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Original Article

Stability Indicating Planar Chromatographic method for Estimation of Minoxidil and Finasteride Combination used in the treatment of Hair loss

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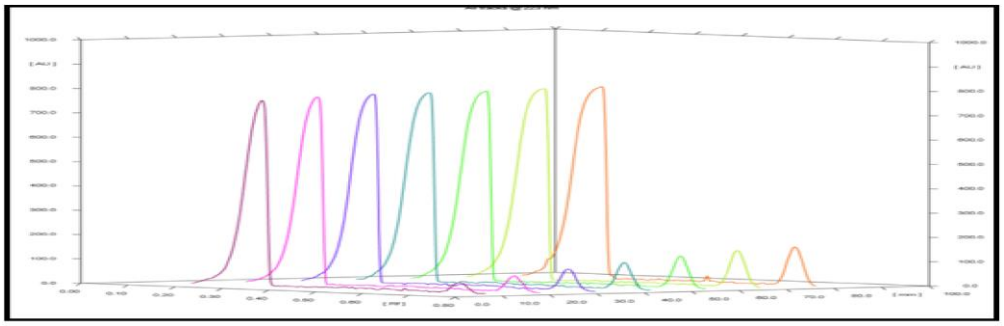
Minoxidil
Stability indicating method
Validation

ABSTRACT

Alopecia areata is a typical non-scarring alopecia that happens in 0.1-0.2% of everybody and records for 0.7-3% of all cases found in dermatology practice. A vital quantitative examination of Minoxidil and Finasteride was created in Bulk and pharmaceutical measurement structure with precise, and sensitive stability indicating high performance thin layer chromatographic (HPTLC) technique. The chromatographic improvement was done on the HPTLC plates precoated with silica gel 60 F_{254} utilizing a mobile phase of n-butanol: TEA (10:0.1v/v) as versatile stage. Detection was completed densitometrically at 223 nm. The R_f estimation of medication was seen as 0.22 ± 0.24 for Minoxidil and 0.63 ± 0.65 for Finasteride. The technique was approved according to the ICH rule concerning linearity, accuracy, precision and robustness. The calibration curve was seen as straight over a scope of 6000-24000 ng/band for Minoxidil and 200-800 ng/band for Finasteride with a regression coefficient of 0.9972 For Minoxidil and 0.998 for finasteride. The % recovery was found 98-101.92% for Minoxidil and 98.42-101.68% for Finasteride. Forced degradation studies like acid hydrolysis, base hydrolysis, oxidation, dry heat and photolytic degradation were performed. The degradation products obtained were well resolved from the pure drugs with significantly different R_f values. The proposed technique could viably isolate the medications from its degradation products just as from excipients. The proposed TLC technique has expected subjective just as quantitative applications for synchronous estimation of minoxidil and finasteride in bulk and pharmaceutical dosage form.

GRAPHICAL ABSTRACT



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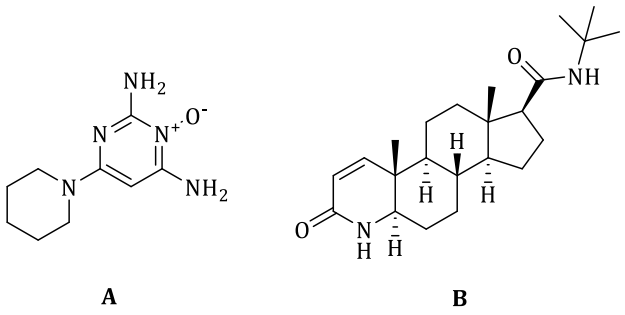
Introduction

Minoxidil and finasteride is anti-alopecia drug which is used for hair growth in men. Where minoxidil is a peripheral vasodilator that directly relaxes the vascular smooth musculature, lowering the systolic and diastolic pressure and its action is linked to activation of calcium channels and also use for hair growth in men. For the finasteride, it is an antiandrogen agent (male hormone) that was developed as a drug for prostatitis. By inhibiting the enzyme 5α-reductase, finasteride blocks male hormone testosterone from transforming into the dihydrotestosterone (DHT), a hormone that shows a much stronger activity than testosterone [1]. Chemically Minoxidil is 6-piperidin-1-ylpyrimidine-2, 4-diamine 3-oxide (Figure 1) and finasteride is 17β-(*N-tert*-butyl carbamoyl)-4-aza-5*α*-androst-1-en-3-one (Figure 1). The molecular formula and molecular weight of Minoxidil is $C_9H_{15}N_5O$ and 209.25 g/mol and for Finasteride $C_{23}H_{36}N_2O_2$ and 372.5 g/mol respectively. Minoxidil freely soluble in methanol and insoluble in water, where finasteride freely soluble in chloroform, methanol and insoluble in water [2-3].



A **B**

Figure 1: Chemical structure of (A) Minoxidil (B) Finasteride

The analytical method such as UV spectrophotometric and high performance liquid chromatography (HPLC) has been reported for the minoxidil and finasteride in the bulk and marketed formulation, [4-8] however there is no high performance thin layer chromatography (HPTLC) till date. Advantages of HPTLC are that it is capable to identify sample using small amount of mobile phase, it minimize time of work and cost of analysis, also help for identification and better separation in herbal formulation. Therefore the HPTLC method was developed with stability study [9]. The propose method was validated according to the ICH guideline, research article of stability indicating method and its updated international convention [10-11].

Material and methods

HPTLC Instrument

Sample application was carried out with the help of Hamilton syringe 100µL syringe which give 6 mm bands width using Camag Linomat 5 (Switzerland) sample applicator on pre-coated silica gel aluminium plate 60 F_{254}, (10 cm x 10 cm with 0.2 mm thickness, E. Merck, Germany). Camag TLC scanner is used for the densitometry scanning of the develop chromatogram.

Chemical and Reagents

Analytically pure active pharmaceutical ingredients, Minoxidil and Finasteride were obtained as a gift samples from Centurion Remedies Pvt Ltd., Vadodara, Gujarat, India. HPLC grade methanol was acquired from the SRL Diagnostic Ltd, Mumbai. AR grade n-butanol was obtained from Astron chemical Pvt. Ltd, Ahmedabad, Gujarat, India.

Chromatographic Condition

Sample Application

Standards and synthetic mixture samples of Minoxidil and Finasteride was applied on the HPTLC plates in the form of narrow bands of 6 mm length, 10 mm from the bottom and left edge, and with 9 mm space between two bands. Samples application was carried out under a continuous drying stream of nitrogen gas.

Mobile phase development

Plates were developed using a mobile phase consisting of n- butanol: TEA (10:0.1 v/v). Linear ascending development was carried out in a twin-trough glass chamber equilibrated through chamber saturation with the mobile phase vapour for 40 min at 25 °C ± 2 °C. 10 mL of the

mobile phase (5 mL in the trough containing the plate and 5 mL in the other trough) was used for each development and was allowed to migrate a distance of 80 mm. Once development was over, the HPTLC plates were dried completely.

Densitometry analysis

Densitometric scanning was performed in the absorbance mode under control by winCATS planar chromatography software. The source of radiation was the deuterium lamp, and bands were scanned at λmax223 nm. The slit dimensions were 5 mm length and 0.45 mm width, with a scanning rate of 20 mm/s. Concentrations of the compound were determined from densitometric the intensity of diffusely reflected light and evaluated as peak areas against concentrations by using a linear regression equation.

3.1 Preparation of standard stock solution

Stock solutions were prepared by accurately weighing 300 mg of Minoxidil, transferring into 10 mL volumetric flask containing 2.0 mL of methanol. The flask was swirled to dissolve the solids and volume was made up to the mark with methanol to yield a solution containing 30000 μg/mL of Minoxidil. For the finasteride 10 mg weigh and transferring into 10 mL volumetric flask containing few mL of methanol. The flask was swirled to dissolve the solids and volume was made up to the mark with methanol to yield a solution containing 1000 μg/mL of Finasteride. Form the above solution 1 mL aliquots take and transfer into 10 mL volumetric flask and make up with methanol which give 3000 μg/mL for minoxidil and 100 μg/mL for the finasteride.

Validation

Validation was carried out for optimized HPTLC method with respect to following parameters:

a) Linearity

Calibration curve were constructed by plotting peak area versus concentration of Minoxidil and Finasteride and the regression equation were calculated. The calibration curves were plotted over 7 different concentration range in the range of Minoxidil (6000-24000 ng/band) and Finasteride (200-800 ng/band) the calibration curve were developed by plotting peak area versus concentration (n = 5).

b) Accuracy

The accuracy of the method was determined by calculating % recoveries of Minoxidil and finasteride at 0, 7200, 9000, 10800 ng/band and 0, 240, 300, 360 ng/band respectively.

The amount of minoxidil and finasteride was estimated by measuring the peak area and by applying these values to the calibration equation. Each concentration applied on TLC plate 3 times on precoated TLC plate F_{254} and mean of area is taken to determine the recovery percentage

c) Precision

Precision was evaluated in terms of intraday, interday and repeatability precisions. For the interday study sample solution of Minoxidil and finasteride were prepared in low, medium and high concentrations 9000, 15000, 21000 ng/band for Minoxidil and 300, 500, 700 ng/band for Finasteride. The solutions were prepared and analyzed for three different days and the results were evaluated. Similarly, for the intraday study three solutions of different concentrations were analysed three times on the same day. The peak areas obtained were used to calculate the mean and RSD values.

Repeatability of measurement of peak area was determined by analysing Minoxidil 15000 ng/band and Finasteride 500 ng/band sample six times without changing the position of plate and by application of same concentration band seven times on TLC plate with use of Hamilton syringe to check preciseness of syringe.

d) Sensitivity

Sensitivity of the method was determined with respect to LOD and LOQ. Noise was determined by scanning a blank band (methanol) six times. A series of concentrations of drug solutions for

Minoxidil 6000–24000 ng/band and Finasteride 200-800 ng/band was applied on a plate and analyzed to determine LOD and LOQ. LOD was calculated as 3 times the noise level, and LOQ was calculated as 10 times the noise level. LOD and LOQ were experimentally verified by diluting the known concentrations of Minoxidil and Finasteride.

e) Robustness

Deliberate changes in proposed method parameter like chamber saturation time, grade of mobile phase and detection wavelength were introduced and the effects on the results were examined. Robustness of the method was determined in triplicate at a concentration level of 15000 ng/band for Minoxidil and 500 ng/band for Finasteride.

f) Analysis of marketed formulation

10 mL solution equivalent to 300 mg of Minoxidil and 10 mg finasteride was transfer to 25 mL volumetric flask make up the volume with Methanol so final concentration was 12000 µg/mL for minoxidil and 400 µg/mL for finasteride. Pipette out 2.5 mL aliquot from above solution and transfer in another 10 mL volumetric flask and make up with methanol, the final concentration of minoxidil get 3000 µg/mL and for finasteride 100 µg/mL. From the above solution 5 µl was applied on TLC plates and analysed. From the developed chromatogram peak area and R_f value and % amount of drug was calculated.

g) Forced degradation study

Stress degradation study using acid and alkali hydrolysis, chemical oxidation, dry heat and photolytic degradation were carried out and interference of the degradation products was investigated. Minoxidil and Finasteride was weighed 10 mg and transferred to 10 mL volumetric flasks and expose to different stress conditions, as demonstrated in Table 1.

Table 1: Stress condition given to both drugs for force degradation study

Sr.No.	Stress Type	Stress Condition
1	Acid hydrolysis	2mL 0.01 M HCl at 60-70 °C for 30 min, neutralized with 0.01M NaOH
2	Alkali hydrolysis	2 mL 0.01 M NaOH at 60-70 °C for 20 min, neutralized with 0.01M HCl
3	Oxidative	3% v/v H_2O_2 at 60-70 °C for 30 min
4	Thermal	60-70 °C for 90 min
5	Photolytic	exposed to UV light for 24 hrs

Result and Dissection

Selection of mobile phase

For detachment of Minoxidil and Finasteride, number of polar and non-polar natural dissolvable have been gone after for mobile phase composition utilizing silica gel GF_{254} as fixed stage. *n*-butanol (10 mL) with 0.1 mL TEA were discovered successful dissolvable for better detachment of two medications with conservative spot. The two medications are dissolvable in methanol henceforth methanol was considered as noteworthy dissolvable for mobile phase composition for additional enhancement.

Selection of detection of wavelength

The solution was applied in the form of a band in concentration of 15000 ng/band of Minoxidil and 500 ng/band of Finasteride were prepared in Methanol. The plate was developed using *n*-butanol: TEA (10:0.1 v/v) and dried in air. It was subjected to Densitometric measurement in scanning mode in UV region of 200-400 nm, and the Overlay spectra was showed that both drugs give good absorbance at 223 nm. Therefore, it was selected as detection wavelength

Validation

Linearity and Range

The method was found to be linear over the range of 6000-24000 ng/band for Minoxidil and 200-

800 ng/band for Finasteride. The three dimensional overlay densitogram of calibration curve are shown in Figure 2. Densitogram of Minoxidil (9000 ng/band) and finasteride (300 ng/band) as shown in Figure 3. The calibration curve for Minoxidil and Finasteride was prepared as shown in Figures 4 and 5. Linearity data of minoxidil and finasteride were shown in Table 2.

Table 2: Linearity data of Minoxidil and Finasteride

Sr No.	Minoxidil			Finasteride		
	Conc. (ng/band)	Peak Area (AU) +/-SD (n=5)	% RSD	Conc. (ng/band)	Peak Area(AU)(n=5) +/-SD	% RSD
1	6000	19721.4+/- 245.68	1.24	200	595.4 +/- 15.76	2.64
2	9000	22274.4+/- 258.20	1.15	300	900+/-7.61	0.84
3	12000	24916.8+/- 575.99	2.31	400	1175+/-28.28	2.40
4	15000	27979.8+/- 410.63	1.46	500	1522+/- 27.87	1.83
5	18000	30558.4+/- 356.28	1.16	600	1814.8+/-32.87	1.81
6	21000	32768+/- 289.90	0.88	700	2205.6+/-17.96	0.81
7	24000	35245.6+/- 428.16	1.21	800	2538+/-44.21	1.74

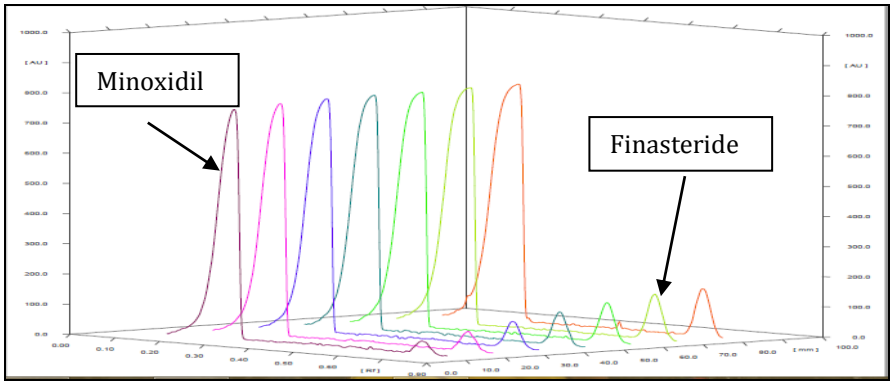


Figure 2: Calibration curve of Minoxidil (6000-24000 ng/band) and Finasteride (200-800 ng/band)

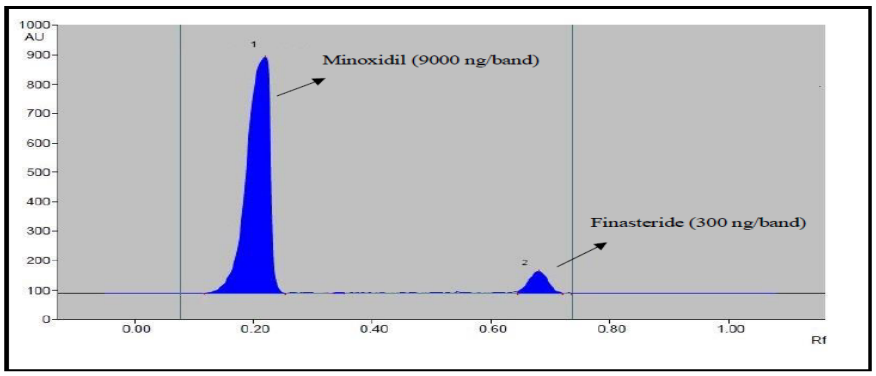


Figure 3: Densitogram of Minoxidil (9000 ng/band) and Finasteride (300 ng/band)

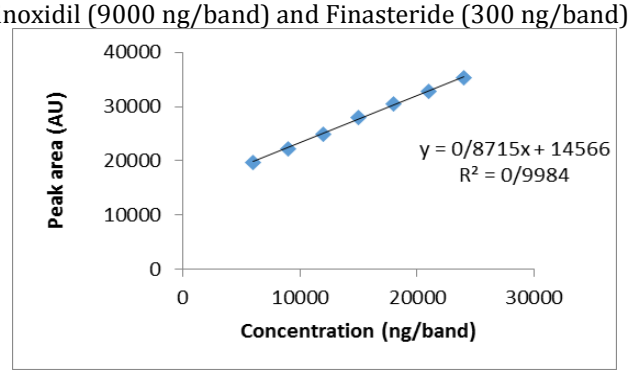


Figure 4: Calibration Curve of Minoxidil at 223 nm

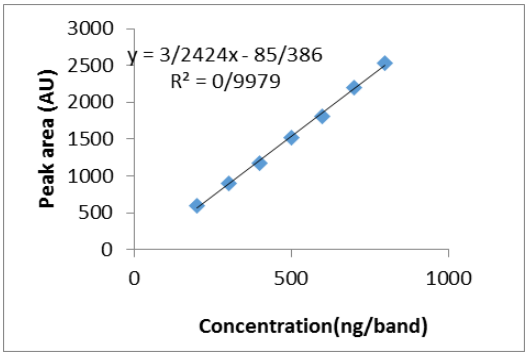


Figure 5: Calibration Curve of Finasteride at 223 nm

Accuracy

Accuracy was determination by calculating the recovery. The method was found to be accurate with % recovery 98-101.92% for Minoxidil and 98.42-101.68% for Finasteride as shown in Table 3.

Table 3: Accuracy study of Minoxidil and Finasteride (n=3)

Minoxidil				Finasteride			
Conc. (ng/band)	% of std. drug added	Amount Found (ng/band)	% recovery (mean+/- SD)	Conc. (ng/band)	% of std. drug added	Amount Found (ng/band)	% recovery (mean +/- SD)
9000	0	9008.10	100.08+/- 1.13	300	0	300.92	100.30+/-0.88
	80	16200.74	100.00+/- 0.66		80	540.45	100.59+/-1.03
	100	17971.84	99.68+/- 1.22		100	597.1	99.00+/-0.37
	120	19684.03	98.71+/- 0.41		120	656.10	98.70+/-0.22

Precision

a) Repeatability:-Repeatability of scanning device and injection was studied by applying and analyzing Minoxidil (15000 ng/band) and Finasteride (500 ng/band) six time. The % RSD value obtained were less than 2%
b) Interday and Intraday:- The method was found to be precise with % RSD less than 2%

Limit of Detection and Limit of Quantification

Detection limit and quantification limit was calculated by the method as described in sensitivity. The LOQ and LOD for Minoxidil were 3772.67ng and 1244.98 ng and for Finasteride, LOQ and LOD were found to be 67.04ng and 22.12ng. This indicates adequate sensitivity of the method.

Robustness

The % RSD Value less than 2% were obtained after introducing small, deliberate changes in parameters of the developed HPTLC method, confirming its robustness show in Table 4.

Table 4: Robustness Data

			Minoxidil		Finasteride	
Parameters	Normal Condition	Deliberate Change	R_f value+/-SD (n=3)	% RSD	R_f value+/-SD (n=3)	% RSD
Chamber saturation time(min)	40	35	0.20+/- 0.01	2.28	0.63+/- 0.01	1.96
		45	0.22+/- 0.01	2.11	0.64+/- 0.02	2.62
Wavelength (nm)	223	221	0.21+/- 0.01	2.17	0.62+/- 0.01	2.00
		225	0.23+/- 0.01	2.02	0.65+/- 0.01	1.89
Mobile Phase grade	AR grade	HPLC grade	0.22+/- 0.01	2.11	0.64+/- 0.01	1.92

Analysis of marketed formulation

The marketed Formulation was analyzed by developed method. The percentage amount of drug was found to be less than 98% for the both drugs. Graph of marketed formulation was show in Figure 6.

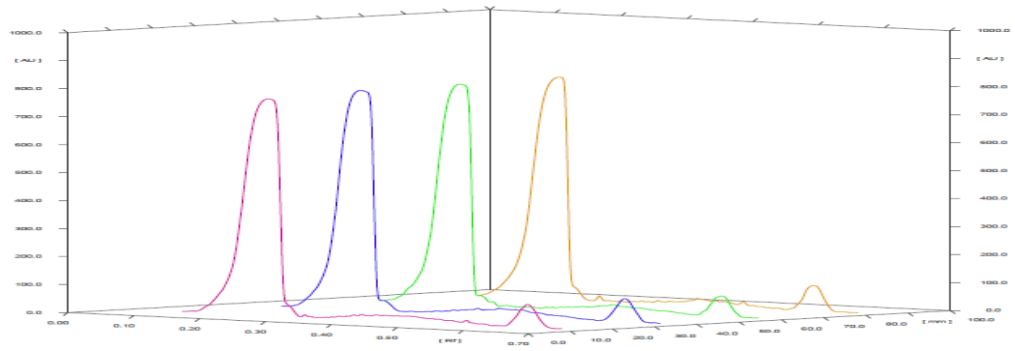


Figure 6: Analysis of marketed formulation with Standard

Forced degradation study

Acid Hydrolysis: Densitogram for acid degradation of standard and Mixture are showed in Figures 7-9.

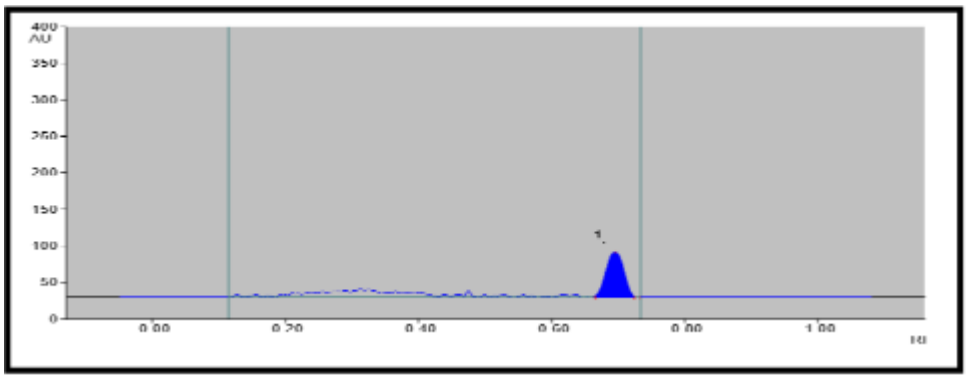


Figure 7: Densitogram of Minoxidil (500 ng/band) treated with 0.01 M HCl under reflux at 60-70 °C for 30 min

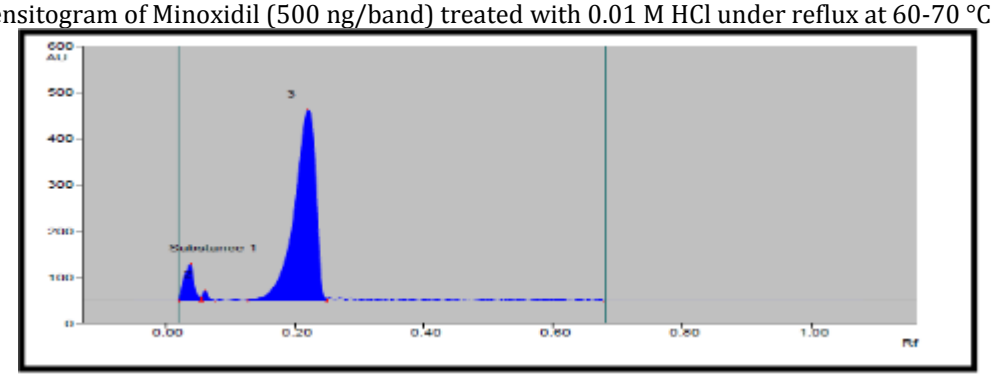


Figure 8: Densitogram of Finasteride (500 ng/band) treated with 0.01 M HCl under reflux at 60-70 °C for 30 min

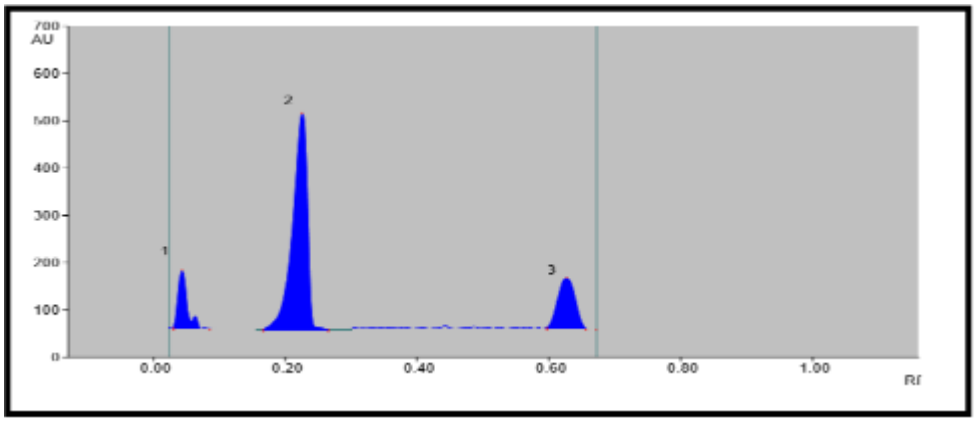


Figure 9: Densitogram of Mixture (500 ng/band) treated with 0.01 M HCl under reflux at 60-70 °C for 30 min

a) *Alkali Hydrolysis:* Densitogram for alkali degradation of standard and Mixture are showed in Figures 10-12.

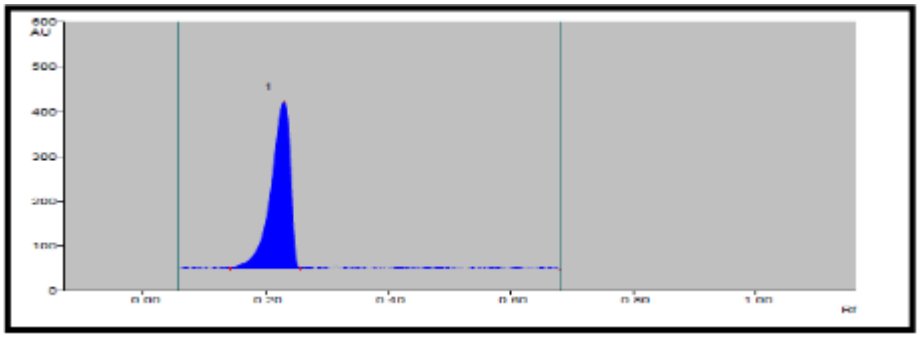


Figure 10. Densitogram of Minoxidil (500 ng/band) treated with 0.01 M NaOH under reflux at 60-70 °C for 20 min

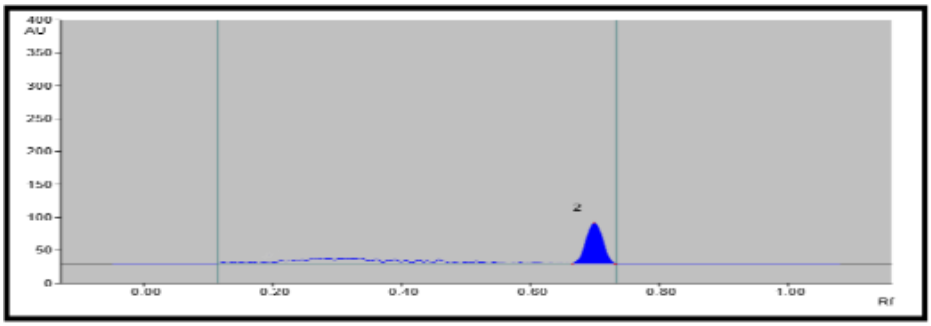


Figure 11: Densitogram of Finasteride (500 ng/band) treated with 0.01 M NaOH under reflux at 60-70 °C for 20 min

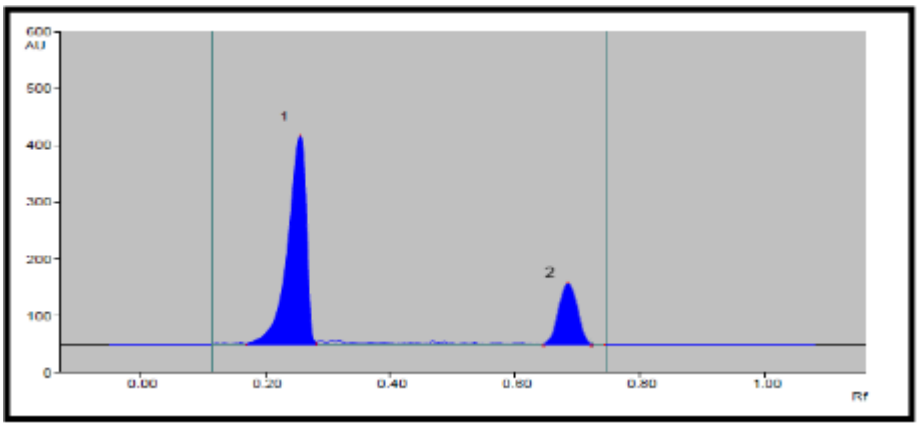


Figure 12: Densitogram of Mixture (500 ng/band) treated with 0.01 M NaOH under reflux at 60-70 °C for 20 min

b) *Photolytic study:* Densitogram for acid degradation of standard and Mixture are showed in Figures 13-15.

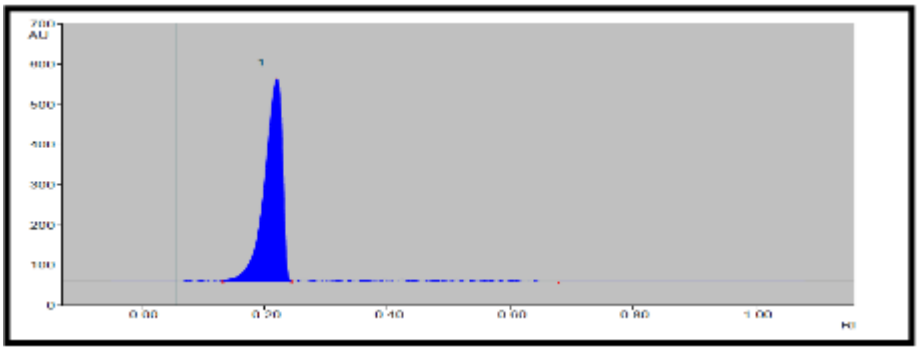


Figure 13: Densitogram of Minoxidil (500 ng/band) treated with UV light for 24 hr

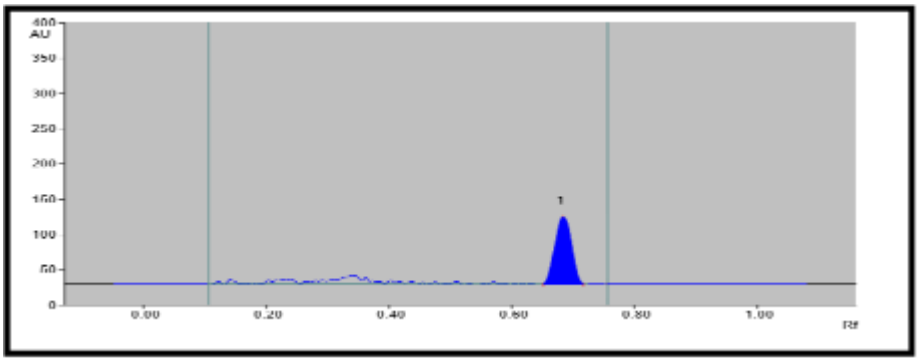


Figure 14: Densitogram of Finasteride (500 ng/band) treated with UV light for 24 hr

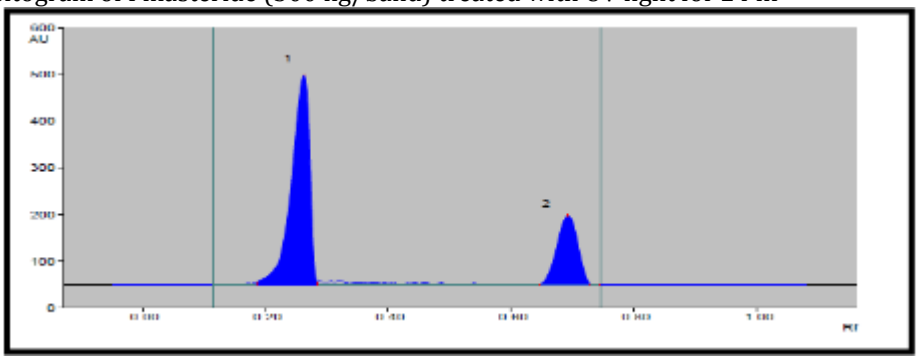


Figure 15: Densitogram of Mixture (500 ng/band) treated with UV light for 24 hr

c) ***Oxidative stress study:*** Densitogram for acid degradation of standard and Mixture are showed in Figures 16-18.

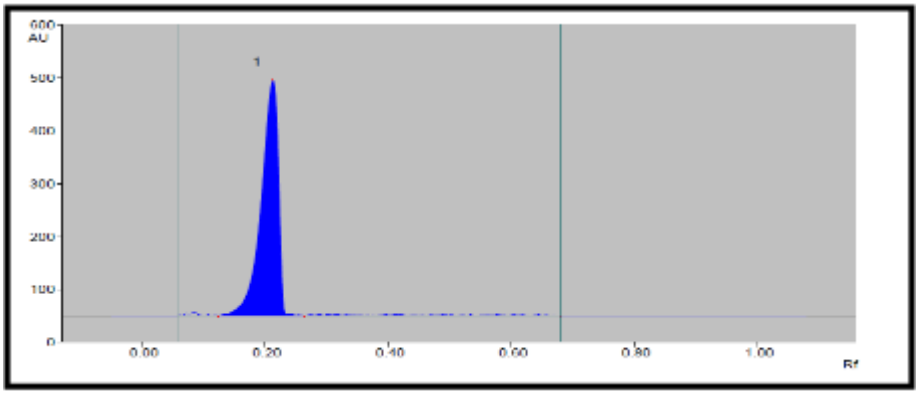
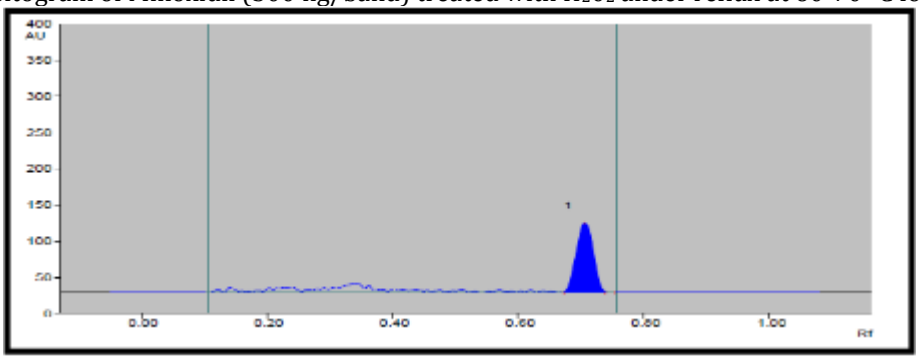


Figure 16: Densitogram of Minoxidil (500 ng/band) treated with H_2O_2 under reflux at 60-70 °C for 30 min



Figurre 17: Densitogram of Finasteride (500 ng/band) treated with H_2O_2 under reflux at 60-70 °C for 30 min

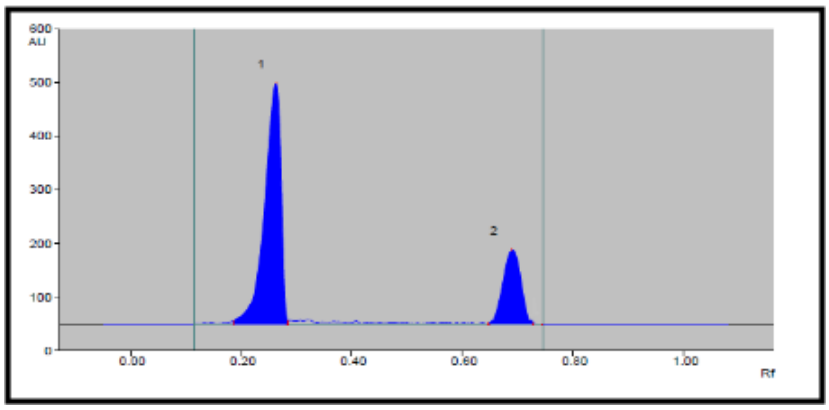


Figure 18: Densitogram of Mixture (500 ng/band) treated with H_2O_2 under reflux at 60-70 °C for 30 min

d) ***Dry heat study:*** Densitogram for acid degradation of standard and Mixture are illustrated in Figure 19-21.

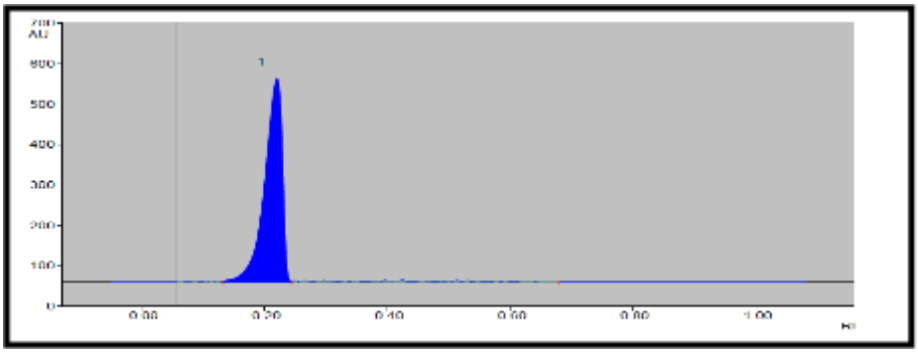


Figure 19: Densitogram of Minoxidil (500 ng/band) treated with Dry heat for 90 min at 60±5 °C

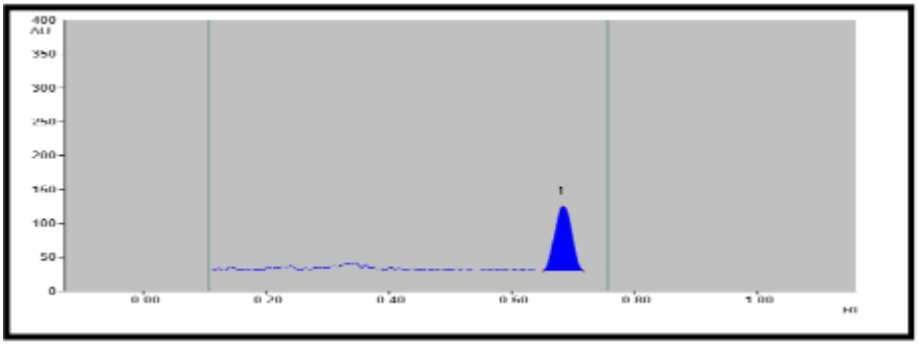


Figure 20: Densitogram of Finasteride (500 ng/band) treated with Dry heat for 30 min. at 60±5 °C

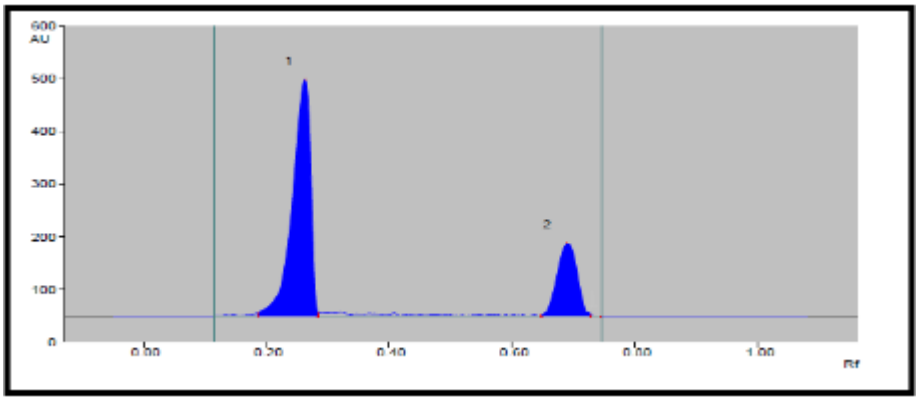


Figure 21: Densitogram of Mixture (500 ng/band) treated with Dry heatat 60±5 °C

From the force degradation study both Minoxidil and finasteride were highly unstable in alkaline hydrolysis stress condition as shown in Table 5.

Table 5: Summary of Forced degradation study of Minoxidil and Finasteride

Sr. No.	Type of degradation	Optimized Condition	Matrix		% recovery	
1	**Acid Hydrolysis**	0.01 N HCl at 60-70°C for 30 min	Minoxidil		90 %	
			Finasteride		85%	
			Minoxidil	Finasteride	92%	84%
2	**Alkaline Hydrolysis**	0.01 N NaOH at 60-70°C for 30 min	Minoxidil		81%	
			Finasteride		82%	
			Minoxidil	Finasteride	82%	83%
3	**Oxidative**	3% v/v H_2O_2 at 60-70°C for 30 min	Minoxidil		84%	
			Finasteride		92%	
			Minoxidil	Finasteride	84%	91%
4	**UV–Visible Light**	UV light 24 hr	Minoxidil		96%	
			Finasteride		92%	
			Minoxidil	Finasteride	95%	92%
5	**Dry Heat**	60-70°C for 90 min	Minoxidil		93%	
			Finasteride		86%	
			Minoxidil	Finasteride	91%	85%

The proposed HPTLC method has well potential qualitative and quantitative application for simultaneous estimation of Minoxidil and Finasteride in bulk and Pharmaceutical dosage form with very good accuracy, precision, repeatability and robustness of method. It showed that both drugs are least stable in alkali condition as they have more degradation compare with the other stability parameters. The proposed studies include the development of HPTLC method for simultaneous estimation of Minoxidil and finasteride in bulk and Pharmaceutical dosage form. The chromatographic improvement was done on the HPTLC plates precoated with silica gel 60 F_{254} utilizing a mobile phase of n-butanol: TEA (10:0.1v/v) as versatile stage. The linearity for Minoxidil and Finasteride was found to be 6000-24000 ng/band and 200-800 ng/band respectively. The R_f estimation of medication was seen as 0.22 ± 0.24 for Minoxidil and 0.63 ± 0.65 for Finasteride. From the accuracy data % recoveries for Minoxidil and Finasteride were found to be 98-101.92% for Minoxidil and 98.42-101.68% for Finasteride respectively. The method was also found to be specific. From the robustness study it's clearly said that minor changes did not effect on the symmetry of peak and R_f of Minoxidil and Finasteride. which confirming the reliability of the method. The percentage amount of drugs were found more than 98 % for the assay of synthetic mixture which specify that the method provides accurate and precise results

Conclusion

The proposed HPTLC method gives linear, accurate, robust and precise results for determination of Minoxidil and finasteride in marketed formulation without prior separation and is easily applied for routine analysis. The most striking feature of the method is its simplicity and rapidity. Utilization of the solvents are also less as Single ratio of solvent used as mobile phase that makes the developed method economic, Method validation has been demonstrated by variety of tests for linearity, accuracy, precision, LOD & LOQ. The proposed method was successfully applied to determination of these drugs in commercial product. Forced degradation study of Minoxidil and Finasteride was performed in which Maximum degradation of both drugs in alkali condition were found. The proposed method was successfully applied to determination of these drugs in commercial product.

Conflict of Interest

We have no conflicts of interest to disclose.

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