International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-2, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Development and Validation of Stability Indicating Method for Minoxidil and Finasteride in its Pharmaceutical Dosage Form

Patel PD*, Surti NI, Upadhyay UM

Sigma Institute of Pharmacy, Bakrol, Vadodara, Gujarat, India.

Manuscript No: IJPRS/V4/I2/00085, Received On: 07/05/2015, Accepted On: 21/05/2015

ABSTRACT

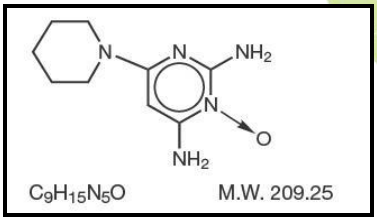
A simple, accurate, precise and specific RPHPLC method has been developed and validated for simultaneous estimation of Finasteride and Minoxidil in its Pharmaceutical dosage form. RPHPLC method was optimized on Hypersil BDS C-18, 250mm x 4.6mm, 5µ particle size with mobile phase Phosphate Buffer (pH 7) : ACN in ratio of (80:20 v/v) and pH 7 adjusted with 1 M NaOH. The mobile phase at flow rate of 1.0 ml/min, Injection volume 20µl and detection wavelength was kept 216 nm. The retention time for Finasteride and Minoxidil was 2.95±0.1 min and 5.74±0.1 min respectively. The linearity was observed in the concentration range of 0.50 to 1.50 mcg/ml and 25 to 75 mcg/ml with correlation coefficient of 0.997 and 0.999 for Finasteride and Minoxidil respectively. The % degradation during force degradation was found to be 10 to 50 % for both Finasteride and Minoxidil in the given condition using developed RP- HPLC method.

KEYWORDS

Minoxidil, Finasteride, RP-HPLC, Stability Indicating, Validation

INTRODUCTION

Minoxidil



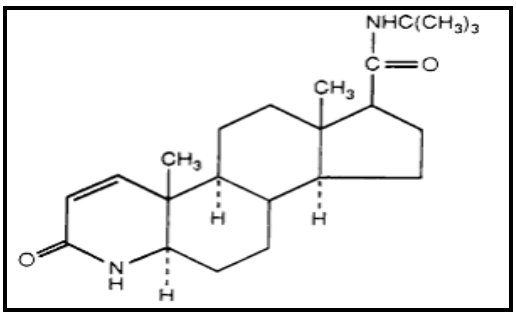
IUPAC Name: 2,6-diamino-4-(piperidin-1-yl)pyrimidin-1-ium-1-olate.

Mechanism of Action: Minoxidil is thought to promote the survival of human dermal papillary cells (DPCs) or hair cells by activating both extracellular signal-regulated kinase (ERK) and Akt and by preventing cell death by increasing the ratio of BCl-2/Bax.

***Address for Correspondence:**
Patel Paritosh D.
Sigma Institute of Pharmacy, Bakrol,
Vadodara, Gujarat, India
E-Mail Id: paritosh4u4ever@gmail.com

Minoxidil may stimulate the growth of human hairs by prolonging anagen through these proliferative and anti-apoptotic effects on DPCs. Minoxidil, when used as a vasodilator, acts by opening adenosine triphosphate-sensitive potassium channels in vascular smooth muscle cells. This vasodilation may also improve the viability of hair cells or hair follicles.

Finasteride



IUPAC Name: (1S,2R,7R,10S,11S,14S,15S)-N-tert-butyl-2,15-dimethyl-5-oxo-6-

azatetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadec-3-ene-14-carboxamide.

Mechanism of Action: Finasteride inhibits Type II 5a-reductase through the formation of a stable complex with the enzyme. Inhibition of Type II 5a-reductase blocks the peripheral conversion of testosterone to DHT, resulting in significant decreases in serum and tissue DHT concentrations, minimal to moderate increase in serum testosterone concentrations, and substantial increases in prostatic testosterone concentrations. As DHT appears to be the principal androgen responsible for stimulation of prostatic growth, a decrease in DHT concentrations will result in a decrease in prostatic volume. In men with androgenic alopecia, finasteride has shown to decrease scalp DHT concentration to the levels found in hairy scalp, reduce serum DHT, increase hair re-growth, and slow hair loss.

MATERIAL AND METHODS

Materials

Sample of Finasteride and Minoxidil were procured from Gitar Laboratory, Ahmedabad. Both the drugs are used as a standard without further purification. HPLC grade Methanol, Acetonitrile, KH2PO4, Ortho-phosphoric acid and Double distilled water were used.

Identification of Drugs

Identification of both the drug should be done by following parameter:

1. Melting point
2. IR identification
3. Solubility

Selection of Wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. A wavelength which gives good response for the drugs to be detected is to be selected. Standard solution of Minoxidil (50 mcg/ml) and Finasteride (1 mcg/ml) were scanned over the range of 200 to 400 nm. Two drugs detection were carried out at different wavelength maxima. But, best responses of two drugs were achieved at 216 nm. So, both drugs were detected at 216 nm wavelength.

Selection of Chromatographic Condition

Proper selection of the HPLC method depends upon the nature of the sample (ionic, ionizable or neutral molecule), its molecular weight and solubility. The drugs selected for the present study are polar in nature and hence either reversed phase or ion-pair or ion exchange chromatography can be used.

Reversed phase HPLC was selected for the initial separations because of its simplicity and suitability. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation.

Preparation of Mobile Phase

Dissolve 6.81 g Potassium dihydrogen phosphate into 1000 ml Water and adjust pH 7.0 with 1 M NaOH solution (7.0 pH Phosphate Buffer). To 800 ml Phosphate Buffer solution, 20 ml ACN was added and mixed properly. Then mobile phase was filtered through 0.45 µm filter paper with vacuum filtration assembly. Mobile Phase was transferred to mobile phase bottle and sonicated for 30 min.

Preparation of Standard Stock Solution

A standard stock solution of mixture of Finasteride and Minoxidil was prepared by accurately weighing 10 mg Finasteride in 1000 ml volumetric flask and 50 mg Minoxidil in 100 ml of volumetric flask and dissolved drugs with the 10 ml of Methanol as a diluents and final volume make up with mobile phase. (Conc. Obtained was 10 ppm Finasteride and 500 ppm Minoxidil).

Preparation of Working Standard Solution

From the mixture of 10 ppm Finasteride and 500

ppm Minoxidil, 1ml was taken and diluted with MeOH to yield a solution with final concentration of 1 ppm Finasteride and 50 ppm Minoxidil.

Sample Preparation (Marketed Formulation)

Preparation Sample of Stock Solution

Take sample equivalent to Finasteride 1 mg and equivalent to Minoxidil 50 mg in to 100 ml volumetric flask and dilute up to mark with diluent.

Preparation Working Sample Solution

Take 1 ml solution from above Stock solution in to 10 ml volumetric flask and dilute up to mark with diluent. (Finasteride 1 mcg/ml and Minoxidil 50 mcg/ml)

Validation

System Suitability Test

Aliquots from each standard solution were combined and diluted with mobile phase to yield a solution with final concentration of 1 mcg/ml and 50 mcg/ml for Finasteride and Minoxidil respectively. The solution was injected six times and system suitability parameters were calculated.

Linearity and Range

From standard stock mixture solution, aliquots of 0.50, 0.75, 1.00, 1.25 and 1.5 ml were transferred to the 10 ml of volumetric flask and volume was made up to mark with diluent to get the concentration of Finasteride 0.5, 0.75, 1.00, 1.25, and 1.50 mcg/ml and 25, 37.5, 50.0, 62.5, and 75 mcg/ml of Minoxidil. 20 µl of each solution was injected under the operating chromatographic conditions described above. Calibration curve was plotted in the concentration range of 0.5-1.25 mcg/ml for Finasteride and 50-75 mcg/ml for Minoxidil. The linear response was observed over a range of 0.5-1.25 mcg/ml for Finasteride and 50-75 mcg/ml for Minoxidil.

Precision

Repeatability

The precision of the method was checked by repeatedly injecting (n = 6) standard solutions of 1mcg/ml Finasteride and 50 mcg/ml Minoxidil under the same chromatographic condition. The peak area was measured. % RSD or CV of area should not be more than 2 %.

Intraday Precision

Three replicates of three concentrations (0.5, 1.0 and 1.5 mcg/ml) of standard solution of Finasteride and (25, 50 and 75 mcg/ml) of standard solution of Minoxidil were analyzed at the same day. % RSD or CV of area should not be more than 2 %.

Interday Precision

Three replicates of three concentrations (0.5, 1.0 and 1.5 mcg/ml) of standard solution of Finasteride and (25, 50 and 75 mcg/ml) of standard solution of Minoxidil were analyzed at three consecutive day. % RSD or CV of area should not be more than 2 %.

Accuracy (% Recovery)

Accuracy of the method was determined by calculating the recovery of Finasteride and Minoxidil by Standard addition method. Middle concentrations for Minoxidil (0.5µl/ml) and Finasteride (25 µl/ml) were selected and standard were spiked at three different levels (80 %, 100 % and 120%).

Limit of Detection and Limit of Quantification

The LOD was estimated from the set of five calibration curves used to determine method linearity. The LOD may be calculated as

$$\mathbf{LOD = 3.3 \times (SD/Slope)}$$

Where, SD = Standard deviation of the Y-intercepts of the five calibration curves Slope = Mean slope of the five calibration curves.

The LOQ was estimated from the set of five calibration curves used to determine method linearity. The LOQ may be calculated as

$$\mathbf{LOQ = 10 \times (SD/Slope)}$$

Where, SD = Standard deviation of the Y-intercepts of the five calibration curves. Slope = Mean slope of the five calibration curves.

Robustness

To confirm robustness, change was done in flow rate (± 0.2 ml/min), Mobile Phase composition (2 %) and pH (± 0.2). % RSD for area was calculated which should be less than 2%.

RESULTS AND DISCUSSION

Melting point Determination

Table 1: Melting point of Minoxidil and Finasteride

Drugs	Melting point range	Observed Melting point
Minoxidil	272-274°C	273°C
Finasteride	252-254°C	253°C

Solubility Study

Table 2: Solubility data for Minoxidil and Finasteride

Solvents	Solubility	
	Minoxidil	Finasteride
Water	Insoluble	Insoluble
Acetonitrile	Slightly soluble	Slightly soluble
Methanol	Soluble	Soluble
0.1 N HCl	Insoluble	Insoluble
0.1 N NaOH	Insoluble	Insoluble

IR Spectroscopy

Finasteride

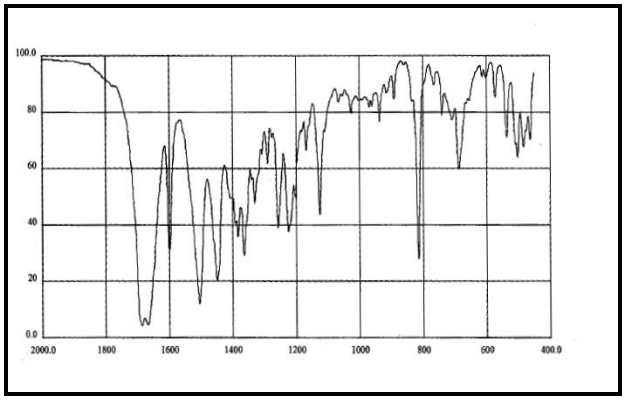


Figure 1: IR Spectra of Finasteride API IP 2014

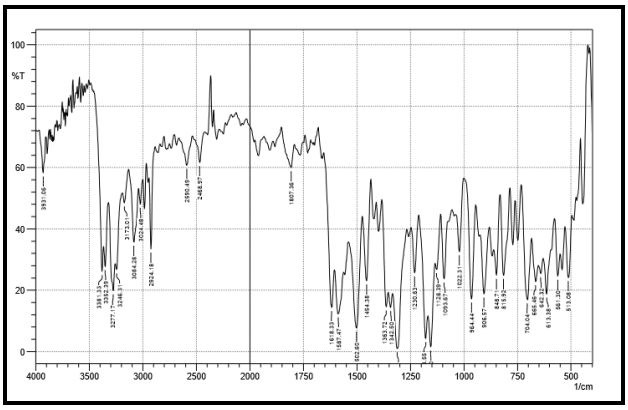


Figure 2: IR Spectra of Finasteride API

Minoxidil

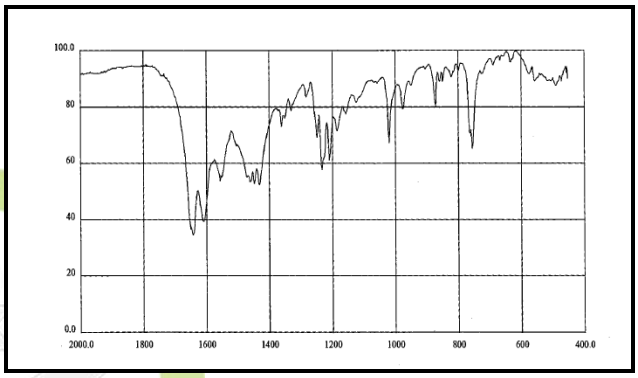


Figure 3: IR Spectra of Minoxidil API IP 2014

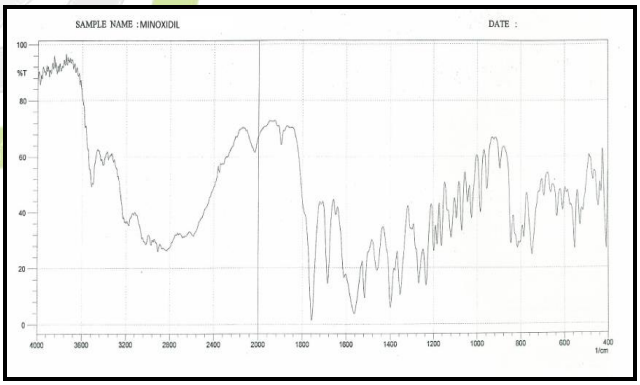


Figure 4: IR Spectra of Minoxidil API

From all identification parameters; M.P., IR, Solubility and UV, both standard drugs were identified as Finasteride and Minoxidil which is going to be used for method development.

Selection of Wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. A wavelength which gives good response for the drugs to be detected is to be selected.

Standard solution of Minoxidil (50 mcg/ml) and Finasteride (1 mcg/ml) were scanned over the range of 200 to 400 nm. Two drugs detection were carried out at different wavelength maxima. But, best responses of two drugs were achieved at 216 nm. So, both drugs were detected at 216 nm wavelength.

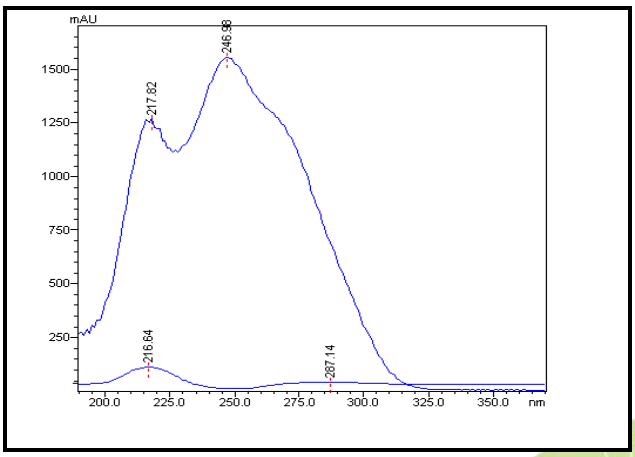


Figure 5: Overlay UV spectrum of Finasteride and Minoxidil showing of wavelength detection

Selection of Mobile Phase

Different type of mobile phase was tried and from chromatogram optimized mobile phase was finalised having the composition as below.

Buffer (7.0 pH): ACN = 80:20 % v/v

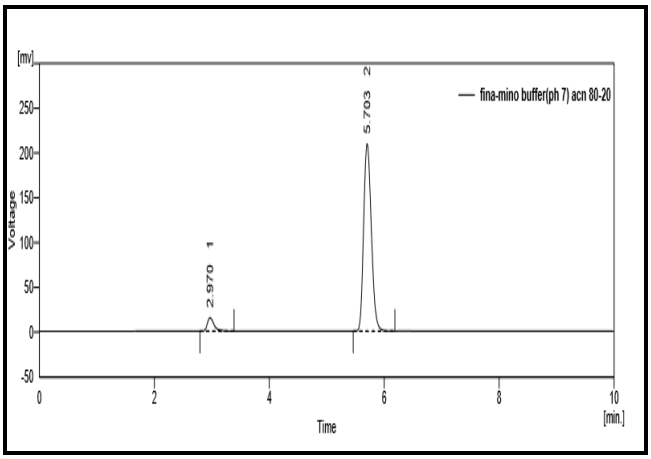


Figure 6: Chromatogram of Finasteride with mobile phase Buffer (pH 7): ACN (80:20 v/v) at 216 nm

Table 3: Data of system suitability

Sr. No.	Theoretical Plates		Retention Time (min)		Tailing Factor		R_s
	Fina	Mino	Fina	Mino	Fina	Mino	
1	3205	8141	2.967	5.750	1.607	1.314	11.984
2	3371	8112	2.960	5.740	1.337	1.314	12.117
3	3371	8122	2.960	5.743	1.667	1.314	12.132
4	3327	8216	2.977	5.777	1.607	1.314	12.056
5	3342	8273	2.983	5.797	1.704	1.314	12.113
6	3256	7963	2.990	5.813	1.704	1.278	12.010
Result			SD=0.0125	SD=0.0305			
			% RSD = 0.4198	% RSD = 0.5288			
Limit	>2000		% RSD < 2		< 2		> 2

Validation of the Development HPLC Method

System Suitability Test

Aliquots from each standard solution were combined and diluted with mobile phase to yield a solution with final concentration of 1 mcg/ml and 50mcg/ml for Finasteride and Minoxidil respectively. The solution was injected six times and system suitability parameters were calculated.

1. Theoretical plate count of Finasteride and Minoxidil is greater than 2000.
2. The tailing factor of six replicate of Finasteride and Minoxidil is less than 2.0.
3. Resolution of the peak is greater than 2.0

Linearity and Range

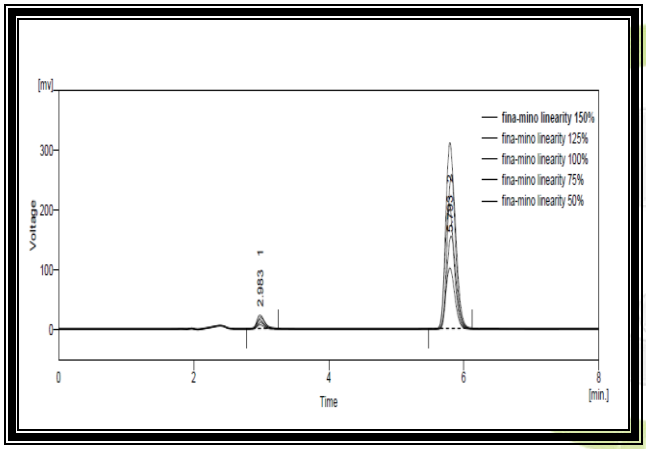


Figure 7: Linearity of Finasteride and Minoxidil

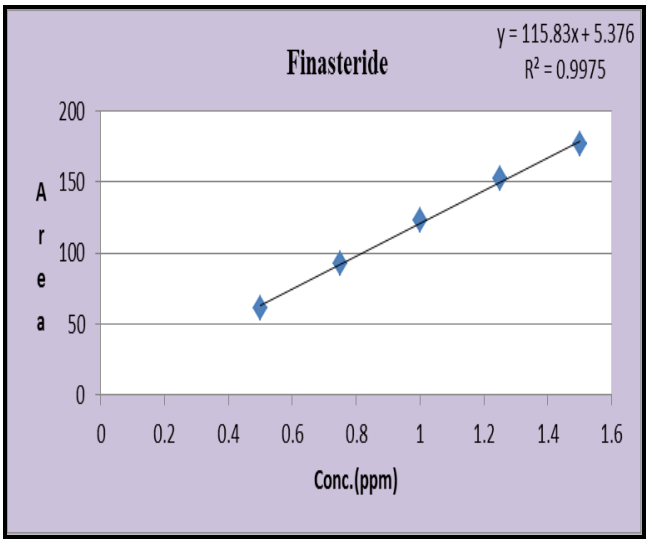


Figure 8: Calibration curve for Finasteride

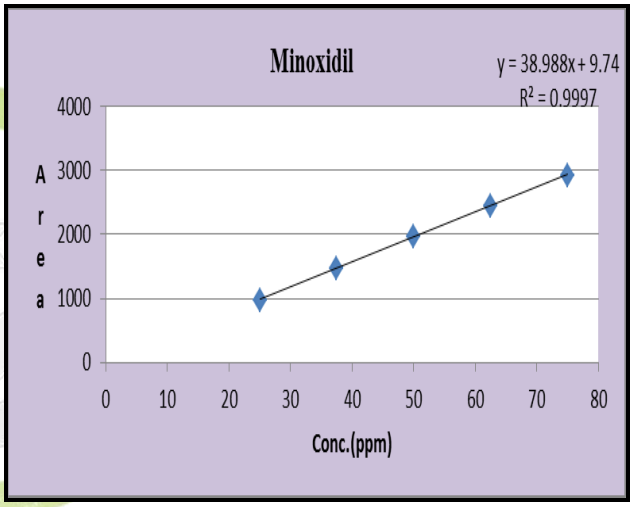


Figure 9: Calibration curve for Minoxidil

Table 4: Data of Calibration Curve

Drug	Conc^{n} (mcg/ml)	Peak area (mv)	Regression equation	Correlation Coefficient(R^2)
Finasteride	0.50	61.438	y = 115.8x + 5.376	0.997
	0.75	92.53		
	1.00	123.4		
	1.25	152.34		
	1.50	176.32		
Minoxidil	25.0	970.218	y = 38.98x + 9.74	0.999
	37.5	1478.055		
	50.0	1972.018		
	62.5	2452.206		
	75.0	2925.133		

For Finasteride, regression equation was found to be **y = 115.8x + 5.376** and correlation co-efficient (R^2) was found to be **0.997**

For Minoxidil, regression equation was found to be **y = 38.98x + 9.74** and correlation co-efficient (R^2) was found to be **0.999.**

Hence the method shows linearity in the range of 0.5 to 1.50 mcg/ml for Finasteride and 25 to 75 mcg/ml for Minoxidil.

Precision

Repeatability

Discussion: The % RSD for Finasteride and Minoxidil was found to be 0.916 and 1.017 respectively.

Table 5: Repeatability Data of Finasteride and Minoxidil

Finasteride		**Minoxidil**	
Concentration (mcg/ml)	**Area of Finasteride (mv)**	**Concentration (mcg/ml)**	**Area of Minoxidil (mv)**
1	123.544	50	1985.344
1	124.403	50	1993.557
1	122.663	50	1965.786
1	122.783	50	1959.507
1	121.063	50	1940.169
1	122.393	50	1953.488
Mean	122.808	Mean	1966.309
SD	1.12468	SD	19.99501
% RSD	0.91580	% RSD	1.01688

Intraday Precision

Table 6: Intraday precision data for Finasteride and Minoxidil

Drug	**Conc. (mcg/ml)**	**Area (mv)**			**Mean Area (mv)**	**SD**	**% RSD**
		Set 1	**Set 2**	**Set 3**			
Fina	0.5	61.650	61.834	62.511	61.998	0.453	0.7313
	1.0	119.536	117.227	121.506	119.423	2.141	1.7934
	1.5	182.863	181.022	178.853	180.912	2.007	1.1095
Mino	25	986.16	994.097	989.592	989.95	3.979	0.4020
	50	1985.34	1955.95	1957.86	1966.38	16.444	0.8362
	75	2918.76	2896.34	2874.26	2896.45	22.254	0.7683

% RSD was found to be 0.731-1.793 and 0.402-0.836 for Finasteride and Minoxidil respectively.

Interday Precision

Table 7: Interday precision data for Finasteride and Minoxidil

Drug	Conc. (mcg/ml)	Area (mv)			Mean Area (mv)	SD	% RSD
		Day 1	Day 2	Day 3			
Fina	0.5	61.832	61.397	60.662	61.297	0.591	0.9647
	1.0	123.792	122.308	119.654	121.918	2.096	1.7195
	1.5	182.489	179.942	177.607	180.012	2.441	1.3564
Mino	25	989.09	982.11	967.38	979.53	11.083	1.1315
	50	1988.72	1959.81	1934.32	1960.95	27.219	1.3880
	75	2932.73	2891.73	2847.98	2890.81	42.384	1.4661

% RSD was found to be 0.965-1.719 and 1.131-1.466 for Finasteride and Minoxidil respectively.

Accuracy (% Recovery)

Concentration of Pre-analysed sample taken for Finasteride = 0.50 µg/ml

Concentration of Pre-analysed sample found for Finasteride = 0.498 µg/ml

Concentration of Pre-analysed sample taken for Minoxidil = 25.00 µg/ml

Concentration of Pre-analysed sample found for Minoxidil = 25.01 µg/ml

Table 8: Data of Accuracy for Finasteride and Minoxidil

Drug	Level	Amount of Std. Spiked (µg/ml)	Total conc. (µg/ml)	Total amount found	Amount Recovery (µg/ml)	% Recovery
Fina	80 %	0.4	0.9	0.899	0.401	100.36
				0.904	0.406	101.68
				0.891	0.393	98.37
	100 %	0.5	1.0	1.001	0.503	100.55
				0.999	0.501	100.01
				1.0	0.502	100.35
	120 %	0.6	1.1	1.107	0.609	101.50
				1.089	0.591	98.61
				1.107	0.608	101.31

Mino	80 %	20	45	45.03	20.02	100.07
				45.30	20.29	101.94
				44.73	19.72	98.94
	100 %	25	50	49.99	24.98	99.91
				50.45	25.44	101.76
				49.95	24.94	99.77
	120 %	30	55	55.44	30.43	101.44
				55.21	30.20	100.68
				55.38	30.37	101.23
Fina	80 %	SD = 1.6816		% RSD = 1.6795		
	100 %	SD = 0.2710		% RSD = 0.2702		
	120 %	SD = 1.6644		% RSD = 1.6068		
Mino	80 %	SD = 1.4290		% RSD = 1.4285		
	100 %	SD = 1.1109		% RSD = 1.1056		
	120 %	SD = 0.3891		% RSD = 0.3848		

SD Accuracy was found to be 100.35% - 101.50% and 99.77% - 101.43% for Finasteride and Minoxidil respectively.

Table 9: Data of % Assay for Finasteride and Minoxidil

Drug	**Conc. (mcg/ml)**	**Area of Sample (mv)**			**Mean of % Assay**	**SD**	**% RSD**
		Set 1	**Set 2**	**Set 3**			
Fina	1	120.90	122.37	119.87			
	% Assay	99.64	100.85	98.79	99.76	1.033	1.036
Mino	50	1963.99	1980.87	1965.67			
	% Assay	101.01	101.88	101.10	101.33	0.478	0.471

% Assay was found to be 99.76% and 101.33% for Finasteride and Minoxidil respectively.

Assay

Preparation sample of stock solution: Take sample equivalent to Finasteride 1 mg and equivalent to Minoxidil 50 mg in to 100 ml volumetric flask and dilute up to mark with diluent.

Preparation Working sample solution:

Take 1 ml solution from above Stock solution in to 10 ml volumetric flask and dilute up to mark with diluent.

Limit of Detection and Limit of Quantification

Table 10: Data of LOD and LOQ

Parameter	Finasteride	Minoxidil
S.D. of the Y-Intercepts of the 5 calibration curves	4.6452	12.9629
Mean slope of the 5 calibration curves	111.1	39.07
LOD= 3.3 × (SD/Slope) (mcg/ml)	0.1379	1.0948
LOQ = 10 × (SD/Slope) (mcg/ml)	0.4181	3.3178

The LOD and LOQ for Finasteride were found to be 0.1379 and 0.4181 respectively. The LOD and LOQ for Minoxidil were found to be 1.0948 and 3.3178 respectively.

Robustness

Table 11: Data for Flow rate change

Drug	Conc. (mcg/ml)	Flow Rate (ml/ min)	Area (mv)			Mean Area (mv)	SD	% RSD
			Set-I	Set-II	Set-III			
Fina	1	0.8	124.44	124.03	123.11	123.86	0.677	0.54
	1	**1.0**	**120.91**	**122.37**	**119.88**	**121.05**	**1.034**	**1.036**
	1	1.2	120.11	119.15	119.51	119.59	0.488	0.40
Mino	50	0.8	1999.84	1998.09	1972.00	1989.98	15.59	0.78
	50	**1.0**	**1963.99**	**1980.87**	**1965.67**	**1970.17**	**0.478**	**0.472**
	50	1.2	1924.88	1909.48	1907.34	1913.90	9.566	0.49

% RSD for area was found to be 0.40-1.036 and 0.49-0.78 for Finasteride and Minoxidil respectively. (Flow rate change)

Table 12: Data for Mobile Phase Ratio change

Drug	Conc. (mcg/ml)	M.P. Ratio (%)	Area (mv)			Mean Area (mv)	SD	% RSD
			Set-I	Set-II	Set-III			
Fina	1	82:18	125.81	126.56	128.08	126.82	1.159	0.91
	1	**80:20**	**120.91**	**122.37**	**119.88**	**121.05**	**1.034**	**1.036**
	1	78:22	118.32	119.27	119.38	118.99	0.580	0.48
Min	50	82:18	2021.85	2028.2	2040.73	2030.29	9.596	0.473
	50	**80:20**	**1963.99**	**1980.87**	**1965.67**	**1970.17**	**0.478**	**0.472**
	50	78:22	1896.10	1903.42	1913.07	1904.19	8.511	0.447

% RSD for area was found to be 0.48-1.036 and 0.447-0.473 for Finasteride and Minoxidil respectively. (Phase Ratio change)

Table 13: Data for Change in pH

Drug	Conc. (mcg/ml)	pH (± 0.2)	Area (mv)			Mean Area (mv)	SD	% RSD
			Set-I	Set-II	Set-III			
Fina	1	6.8	125.434	126.06	123.11	124.87	1.554	1.24
	1	**7.0**	**120.91**	**122.37**	**119.88**	**121.05**	**1.034**	**1.036**
	1	7.2	124.20	124.70	126.58	125.16	1.252	1.00
Mino	50	6.8	2009.89	2025.68	2042.13	2025.90	16.12	0.79
	50	**7.0**	**1963.99**	**1980.87**	**1965.67**	**1970.17**	**0.478**	**0.472**
	50	7.2	1990.42	1998.24	2020.13	2002.93	15.39	0.76

% RSD for area was found to be 1.00-1.24 and 0.447-0.79 for Finasteride and Minoxidil respectively. (Change in pH)

Table 14: Summary of Validation

Sr. No.	Parameters	Results	
		Finasteride	**Minoxidil**
1.	Linearity Range (n=5) (mcg/ml)	0.5 to 1.5	25 to 75
2.	Regression equation	y = 115.8x + 5.376	y = 38.98x + 9.74
3.	Correlation coefficient (R^2)	0.997	0.999
4.	Limit of detection(n=5) (µg/ml)	0.1379	1.0948
5.	Limit of quantification (n=5) (mcg/ml)	0.4181	3.3178
6.	Precision		
	Repeatability (% RSD) (n=6)	0.9158	1.0168
	Intraday (% RSD) (n=3)	1.2114	0.6688
	Interday (% RSD) (n=3)	1.3468	1.3285
7.	Robustness (% RSD)	< 1.3	< 1.3
8.	Accuracy (Mean ± SD) (%, n=3)	100.50 ± 1.0	100.50 ± 1.0

Stability Indicating Method

Table 15: Data for Minoxidil and Finasteride Standard for stability

Drug	Retention time (min)	Area (mv)	Theoretical plates	Tailing Factor	Resolution
Finasteride	2.940	138.125	3325	1.667	12.350
Minoxidil	5.773	1936.108	8207	1.314	

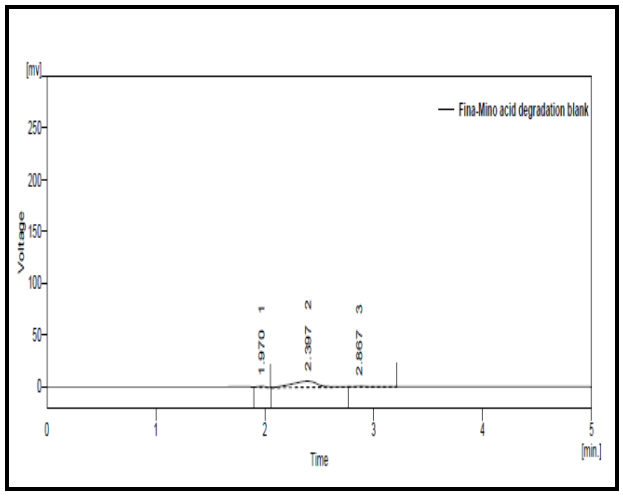


Figure 10: Minoxidil and Finasteride Standard for stability

Acid Degradation

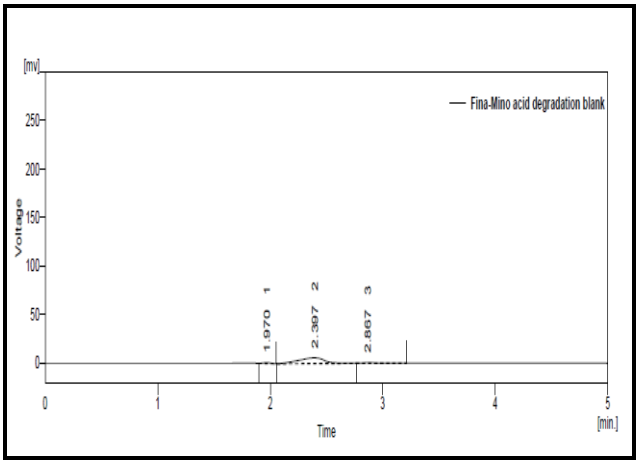


Figure 11: Blank for Acid Degradation

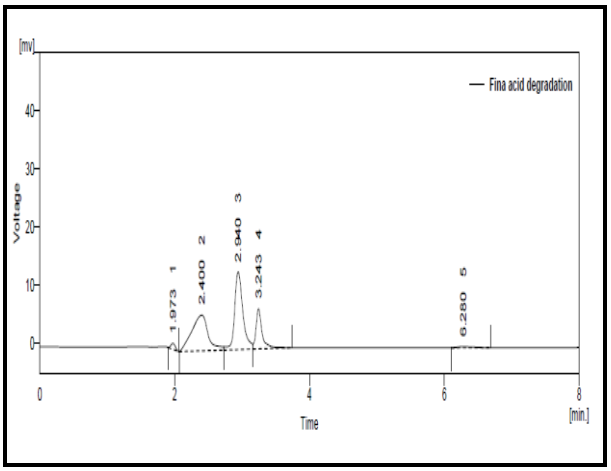


Figure 12: Finasteride API Acid Degradation

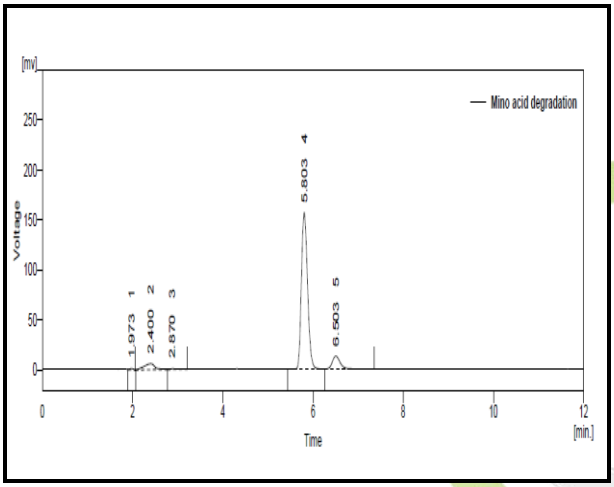


Figure 13: Minoxidil API Acid Degradation

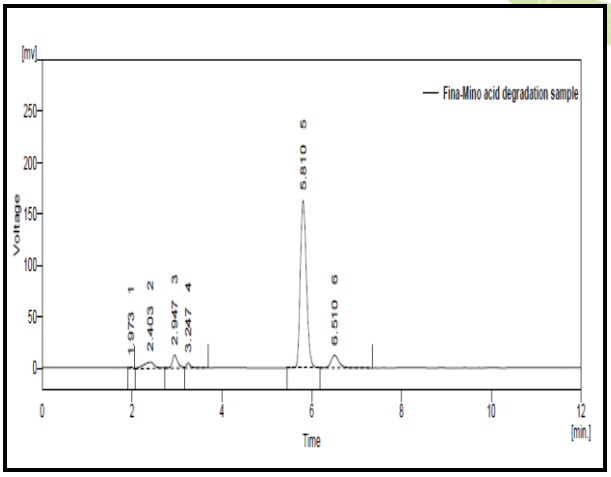


Figure 14: Finasteride and Minoxidil Sample Acid Degradation

Impurities generated from acid degradation were well separated by this method with good resolution

Base Degradation

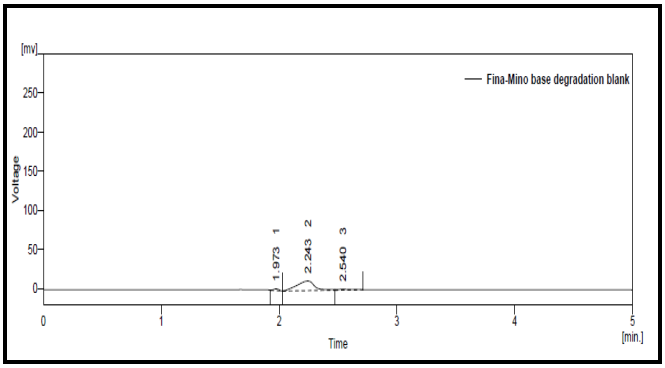


Figure 15: Blank for Base Degradation

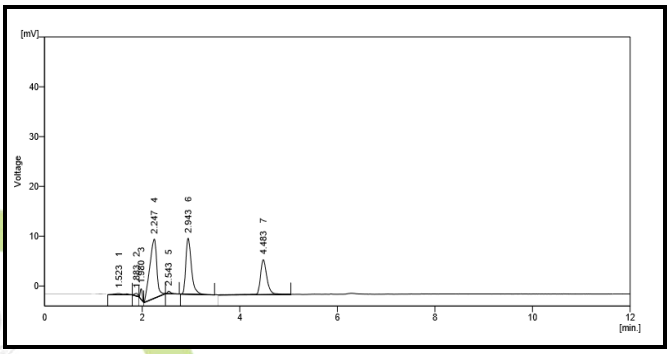


Figure 16: Finasteride API Base Degradation

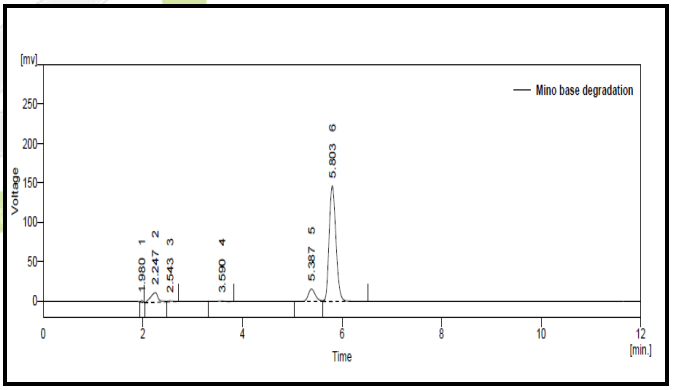


Figure17: Minoxidil API Base Degradation

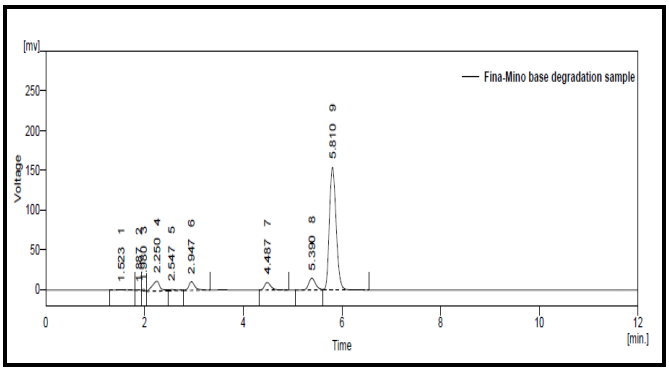


Figure 18: Finasteride and Minoxidil Sample Base Degradation

Impurities generated from base degradation were well separated by this method with good resolution.

Oxidative Degradation

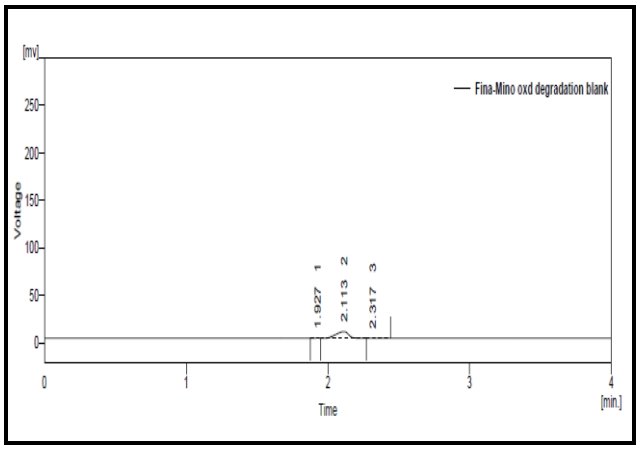


Figure 19: Blank for Oxidative Degradation

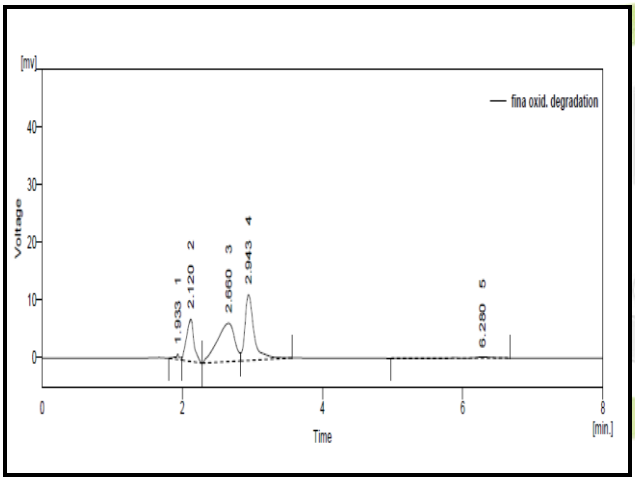


Figure 20: Finasteride API Oxidative Degradation

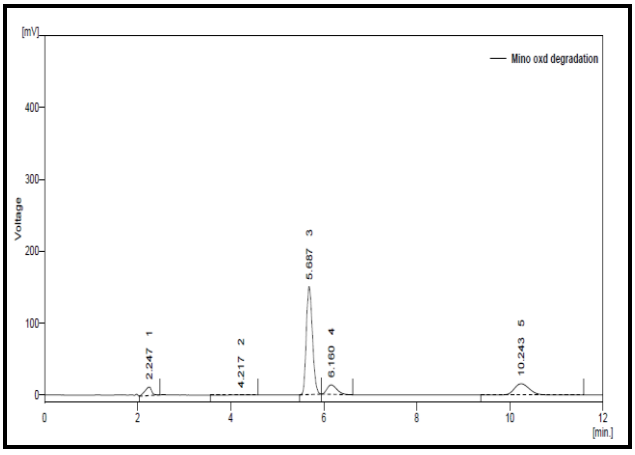


Figure 21: Minoxidil API Oxidative Degradation

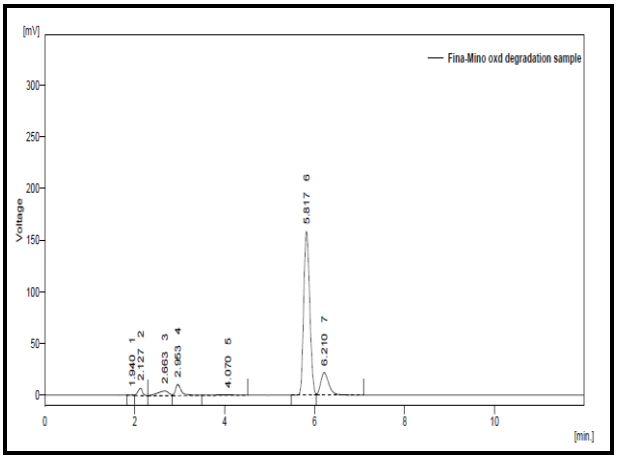


Figure 22: Finasteride and Minoxidil Sample Oxidative Degradation

Impurities generated from oxidative degradation were well separated by this method with good resolution.

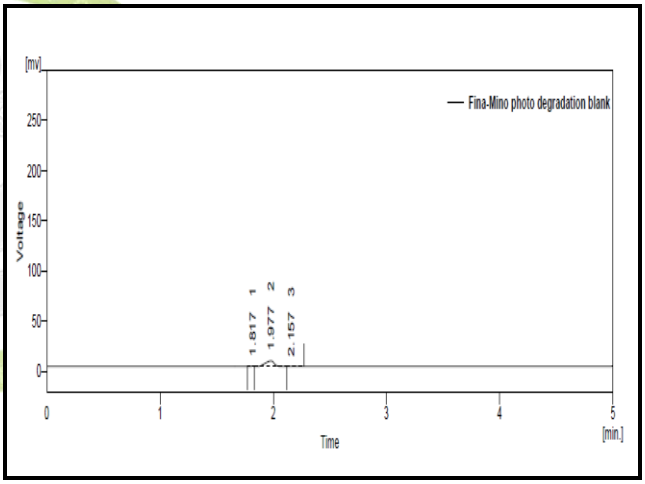


Figure 23: Blank for Photolytic Degradation

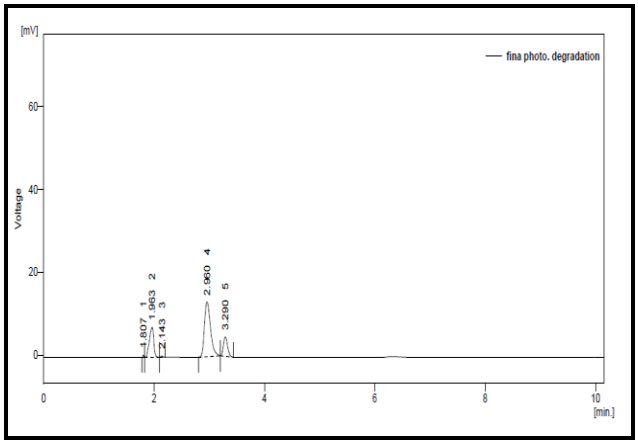


Figure 24: Finasteride API Photolytic Degradation

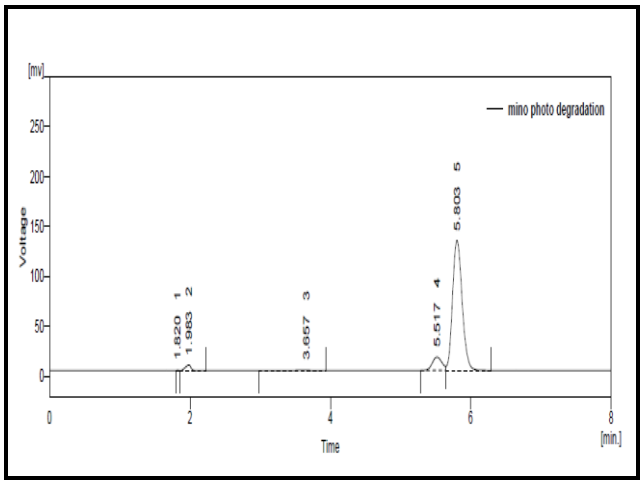


Figure 25: Minoxidil API Photolytic Degradation

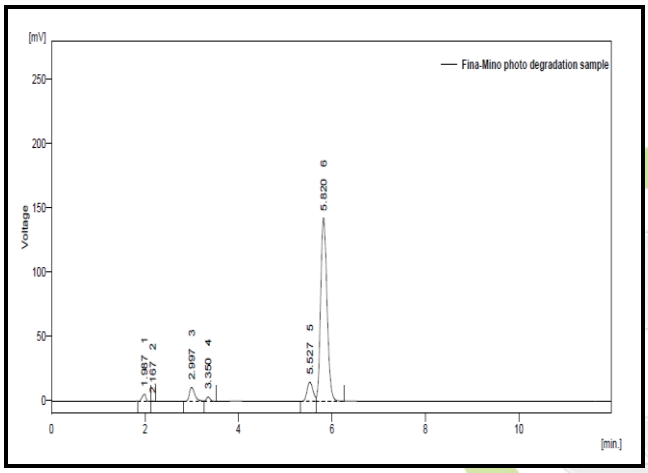


Figure 26: Finasteride and Minoxidil Sample Photolytic Degradation

Impurities generated from photolytic degradation were well separated by this method with good resolution.

Thermal Degradation

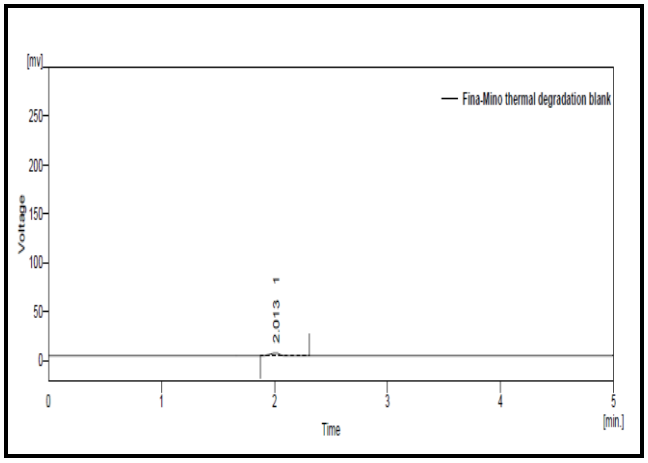


Figure 27: Blank for Thermal Degradation

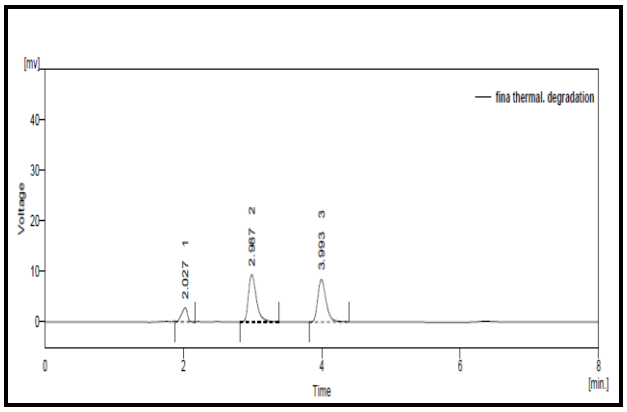


Figure 28: Finasteride API Thermal Degradation

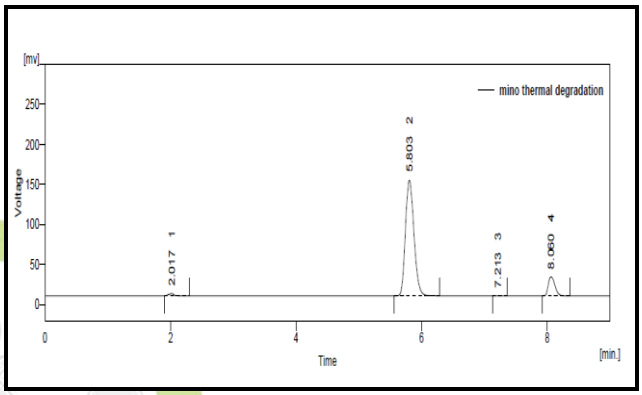


Figure 29: Minoxidil API Thermal Degradation

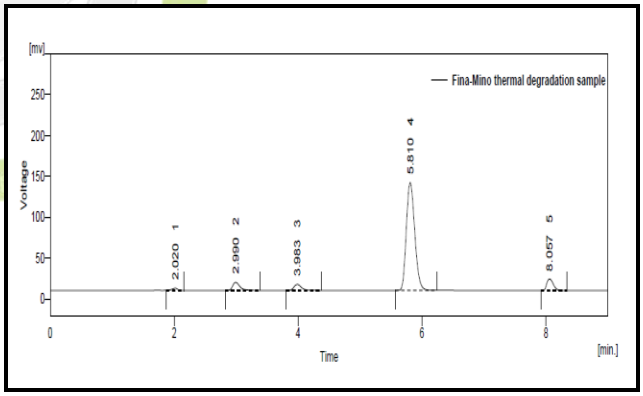


Figure 30: Finasteride and Minoxidil Sample Thermal Degradation

Impurities generated from thermal degradation were well separated by this method with good resolution.

Specificity

The method which is developed is specific because from the degradation study, sample peak is separate from the degradant peak. Both the drug peak not interfere by the degradant peak.

Table 16: Data for Finasteride % Degradation

Finasteride		
Parameter	Standard	Sample
	% Degradation	% Degradation
Acid	17.39	21.59
Base	35.39	31.65
Thermal	45.42	41.80
Oxidation	25.00	25.86
Photolytic	26.50	32.02

Table 17: Data for Minoxidil % Degradation

Minoxidil		
Parameter	Standard	Sample
	% Degradation	% Degradation
Acid	23.43	20.44
Base	28.15	24.11
Thermal	29.54	35.59
Oxidation	28.76	23.65
Photolytic	35.90	29.87

CONCLUSION

A simple, specific, accurate and precise RP-HPLC method has been developed and validated for estimation of Finasteride and Minoxidil in its Pharmaceutical dosage form. Finasteride and Minoxidil were estimated on Hypersil BDS C-18 (250 x 4.6 mm, 5 µm) column using Buffer (pH 7): ACN (80:20 v/v) as mobile phase with flow rate 1 ml/min and detection was carried out at 216 nm. The linearity and range was found to be 0.5 to 1.5 mcg/ml for Finasteride and 25 to 75 mcg/ml for Minoxidil. The co-relation coefficient was found to be 0.997 and 0.999 for Finasteride and Minoxidil respectively. % RSD of repeatability, intraday and intermediate precision was found to be less than 2%. % RSD for Robustness parameters (Flow rate change, pH change, Mobile phase ratio change) was found to be less than 2%. So the developed method was precise and robust. The % Recovery of Finasteride and Minoxidil at different levels were found in the range of 100.37% to 101.50% and 99.77% to 101.43% respectively. The assay value for Finasteride and Minoxidil was found to be 98.79% to 100.85% and 101.01% to 101.81% respectively. So, the developed method was accurate. The % degradation was found to be 10 to 50 % for Finasteride and minoxidil in the given condition using developed HPLC method.

REFERENCES

1. Gillian M. Analytical Instrumentation-A Guide to Laboratory, Portable and Miniaturized; 1st Edn; A John Wiley & Sons, Ltd., England, 2007, pp 1-55.
2. Skoog Douglas, A., West Donald, M., & Holler, F. J. (2014). Fundamentals of analytical chemistry. *Chem. Listy*, *108*, 973-992.
3. Currell, G. (2008). *Analytical instrumentation: performance characteristics and quality* (Vol. 27). John Wiley & Sons., 2000, pp 1-43.
4. Gary, D. C. (2003). *Analytical Chemistry*. 6th Edn; A John Wiley & Sons, Inc., New Jersey, 2003, pp 457-511, 555-573.
5. Donald, L. P., Gary, M. L., Goerge, S. K., and James, R. V. (2009). Introduction to Spectroscopy; 4th Edn; Brooks/Cole, USA, 2009, pp 293-335.
6. Lena, O., and Antony, J. S. (2002). *Handbook of Pharmaceutical Analysis*. Marcel Dekker, Inc., New York, 2002, pp 75-166, 201-239.
7. Ashutosh, K. (2005). *Pharmaceutical Drug Analysis*; 2nd Edn; New age International Publisher, New Delhi, 2005, pp 293-338, 452-475.
8. Yuri, K., and Rosario, L. (2007). *HPLC for Pharmaceutical Scientists*; A John Wiley & Sons, Inc., New Jersey, 2007, pp 8-257.

9. Snyder, L. R., Kirkland, J. L., and Glajch, J. L. (1997). *Practical HPLC Method Development*; Wiley, New York, 1997, pp 34-39.
10. Lloyd, R. S., Joseph, J. K., and John, W. D. (2010), *Introduction to Modern Liquid Chromatography*; 3rd Edn; A John Wiley & Sons, Inc., New Jersey, pp 284-497, 531-560.
11. David, G. W. (2000). Pharmaceutical Analysis A textbook for Pharmacy Students and Pharmaceutical Chemists; 1st Edn; Churchill Livingstone, UK, 2000, pp 75-116, 195-275.
12. Ahuja, S. (2001). Pharmaceutical Analysis: An overview In; Handbook of Modern Pharmaceutical Analysis; Academic Press, United States Of America, 2001, Vol-III, pp 95-119, 349.
13. Bose, A. (2014). HPLC Calibration Process Parameters in terms on System Suitability Test. *Austin Publi. gro.*2014, 1 (2), 1-4.
14. Robert AN., and Alfred HW. Pharmaceutical Process Validation; 3rd Edn; Marcel Dekker, Inc., New York, 2003, pp 542-557.
15. Chung, C. C., Herman, L., Lee, Y. C., and Xueming, Z. (2004). *Analytical Method Validation and Instrument Performance Verification*. A John Wiley & Sons, Inc., New Jersey, 2004, pp 153-172, 173-186.
16. Breaux, J., Jones, K., and Boulas, P. (2003). Understanding and Implementing Efficient Analytical Methods Development and Validation. *Pharmaceutical Technology Analytical Chemistry & Testing*2003, 6-13.
17. www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf on 26th September 2014.
18. Singh, R., & Rehman, Z. (2012). Current trends in forced degradation study for pharmaceutical product development. *Journal of Pharmaceutical Education and Research*, *3*(1), 54-64.
19. Sean, C. S. (2009). *Martindale The Complete Drug Reference*; 36th Edn; RPS Publishing, USA, 2009, pp 1343, 1577.
20. Glyn, V., and Heather, W. (2003). Drugs Handbook; 24th Edn; Palgrave Macmillan, New York, 2003, pp 90, 192.
21. Laurence, B., Keith, P., Donald, B., and Iain, B. *Goodman and Gilman's Manual of Pharmacology and Therapeutics*; 11th Edn; McGraw-Hill Professional, New York, 2007, pp 1027, 1093-1994.
22. http://www.regrowth.com/remedies/localapplication-of-combination-of-minoxidil-andfinesteride/#sthash.irWmxKJJ.dpuf on 26th September 2014.
23. Drug Profile of Minoxidil http://www.drugbank.ca/drugs/DB00350 on 1st Oct 2014.
24. *Indian Pharmacopoeia*, Ministry of Health & Family Walfare, Government of India, 7th edition, Published by the Indian Pharmacopoeia Commission, Ghaziabad, II, 2014, 551, 2236-2239.
25. *British Pharmacopoeia*, General Medicine Council, 7th edition, 2014, I & II, 1059.
26. United States Pharmacopoeia, National Formulary, USP 34 NF 29, The United State Pharmacopoeial Convention 12601, Rockville, MD 20852, August 1, 2014, 3847, 3848
27. Drug Profile of Finasteride http://www.drugbank.ca/drugs/DB01216 on 1st Oct 2014
28. Indian Pharmacopoeia, Ministry of Health & Family Walfare, Government of India, 7th edition, Published by the Indian Pharmacopoeia Commission, Ghaziabad, II, 2014, 449, 1759.
29. British Pharmacopoeia, General Medicine Council, 7th edition, 2014, I & II, 656
30. United States Pharmacopoeia, National Formulary, USP 34 NF 29, The United State Pharmacopoeial Convention 12601,

Rockville, MD 20852, August 1, 2014, 2986, 2987.

31. Zaheer, Z. A., Mirza, S., Moazzam, I., & Sayad, I. (2012). UV-spectrophotometric determination of minoxidil and its application to the assay in pharmaceutical dosage forms. *Der Pharma Chemica*, *4*(1), 568-573.
32. Zarghi, A., Shafaati, A., Foroutan, S. M., & Khoddam, A. (2004). Rapid determination of minoxidil in human plasma using ion-pair HPLC. *Journal of Pharmaceutical and Biomedical Analysis*, *36*(2), 377-379.
33. Gaidhane, H. K., Bidada, J. P., Bhusari, A. S., Navkhare, M. S., Diwanka, G. P., & Tiwari, A. H. (2011). Development and Validation of Stability Indicating HPLC Method for the estimation of Minoxidil and related substance in topical formulation. *Journal of Pharmacy Research*, *4*(12), 4481-4484.
34. Bansal, M., Rathore, P., & Goel, B. Quantitative Determination of Finasteride from Tablet Formulation by UV-Spectrophotometry. *Journal of Pharmaceutical and BioSciences*, 2013, 1, 48-50.
35. Vijaya Lakshmi, N., Rao, G. K., Rani, B. R., Manasa, K., & Bhavani, V. (2013). Development and Validation of UV Spectrophotometric Method for the Estimation of Finasteride in Tablets. *International Journal of Pharmaceutical Sciences*, *3*(1), 123-125.
36. Thimmaraju, M. K., Rao, V., & Gurrala, S. (2011). RP HPLC method for the determination of finasteride and tamsulosin in bulk and pharmaceutical formulations. *Der Pharmacia Lettre*, *3*(5), 79-86.
37. Srinivas, G., Kishore, K. K., Reddy, Y. R. K., Mukkanti, K., Gangaram, V. K., & Madhavan, P. (2011). A validated stability indicating LC method of assay and related substances for finasteride. *Journal of Chemical and Pharmaceutical Research*, *3*(6), 987-96.
38. Sankar, D. G., Rao, B. D., & Kishore, V. S. (2007). Simultaneous determination of tamulosin hydrochloride and finasteride in formulations by reverse-phase HPLC. *Asian Journal of Chemistry*, *19*(2), 1375.
39. Basavaiah, K., & Somashekar, B. C. (2007). Determination of finasteride in tablets by high performance liquid chromatography. *Journal of Chemistry*, *4*(1), 109-116.
40. Hulya, D. D., Aysen, K. C., Serap, S. (2004). Determination of Finasteride by HPLC & Its Analytical Method Validation. *Adnan Mender. Univer. 4th AACD Congre*. 2004, 109-116.
41. Thomas, J. B., and Paris, D. N. S. (2011). *Handbook of basic tables for Chemical Analysis*; 3rd Edn; CRC Press Taylor and Francis Group, USA, 2011, pp 137-208, 351-474.
42. www.fda.gov/downloads/drugs/guidancecompliancegulatoryinformation/guidances/ucm073384.pdf on 9th October 2014.

