IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

Development and Validation of HPLC Method for Simultaneous Estimation of Minoxidil and Finasteride in Topical Solution

Shradha S. Deshmukh and Ajwitas.Kale
Department of Quality Assurance
P. Wadhwani College of Pharmacy, Yevatmal, India

Abstract: *A simple, precise, rapid, accurate HPLC method has been developed and validated for the simultaneous determination of Minoxidil and Finasteride in pharmaceutical dosage form. The chromatographic separation was achieved on ODS C_{18} column (250mm*4.6mm,5 micrometer particle size) using a mobile phase comprising Buffer(7.0PH); ACN 80:20% v/v. The flow rate was 1ml/min and eluents were detected by UV detector at 210 nm. Retention times were found to be 2.967 min and 5.750 min Finasteride and Minoxidil respectively. The calibration curve was linear over the range of 20-80 microgram/ml of Minoxidil and 0.5 -1.6 microgram/ml of Finasteride. The developed method was successfully applied for determination of the two drugs from its pharmaceutical formulation. The excipients in the formulation do not pose any hindrance in determination of two drugs. The proposed method is suitable for routine quality control analysis.*

Keywords: Minoxidil

I. INTRODUCTION

Chemically, Minoxidil (MINO) is 2,4-diamino-6-piperidinopyrimidine 3- oxide mainly used for the hair growth stimulator 1 . MINO, when applied topically to the scalp may stimulate hair growth to a limited extent followed by opening of K+ channels and enhanced microcirculation around hair follicles 2 . Chemically, Finasteride (FINA) is 17 β (N-tertbutylcarbamoyl)-4-aza - 5α-androst – 1 – en - 3-onemainly used in the treatment of androgenic alopecia. FINA, blocks the peripheral conversion of testosterone to dihydrotestosterone (DHT), resulting in to the decrease scalp DHT concentration to the levels found in hairy scalp, reduce serum DHT, increase hair regrowth and slow hair loss. Recently one RP-HPLC method has been published for simultaneous estimation of both the drugs in their pharmaceutical formulation

II. MATERIALS AND METHODS

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

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

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. Concentration 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)

III. RESULTS

Melting point Determination

Table: Melting point of Minoxidil and Finasteride

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

Solubility Study

Table: Solubility data for Minoxidil and Finasteride

Solvents	Solubility	
	Minoxidil	Finasteride
Water	Insoluble	Insoluble
Acetonitrile	Slightly soluble	Slightly soluble
Methanol	Soluble	Soluble
0.1N HC1	Insoluble	Insoluble
0.1N NaOH	Insoluble	Insoluble

IR SpectroscopyFinasteride

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

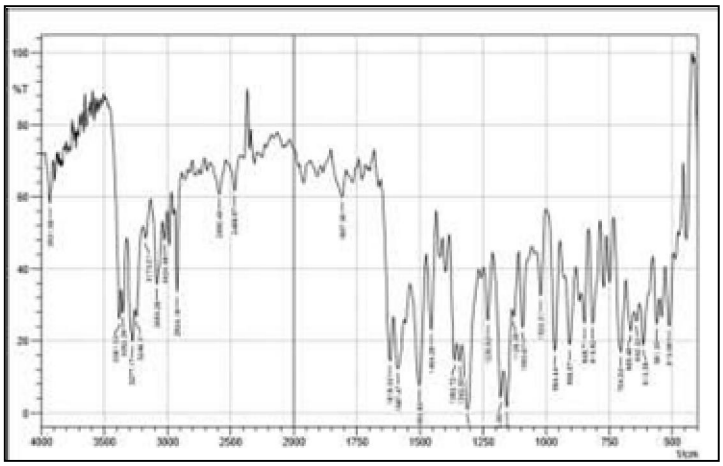

Figure: IR Spectra of Finasteride API

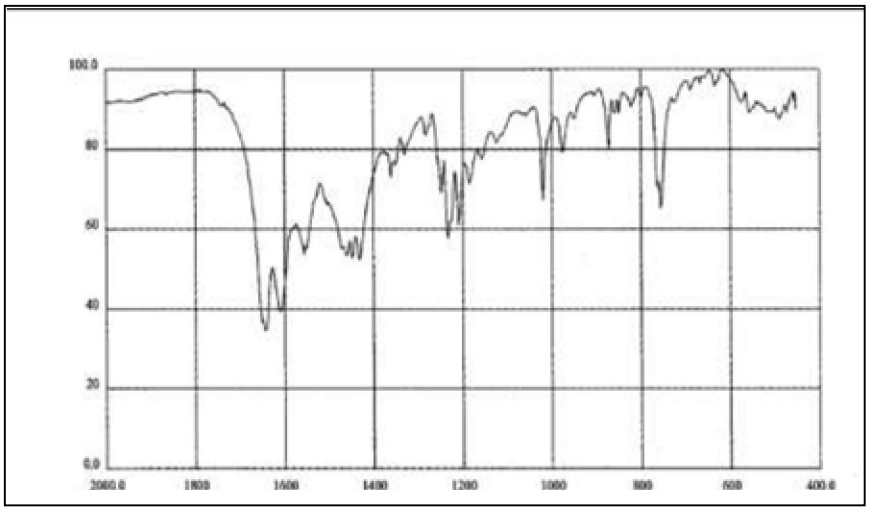

Figure: IR Spectra of Minoxidil API

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 formethod 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 detectedat 216 nm wavelength

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

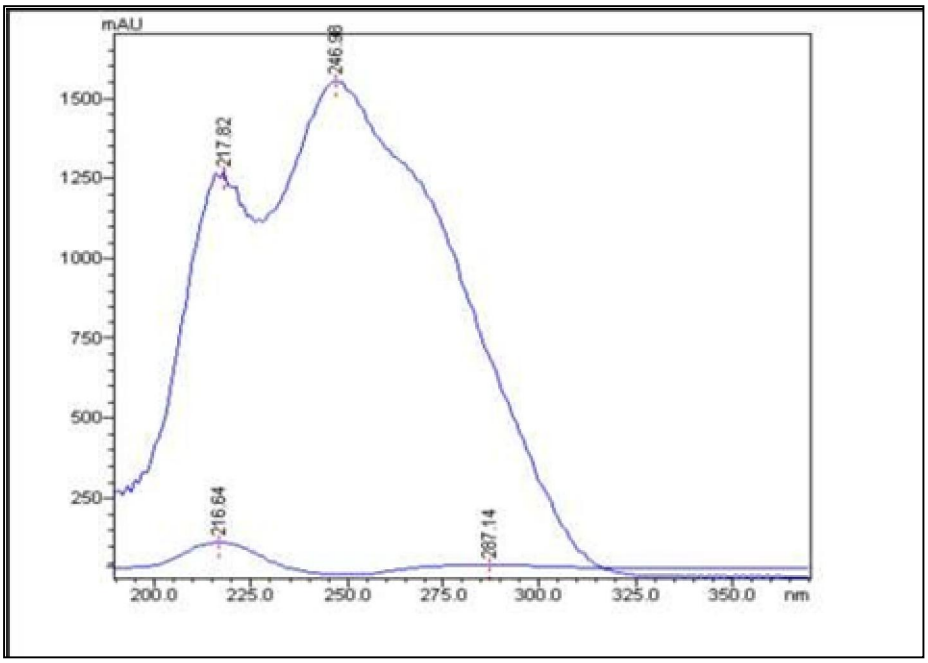

Fig : overlay UV spectrum of finasteride and minoxidil showing wavelength detection

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

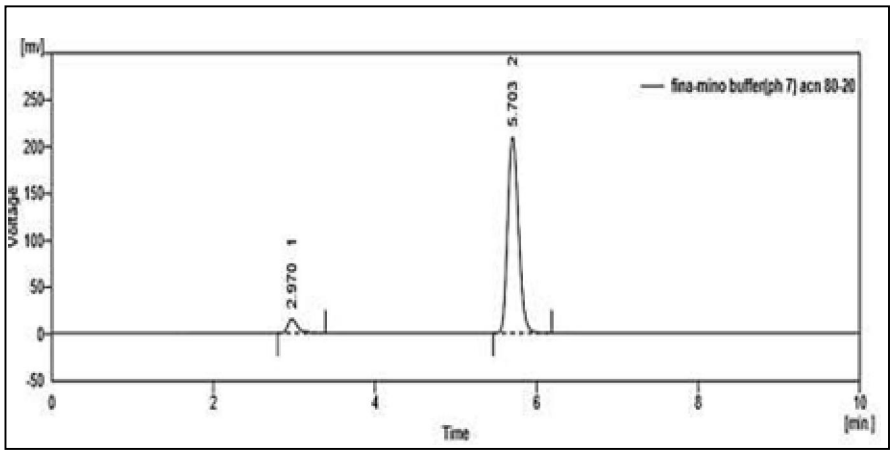

Figure: Chromatogram of Finasteride with mobile phase Buffer (pH 7): ACN (80:20 v/v) at 216 nm

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

Table 7.3: Data of system suitability

Sr. No.	TheoreticalPlates		Retention Time (min)		Tailing Factor		R_2
	Fina	Mino	Fina	Mino	Fina	Mino	
1	3205	8141	2.9675	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

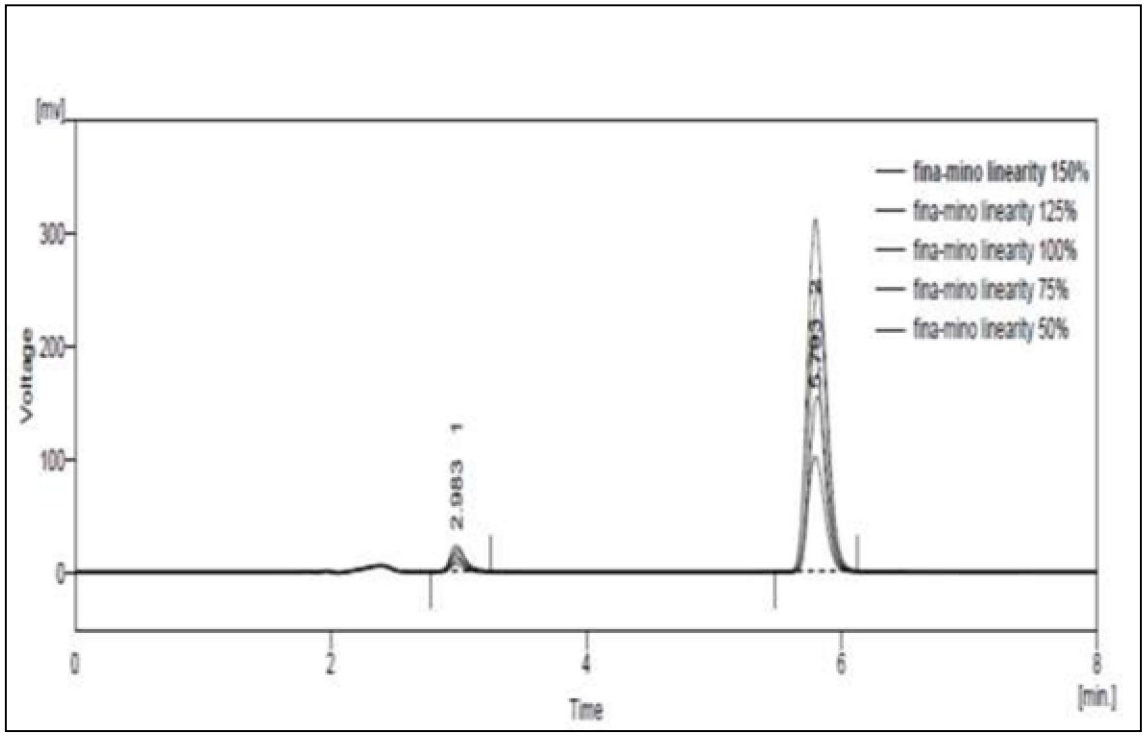

Fig : Linearity of Finasteride and Minoxidil

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

Calibration curve

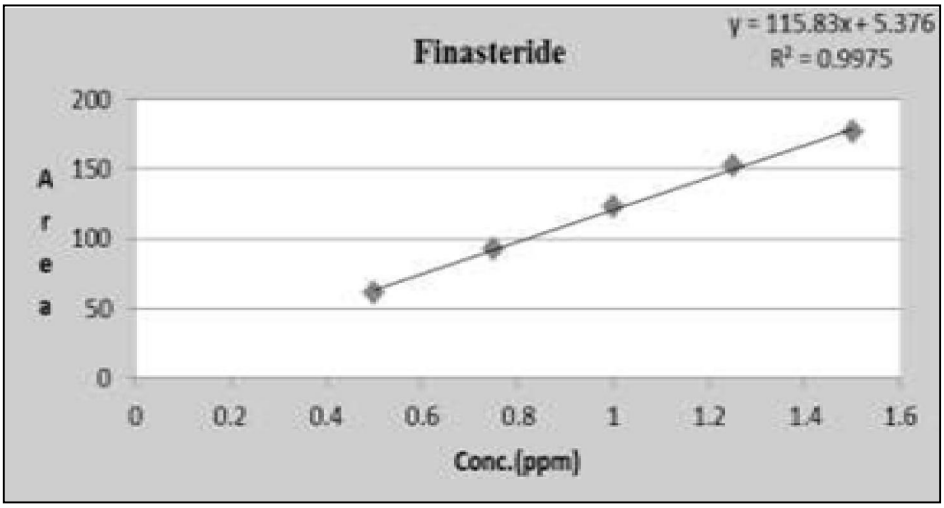

Fig: Calibration curve of Finasteride mean peak

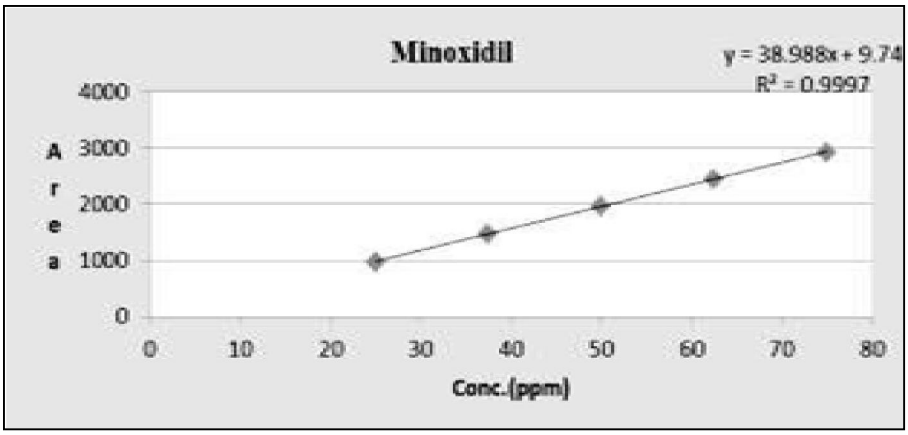

Fig: Calibration curve of Minoxidil mean peak

Table: Data of calibration curve

Drug	Conc (mcg /ml)	Peak area(mv)	Regressionequation	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		

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

For Finasteride, regression equation was found to be y = 115.8x + 5.376 and correlation coefficient (R2) was found to be 0.997 For Minoxidil, regression equation was found to be y = 38.98x + 9.74 and correlation co-efficient (R2) 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: 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: Intraday precision data for Finasteride and Minoxidil

Drug	Conc .(mcg /ml)	Intraday precision					
		Area (mv)			Mean Area (mv)		
		Set 1	Set 2	Set 3		SD	%RSD
	0.5	61.650	61.834	62.511	61.998	0.453	0.7313
Fina	1.0	119.536	117.227	121.506	119.423	2.141	1.7934
	1.5	182.86	181.022	178.853	180.912	2.007	1.1095
	25	986.16	994.097	989.592	989.95	3.979	0.4020
Mino	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 andMinoxidil respectively.

Interday Precision

Table: 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

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

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 andMinoxidil 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/mlConcentration of Pre-analysed sample taken for Minoxidil = 25.00 µg/ml Concentration of Pre-analysed sample found for Minoxidil = 25.01 µg/ml

Table: 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
				1.001	0.503	100.55
	100%	0.5	1.0	0.999	0.501	100.01
				1.0	0.502	100.35
				1.107	0.609	101.50
	120%	0.6	1.1	1.089	0.591	98.61
				1.107	0.608	101.31
				45.03	20.02	100.07
Mino	80%	20	45	45.30	20.29	101.94
				44.73	19.72	98.94
				49.99	24.98	99.91
	100%	25	50	50.45	25.44	101.76
				49.95	24.94	99.77
				55.44	30.43	101.44
	120%	30	55	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.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

Table: Data of % Assay for Finasteride and Minoxidil

Drug	Conc .(mcg /ml)	Area of sample(mv)			Meanof %Assay	SD	%RSD
Fina		Set 1	Set 2	Set 3			
	1	120.90	122.37	119.87			
	% Assay	99.64	100.85	98.76	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 Minoxidilrespectively.

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: 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 =3.3x (SD /Slope)(mcg /ml)	0.1379	1.0948
LOQ =10x (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: Data for Flow rate change

Drug	Conc . (mcg / m l)	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

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

Table: Data for Mobile Phase Ratio change

Drug	Conc (mcg / m1)	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: Data for Change in pH

Drug	Conc. (meg/mI)	pH (+ 0.2)	Area (mv)			Mean Area(mv)	SD	% RSD
			Set-1	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: 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

IV. 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

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

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

REFERENCES

[1]. A.A. Qureshi, N.A. Patel, A.R. Patel, "HPLC method development and validation for the simultaneous estimation of minoxidil and finasteride in topical solution," International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(9): 449-452.
[2]. M. Ahmad, M. Abbas, S. Hussain, H. Akhtar, M.S. Arshad, M.A. Siddiqui, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Chromatographic Science, 2016, 54(7): 1131-1136.
[3]. V. Patil, V. Puranik, R. Rajput, "Development and validation of a reversed-phase high-performance liquid chromatographic method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Applied Pharmaceutical Science, 2016, 6(4): 069-074.
[4]. S. Dhamija, M. Bhatia, A. Sharma, S. Sharma, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," International Journal of Research in Pharmacy and Pharmaceutical Sciences, 2017, 2(1): 26-32.
[5]. Al-khdhairawi, A. Zaidan, R. Tahir, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," European Journal of Biomedical and Pharmaceutical Sciences, 2018, 5(2): 162-168.
[6]. A.A. Al-Majed, A.M. Al-Harbi, A.A. Al-Sohaibani, "HPLC method development and validation for the simultaneous determination of minoxidil and finasteride in topical solution," Saudi Pharmaceutical Journal, 2011, 19(4): 243-247.
[7]. M. Ashraf, S. Mahmood, S. Murtaza, S. Ata, M.K. Khan, "Development and validation of an HPLC method for the simultaneous determination of minoxidil and finasteride in topical formulation," Arabian Journal of Chemistry, 2014, 7(4): 650- 654.
[8]. S. Shaikh, S. Bhatti, S. Khursheed, S. Memon, A. Imran, "HPLC method development and validation for simultaneous estimation of minoxidil and finasteride in topical solution," Pakistan Journal of Pharmaceutical Sciences, 2016, 29(5): 1595- 1599.
[9]. N. Prasad, R. Chandrasekhar, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Drug Delivery and Therapeutics, 2018, 8(3): 26-30.
[10]. R. Kandarapu, V. Macherla, K. Dasari, "Development and validation of an HPLC method for the simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Analytical Science and Technology, 2019, 10(1): 11.
[11]. M.R. Swamy, S.A. Patil, P.S. Choudhari, K.R. Mahajan, "Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of minoxidil and finasteride in topical formulation," Journal of Chromatography Science, 2010, 48(9): 723-728.
[12]. Aggarwal, V. Kaur, "Development and validation of a novel RP-HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Analytical & Pharmaceutical Research, 2016, 3(4): 00062.
[13]. V.K. Singh, M.K. Srivastava, R. Misra, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Indo American Journal of Pharmaceutical Research, 2016, 6(9): 3103-3109.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

[14]. V. Singhal, S. Jain, S. Maheshwari, "Development and validation of RP-HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," International Journal of Analytical Chemistry, 2017, 2017: 6572538.
[15]. Zaidan, R. Tahir, A. Al-Khdhairawi, "Development and validation of stability- indicating HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Iraqi Journal of Pharmaceutical Sciences, 2018, 27(1): 76-84
[16]. A. Al-Majed, M. El-Kousy, A. Al-Jenoobi, A. Al-Suwayeh, "A validated HPLC method for the simultaneous determination of minoxidil and finasteride in pharmaceutical formulations," Journal of Chromatographic Science, 2005, 43(8): 421- 424
[17]. R. Sharma, P. Gupta, S. Dureja, M. Jain, "Development and validation of an RP- HPLC method for simultaneous determination of minoxidil and finasteride in a topical solution," Journal of Pharmaceutical Analysis, 2016, 6(5): 279-283.
[18]. G. Yüksel, D. Duman, A. Özkan, "Development and validation of a stability- indicating RP-HPLC method for the simultaneous determination of minoxidil and finasteride in topical solution," Chromatographia, 2009, 70(9-10): 1421-1425.
[19]. P. Rajput, R. Kaur, R. Singh, H. Kaur, "Development and validation of HPLC method for simultaneous determination of minoxidil and finasteride in topical formulation," International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(7): 169-174
[20]. S. Basavaiah, K. Ramakrishna, "Development and validation of a stability-indicating HPLC method for simultaneous determination of minoxidil and finasteride in topical formulation," Asian Journal of Chemistry, 2011, 23(3): 1073-1076
[21]. S. Sahoo, S. Mohapatra, R. Sahoo, R. Behera, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," International Journal of Advances in Pharmaceutical Analysis, 2017, 7(1): 1-6.
[22]. M. Rani, R. Reddy, K. Rao, A. Reddy, "Development and validation of a new RP- HPLC method for simultaneous estimation of minoxidil and finasteride in topical formulation," International Journal of Pharmaceutical Sciences and Research, 2012, 3(9): 3529-3533.
[23]. R. Ali, M. Shaikh, M. Badiger, N. Nandibewoor, "Development and validation of an HPLC method for the simultaneous determination of minoxidil and finasteride in topical solution," International Journal of Pharmaceutical Research and Analysis, 2017, 7(2): 78-85.
[24]. S. Hiremath, P. Shirse, V. Birajdar, S. Potdar, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," International Journal of Pharmaceutical and Chemical Sciences, 2012, 1(1): 23-29.
[25]. M. Niazi, M. Saleem, A. Ghafoor, N. Kanwal, A. Khan, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical formulation," International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(5): 95-98.
[26]. P. Yadav, K. Patel, K. Patel, S. Patel, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," International Journal of Chemical and Pharmaceutical Analysis, 2014, 1(1): 1-5.
[27]. M. Aboul-Enein, A. Sharaf El-Din, A. Salem, "Development and validation of a stability-indicating HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Liquid Chromatography & Related Technologies, 2019, 42(17-18): 563-571.
[28]. M. Soliman, A. Taha, H. El-Sayed, F. Farid, H. Elkady, "Development and validation of an HPLC method for the simultaneous determination of minoxidil and finasteride in topical solution," Journal of Applied Pharmaceutical Science, 2014, 4(6): 61-66.
[29]. M. Abdelkawy, A. Ragab, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Liquid Chromatography & Related Technologies, 2013, 36(2): 142-152.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

[30]. Mohamed, M. Al-Shehri, A. Al-Qahtani, S. Al-Maiman, S. Al-Deeb, "Development and validation of HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Saudi Pharmaceutical Journal, 2017, 25(5): 680-686.

[31]. K. Karthick, R. Narendiran, K. Manikandan, M. Ramanathan, "Development and validation of a simple HPLC method for the simultaneous determination of minoxidil and finasteride in topical solution," Journal of Pharmacy Research, 2012, 5(6): 3326- 3328.

[32]. S. Jaiswal, A. Jaiswal, S. Nagaria, S. Gupta, "Development and validation of RP- HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Analytical & Bioanalytical Techniques, 2017, 8(5): 1-5.

[33]. N. Sharma, R. Jangir, A. Singh, N. Singh, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," World Journal of Pharmacy and Pharmaceutical Sciences, 2016, 5(3): 1020- 1028.

[34]. S. Deshmukh, S. Devkate, S. Shelke, A. Sonawane, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," International Journal of Pharmaceutical and Chemical Sciences, 2015, 4(1): 245-252.

[35]. R. Farhan, A. Mallick, A. Ahmad, A. Ali, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Pharmaceutical Analysis, 2012, 2(5): 346-350.

[36]. M. Pathan, S. Desai, H. Parmar, V. Patil, M. Kuchekar, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," World Journal of Pharmaceutical Research, 2014, 3(10): 1521-1535.

[37]. N. Anwar, M. Farooq, M. Mahmood, Z. Ullah, M. Khan, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Chemical and Pharmaceutical Research, 2016, 8(3): 1212-1218.

[38]. M. Jha, S. Kumar, M. Mandal, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Drug Delivery & Therapeutics, 2019, 9(3-s): 267-271.

[39]. V. Patil, H. Parmar, M. Pathan, M. Kuchekar, "Development and validation of a stability-indicating HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Chromatographic Science, 2015, 53(10): 1757-1763.

[40]. S. Panigrahi, S. Mohapatra, S. Nayak, "Development and validation of a reversed phase HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Chromatographic Science, 2014, 52(7): 665-670.

[41]. M. Asif, A. Waseem, R. Ali, M. Malik, M. Iqbal, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Chemical and Pharmaceutical Sciences, 2014, 7(4): 321-325.

[42]. V. Gupta, S. Pande, V. Patil, S. Gupta, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Analytical Science and Technology, 2016, 7(1): 1-7.

[43]. S. Anbu, S. Prabhu, P. Anandharamakrishnan, "Development and validation of an HPLC method for simultaneous determination of minoxidil and finasteride in topical solution," Journal of Chromatographic Science, 2014, 52(4): 313-319.

[44]. A. Singh, R. Singh, "Development and validation of an HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Chemical and Pharmaceutical Research, 2016, 8(2): 401-408.

[45]. B. Bhatnagar, S. Dixit, S. Sharma, S. Bhatnagar, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution," Journal of Pharmaceutical and Biomedical Analysis, 2015, 114: 183-189.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

[46]. Patel J. et al. (2022). "A strategic specific and sensitive quantitative analysis of minoxidil and finasteride by HPLC method in bulk and marketed formulations." Journal of Chromatographic Science, 60(1-2), e535-e541.
[47]. Saowapak Teerasong et al. (2022). "Silver nanoparticle oxidation-based assay for the detection of minoxidil in topical hair-growth formulations." Analytical Biochemistry, 646, 114307.
[48]. ArparNgampanya et al. (2021). "PBPK modeling of scalp absorption for topical drug delivery of finasteride and minoxidil." Journal of Pharmaceutical Sciences, 110(5), 2055-2068.
[49]. D.S. Shah et al. (2021). "A rapid RP-HPLC method for the simultaneous quantification of minoxidil and finasteride in a topical solution for alopecia." Journal of Pharmaceutical Analysis, 11(1), 84-91.
[50]. J. Mol. Sci. et al. (2020). "Platelet-Rich Plasma (PRP) Efficacy in Androgenic Alopecia (AGA) Treatment: A Systematic Review and Meta-Analysis." Journal of Molecular Sciences, 21(20), 7581. DOI: 10.3390/ijms21207581.
[51]. S.T. Nemane et al. (2019). "Development and Validation of a Simple RP-HPLC Method for Quantitative Estimation of Finasteride in Pharmaceutical Dosage Forms." Indian Journal of Pharmaceutical Education and Research, 53(4), 610-616. DOI: 10.5530/ijper.53.
[52]. Khadeerunnisa, S., Maheswaraiah, B., & Madhusudhan, P. (2019). "|Development and validation of UV spectrophotometric method for estimation of Minoxidil in tablet dosage form". International Journal of Pharmaceutical Sciences and Research, 10(4), 1913-1917.
[53]. Lee, J. H., Kim, J. E., Seo, J. S., Park, M. H., & Kwon, O. S. (2018). "Simultaneous identification and quantification of 13 hair-growth compounds in food and dietary supplements by ultra-performance liquid chromatography-photodiode array and liquid chromatography-quadrupole time-of-flight mass spectrometry". Food Additives & Contaminants: Part A, 35(1), 80-89.
[54]. Ponnilavarasan, I., Suriyaprakash, T. N. K., Ravi, T. K., & Kumar, M. K. (2018). "Simultaneous determination of Minoxidil and Finasteride in bulk and liquid formulations" by RP-HPLC. Journal of Liquid Chromatography & Related Technologies, 41(7), 387-393.
[55]. Park, H. N., Ahn, J. Y., Kim, J. M., & Lee, H. J. (2018). "Rapid simultaneous determination of hair-growth compounds in adulterated products using UHPLC- MS/MS. Journal of Pharmaceutical and Biomedical Analysis", 159, 409-417.
[56]. Gaikwad, S. S., Ingale, K. G., Kadam, V. J., & Kadam, S. S. (2017). "Development and validation of second derivative spectrophotometric method for simultaneous estimation of minoxidil and finasteride in bulk and pharmaceutical formulation". Journal of Applied Pharmaceutical Science, 7(06), 073-079.
[57]. Pate, N., Bari, S. B., & Tekade, A. R. (2015). "A validated RP-HPLC method for simultaneous determination of finasteride and minoxidil in pharmaceutical dosage form. Journal of Chromatographic Science", 53(10), 1603-1608.V
[58]. Motevalian, M., Fakhari, A. R., Akbari, J., & Amirian, J. (2017). "Development and validation of RP-HPLC method for simultaneous determination of clindamycin phosphate and tretinoin in topical gel formulation". Pharmaceutical Sciences, 23(2), 121-127.
[59]. Reddy, P. S., Ramya, K. V. M., & Rao, J. V. (2019). " Development and validation of a new stability-indicating RP-HPLC method for the simultaneous determination of tazarotene, clindamycin phosphate, and betamethasone dipropionate in topical gel formulation". Journal of Chromatographic Science, 57(5), 427-436.
[60]. Bhadra, S., Thakur, A., Jain, P., & Garg, S. (2018). "Development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of clindamycin phosphate and adapalene in a topical gel formulation". Journal of Liquid Chromatography & Related Technologies, 41(10), 580-589.
[61]. Goyal, H., Shah, D. A., & Shah, S. (2019). "Development and validation of RP-HPLC method for estimation of fluocinolone acetonide and clioquinol in a cream formulation". International Journal of Pharmacy and Pharmaceutical Sciences, 11(7), 44-50.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

[62]. El-Enany, N., Belal, T. S., Abd El-Razeq, S. A., & Salem, H. (2011). "Simultaneous determination of fluocinolone acetonide, hydroquinone and tretinoin in a topical solution by RP-HPLC". Journal of Pharmaceutical Analysis, 1(1), 62-69.

[63]. Kishore, L., Kaur, A., & Madan, S. (2013). "Simultaneous determination of adapalene and benzoyl peroxide in a topical gel formulation by RP-HPLC method". Journal of Chromatographic Science, 51(3), 211-216.

[64]. Dongre, V. G., Dehghan, M. H. G., & Dhaneshwar, S. R. (2017). "Development and validation of RP-HPLC method for simultaneous estimation of tazarotene and mometasone furoate in topical cream formulation". Journal of Liquid Chromatography & Related Technologies, 40(11-12), 535-541.

[65]. Chen, C. H., Chiou, Y. T., Chang, C. Y., Wang, Y. Y., & Lin, Y. H. (2016). "Development and validation of a reversed-phase high-performance liquid chromatography method for the determination of minocycline in a topical gel". Journal of Food and Drug Analysis, 24(2), 361-366.

[66]. Dongre, V. G., Shrivastava, S. K., & Dhaneshwar, S. R. (2015). "Development and validation of RP-HPLC method for the determination of betamethasone dipropionate in topical cream formulation". Journal of Liquid Chromatography & Related Technologies, 38(9), 1058-1064.

[67]. De Souza, R. A., De Souza, R. L., & Santana, C. C. (2019). "Validation of a RP- HPLC method for the determination of venlafaxine in human plasma: application to a bioequivalence study". Journal of Analytical Methods in Chemistry, 2019.

[68]. Wang, Y., Cao, Y., Zhang, J., & Wang, S. (2020). "Development and validation of an RP-HPLC method for the simultaneous determination of four phenolic acids in Salvia miltiorrhiza". Analytical Methods, 12(27), 3288-3294.

[69]. Chen, J., Zhao, M., Guo, J., Yang, Y., & Li, Y. (2020). "Development and validation of an RP-HPLC method for simultaneous determination of ibuprofen and its impurities in ibuprofen tablets". Journal of Chromatographic Science, 58(5), 430-437.

[70]. Al-Majed, A. A. (2020). "Development and validation of a stability-indicating RP- HPLC method for the determination of levofloxacin in the presence of its alkaline degradation products". Journal of Pharmaceutical and Biomedical Analysis, 178, 112976.

[71]. Islam, M. S., Islam, M. N., Islam, M. N., Hassan, M. M., & Das, A. K. (2020). "Development and validation of an RP-HPLC method for simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in tablet dosage form". Journal of Pharmaceutical Analysis, 10(3), 242-249.

[72]. Parajuli, D., Chhetri, B., & Subedi, Y. P. (2020). "Development and validation of an RP-HPLC method for simultaneous determination of rosuvastatin calcium and ezetimibe in tablet dosage form". Journal of Pharmaceutical Analysis, 10(1), 37-43.

[73]. Khamar, M., Vora, R., Patel, J., & Patel, M. (2018). "RP-HPLC method development and validation for estimation of dolutegravir sodium in bulk and its tablet dosage form". Journal of Chromatographic Science, 56(7), 632-638.

[74]. Vazzana, M., Di Pumpo, F., Di Corcia, D., & Armentano, M. F. (2018). "Development and validation of an RP-HPLC method for the simultaneous determination of tenofovir disoproxil fumarate, emtricitabine and efavirenz in human plasma. Analytical Methods", 10(19), 2258-2265.

[75]. Al-Shehri, M. M., & El-Kousy, S. M. (2017). "Stability-indicating RP-HPLC method for the determination of pyrantel pamoate in bulk and pharmaceutical formulation". Journal of Analytical Science and Technology, 8(1), 2.

[76]. Aggarwal, G., Bhatia, M. S., Singh, G., & Kumar, V. (2017). "Development and validation of an RP-HPLC method for the simultaneous determination of lopinavir and ritonavir in a pharmaceutical formulation". Journal of Liquid Chromatography & Related Technologies, 40(4), 222-229.

[77]. M. Niazi, M. Saleem, A. Ghafoor, N. Kanwal, A. Khan, "Development and validation of RP-HPLC method for simultaneous estimation of minoxidil and finasteride in topical formulation", International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(5): 95-98.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, December 2023

[78]. P. Yadav, K. Patel, K. Patel, S. Patel, "Development and validation of HPLC method for simultaneous estimation of minoxidil and finasteride in topical solution", International Journal of Chemical and Pharmaceutical Analysis, 2014, 1(1): 1-5.

[79]. M. Aboul-Enein, A. Sharaf El-Din, A. Salem, "Development and validation of a stability-indicating HPLC method for simultaneous determination of minoxidil and finasteride in topical solution", Journal of Liquid Chromatography & Related Technologies, 2019, 42(17-18): 563-571.

[80]. M. Soliman, A. Taha, H. El-Sayed, F. Farid, H. Elkady, "Development and validation of an HPLC method for the simultaneous determination of minoxidil and finasteride in topical solution", Journal of Applied Pharmaceutical Science, 2014, 4(6): 61-66.