2(M, E) = 0.9988$. Good correlation ($r^2 = 0.9989$) was also obtained between standard and sample spectra of Naftopidil (Figure 3).

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low values of %R.S.D. values as indicated are shown in TABLE 3 indicated robustness of the method.

Analysis of the marketed formulation

A single spot at R_f 0.43 was observed in the chromatogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablet. The % drug content and % RSD were calculated. The low % RSD value indicated the suitability of this method for the routine analysis of Naftopidil in pharmaceutical dosage forms.

Force degradation

The chromatogram of the acid degraded samples

for Naftopidil showed additional peak at R_f value of 0.25 (Figure 4), base degraded drug shows at 0.15 (Figure 5), hydrogen peroxide shows at 0.16 and 0.51 (Figure 6) respectively. The chromatograms of photo-degraded drugs shows at 0.81 (Figure 7) and dry heat degraded drug shows at 0.26 and 0.79 (Figure 8) samples of Naftopidil showed only the spots of the pure drug. The spot of the degraded product was well resolved from the Naftopidil spot. In both cases, the concentration of the drug was changing from the initial concentration, indicating that Naftopidil undergoes degradation under acidic, basic and oxidative conditions.. The lower R_f values of degraded components indicated that they were less polar than the analyte itself. The results are listed in TABLE 5.

CONCLUSION

The developed HPTLC method was precise, specific, accurate and stability indicating and validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of Naftopidil as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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