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Simultaneous Estimation of Minoxidil and Aminexil In Bulk and Pharmaceutical Formulations by RP-HPLC Method

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of minoxidil and aminexil in bulk and pharmaceutical formulations. Separation of minoxidil and aminexil was successfully achieved on a Agilent C18 (150 mm x 4.6 mm x 5µ Make: Waters) or equivalent in an isocratic mode utilizing 0.1% orthophosphoric acid and methanol in the ratio of 60:40 *v/v* at a flowrate of 1 ml/min. The developed method was found to be linear in the concentration range of 50µg/ml to150 µg/ml for minoxidil and 50 µg/ml to 150 µg/ml for aminexil. The value of the correlation coefficient was found to be 0.999 for both minoxidil and aminexil. The LOD and LOQ for aminexil were found to be 0.0146 and 0.0486 mg/ml, respectively, whereas for minoxidil the values are 0.046 mg/ml and 0.155 mg/ml, respectively. This method was found to be good percentage recovery for minoxidil and aminexil were found to be 99.00 and 100.00, respectively indicates that the proposed method is sufficiently accurate. The specificity of the method shows good correlation between retention times of standard with the sample. Therefore, the method specifically determines the analyte in the sample without interference from excipients that are commonly present in the pharmaceutical dosage forms. The method was validated according to ICH guidelines for linearity, range, accuracy, precesion, specificity and robustness.

Key words: RP-HPLC, Minoxidil, Aminexil.

INTRODUCTION

Minoxidil

Minoxidil, chemically known as 6-Piperidin-1-ylpyrimidine-2,4-diamine 3-oxide (Figure 1), is a potent direct-acting peripheral vasodilator that reduces peripheral resistance and produces a fall in blood pressure[1]. Minoxidil is widely used for the treatment of hair loss. It has been proven clinically effective in both the prevention of loss and in establishing varying degrees of hair re-growth in males and females

suffering pattern baldness. Minoxidil must be used indefinitely for continued support of existing hair follicles and the maintenance of any experienced hair regrowth[2,3].

The minoxidil is official in US pharmacopeia, which describes a liquid chromatographic method for its quantification[4]. In the literature different methods have been proposed for its determination in pharmaceutical formulations and biological samples, which include high-performance liquid chromatography with UV detection[5,6], electrochemical detection[7,8], GC[9], and radioimmunoassay[10].

Aminexil

Aminexil is the trade name for kopexil. Kopexil, chemically known as 2,4 diamino pyridine 3 oxide (Figure 2) is an altered form of minoxidil without the side effects. It is a genuine anti-hair-loss innovation that fights against the stiffening of roots. In both men and women hair loss is connected to the deterioration of the roots. Kopexil increases the volume of hair in the growth stage by working on the deep structure of the roots. It rejuvenates the hair roots so that healthy hair growth can persist. Fibrosis condition of the hair roots causes blood vessels to compress and shorten the life span of the hair follicle. This problem can be corrected by kopexil[11].

The detailed literature survey has indicated that there is no report on the simultaneous determination of minoxidil and aminexil by HPLC with UV detection. Therefore, in the present investigation a simple, sensitive, precise and accurate HPLC method for the simultaneous determination of minoxidil and aminexil was developed and validated.

EXPERIMENTAL

Instrumentation

The chromatographic separation was carried out on a HPLC system with Waters 2695 alliance equipped with binary HPLC pump, Waters 2998 PDA detector and Waters Empower2 software.

Pure form of drugs and solvents

1. Minoxidil and Aminexil was obtained as a gift sample from Lara drugs Pvt Ltd., Hyderabad.
2. Ortho phosphoric acid of analytical grade was obtained from Sd Fine Chemicals Ltd., Mumbai.
3. HPLC grade methanol was purchased from Merck (India) Ltd., Mumbai.

Preparation of mobile phase

The mobile phase was prepared by mixing 0.1% orthophosphoric acid and methanol in the ratio of 60:40 *v/v*. The mobile phase was also used as diluent.

HPLC Conditions

Agilent C18, (150 mm × 4.6 mm; 5µm) analytical column was used for separation of minoxidil and aminexil. The chromatographs were recorded using Empower2 software. The mobile phase was pumped at a flow rate of 1 ml/min. It was filtered through 0.45 ¼m filter and degassed before use. The elution was monitored at 223 nm and the injection volume was 10 ¼L. The oven temperature was 30°C. the run time was 6 minutes.

Preparation of standard solution

Accurately weighed quantity, 2.5 mg of minoxidil and 0.75 mg of aminexil was transferred into 200 ml of volumetric flask and add 20 ml of diluent and sonicate for 15 min. Make up the volume with mobile phase.

Preparation of Sample Solution

Commercially available solution of 50 ml sample was measured in to 100 ml volumetric flask added 20ml of Diluent, Sonicate 20minutes Make up the volume with mobile phase.

Method validation

System Suitability Studies

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ±2 % Relative standard deviation range during routine performance of the method.

Specificity

Specificity is the ability to assess

unequivocally the analyte in the presence of components which may be expected to be present (Figures 3 and 4). Typically these might include impurities, degradants, matrix, etc.

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out in triplicate and the percentage recovery and standard deviation of were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table 2 & 3). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Figures 5, 6 & 7. From the data obtained, the developed RP-HPLC method was found to be precise (Table 4).

Linearity range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting peak area¼ against concentration of drugs. The slope and intercept value for calibration curve was y = 44363 x (R^2=0.999) for minoxidil and y = 44600x (R^2=0.999) for aminexil. The results shows that an excellent correlation exists between the peak areas and concentration of drugs within the concentration range indicated above. The linearity curves for minoxidil and aminexil are shown in Figs 8 and 9.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms (figures 10 and 11), which demonstrated that the developed RP HPLC

Limits of quantification and detection (LOD and LOQ)

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of aminexil and minoxidil. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels.

(a) LOQ = 10 σ / S

Table 1: System suitability parameters

Parameters	Minoxidil	Aminexil
Correlation Coefficient	0.999	0.999
Regression Equation	y =43363x	y = 44600x
LOD	0.046	0.0146
LOQ	0.155	0.0486
Theoretical plates	4055	6908
Tailing	1.17	1.12

Table 2: Accuracy for minoxidil

Spiked Level	Sample Weight	Sample Area	µg/ml Added	µg/ml Found	% Recovery	Mean
50%	25	2071238	6.188	6.15	99	98
	25	2025682	6.188	6.02	97	
	25	2034012	6.188	6.04	98	
100%	50	4117610	12.375	12.23	99	100
	50	4128874	12.375	12.26	99	
	50	4193216	12.375	12.45	101	
150%	75	6266170	18.563	18.61	100	100
	75	6267048	18.563	18.61	100	
	75	6260495	18.563	18.59	100	

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response; s = slope of the calibration curve.

Table 3: Accuracy for aminexil

Spiked Level	Sample Weight	Sample Area	µg/ml Added	µg/ml Found	% Recovery	Mean
50%	25	2210444	1.875	1.86	99	100
	25	2214963	1.875	1.87	100	
	25	2231841	1.875	1.88	100	
100%	50	4438236	3.750	3.74	100	100
	50	4478563	3.750	3.77	101	
	50	4486034	3.750	3.78	101	
150%	75	6650104	5.625	5.60	100	100
	75	6685719	5.625	5.63	100	
	75	6647304	5.625	5.60	100	

Table 4: Precision Studies

Sample No.	Sample Wt (mg)	Area (Aminexil)	Area (minoxidil)	%Assay (Aminexil)	%Assay (minoxidil)
1	50	4151071	4426383	99	99
2	50	4193859	4457056	100	100
3	50	4138023	4481933	98	101
4	50	4105298	4452708	98	100
5	50	4137563	4413639	98	99
6	50	4190028	4463492	100	100

Table 5: Robustness for minoxidil

Parameter	Inj	RT	Area	USP Tailing	USP Plate count
TEMP-1	1	3.380	5073841	1.24	4106
TEMP-2	1	2.253	3336814	1.15	3852
FLOW-1	1	3.374	5058205	1.26	4196
FLOW-2	1	2.250	3337216	1.16	3777

Table 6: Robustness for aminexil

Parameter	Inj	RT	Area	USP Tailing	USP Plate count
TEMP-1	1	4.993	5466989	1.17	7347
TEMP-2	1	3.339	3635579	1.12	6282
FLOW-1	1	4.983	5444818	1.17	7493
FLOW-2	1	3.340	3639412	1.12	6467

RESULTS AND DISCUSSION

System suitability results were given in Table 1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1. Therefore the proposed method is suitable for analysis with good precision. The method specificity was confirmed by Figures 3 and 4. Those figures are minoxidil and aminexil standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves that the method is selective. The result given in Table 4 indicates that the method precision passed for both minoxidil and aminexil studies. The method accuracy was evaluated by recovery studies. Minoxidil and aminexil recovery was found to be 99% & 100% as per ICH (97% - 103%) and very low percentage RSD shown that the method

Table 7: LOD and LOQ of minoxidiland aminexil

S.No.	Sample type	inj	Name of sample	RT	Area
1	LOD	1	Minoxidil	2.728	3.590351
2	LOQ	1	Minoxidil	2.717	10.58319
1	LOD	1	Aminexil	4.032	3.469054
2	LOQ	1	Aminexil	4.013	9.682998

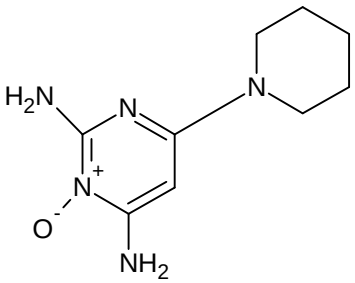

Fig. 1: Chemical structure of minoxidil

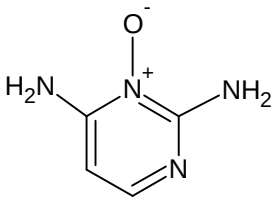

Fig. 2: Chemical structure of aminexil

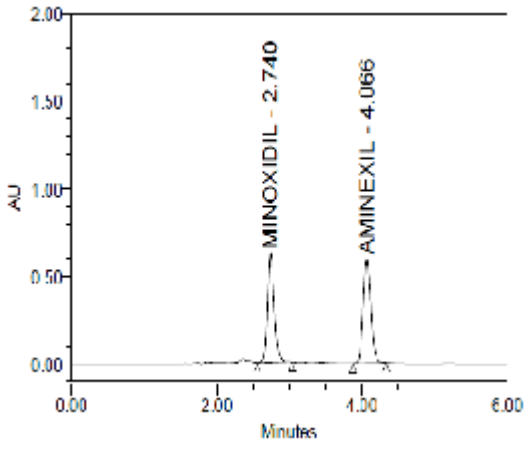

Fig. 3: Chromatogram of standard minoxidil and aminexil

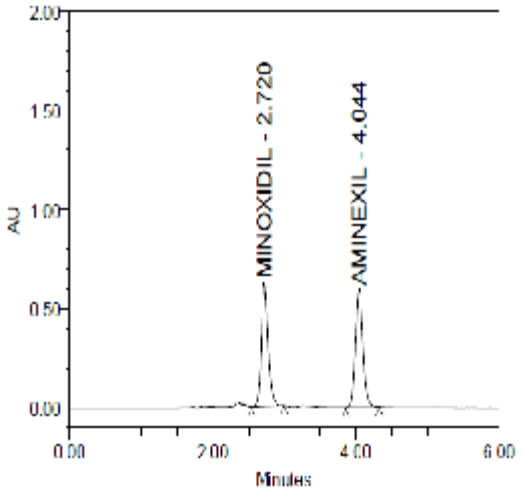

Fig. 4: Chromatogram of minoxidil and aminexil in formulation

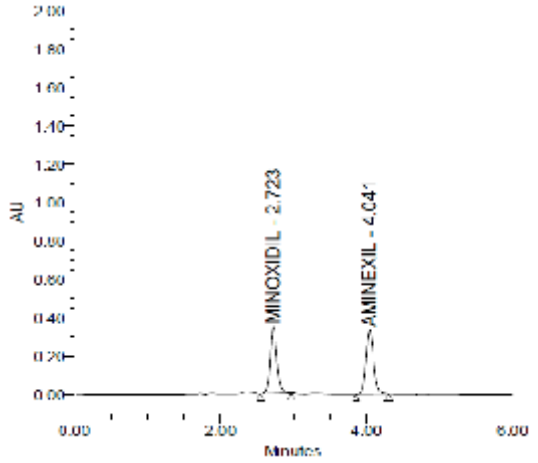

Fig. 5: Chromatogram of minoxidil and aminexil at 50% accuracy level

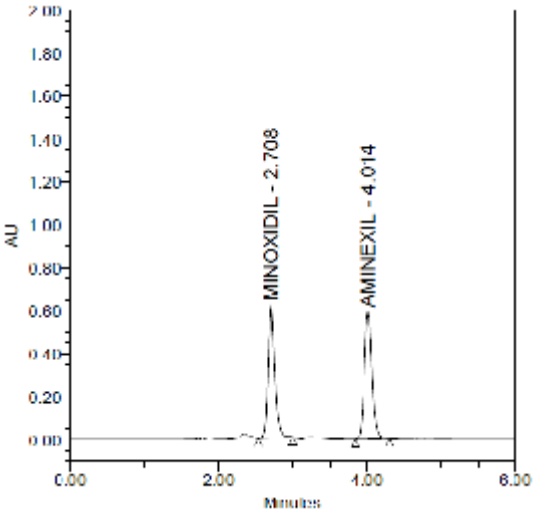

Fig. 6: Chromatogram of minoxidil and aminexil at 100% accuracy level

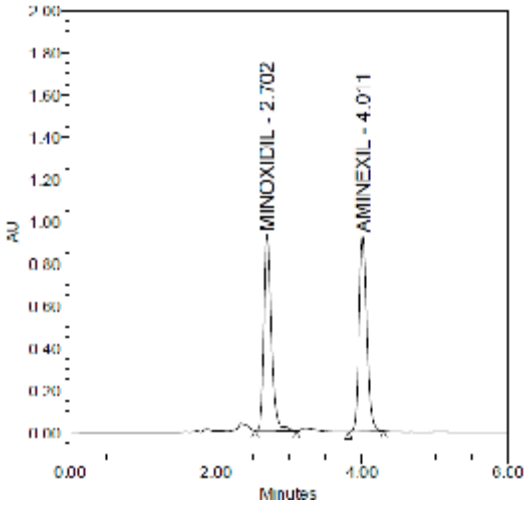

Figure 7: Chromatogram of minoxidil and aminexil at 150% accuracy level

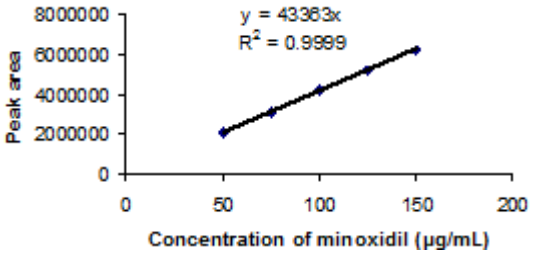

Fig. 8: Linearity curve for minoxidil

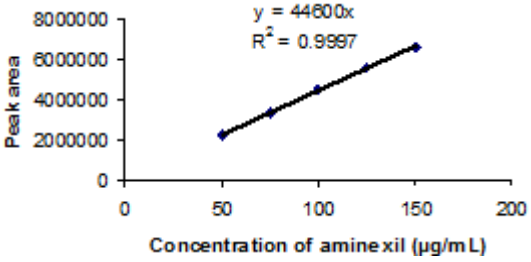

Fig. 9: Linearity curve for aminexil

is accurate the results are shown in Tables 2 and 3. Linearity calibration curve was given in Figures 8 and 9. The graph was plotted by taking five different concentrations versus peak areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be Y= 44363 for minoxidil and y = 44600 for Aaminexil. Method robustness results were given in Tables 5 & 6. LOQ and LOD results were given in Table 7. The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of minoxidil and aminexil in pharmaceutical dosage forms.

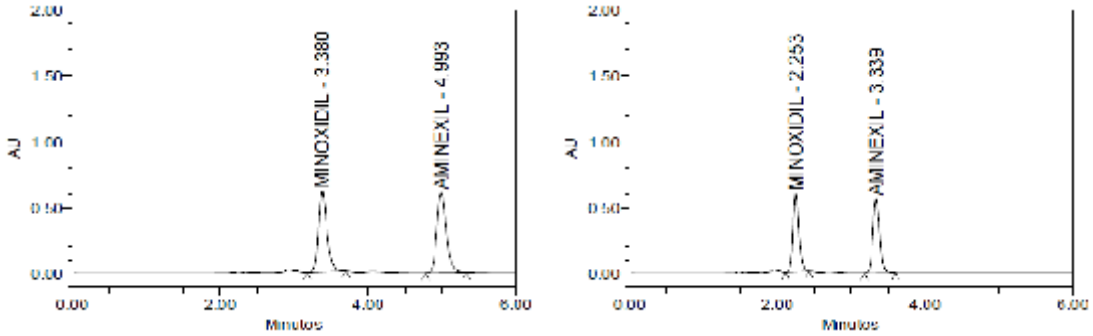

Fig. 10: Effect of flow rate of mobile phase

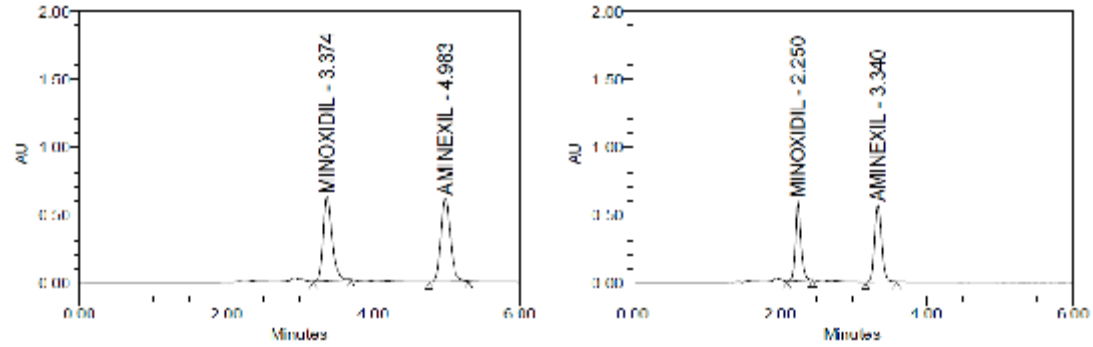

Fig. 11: Effect of column temperature

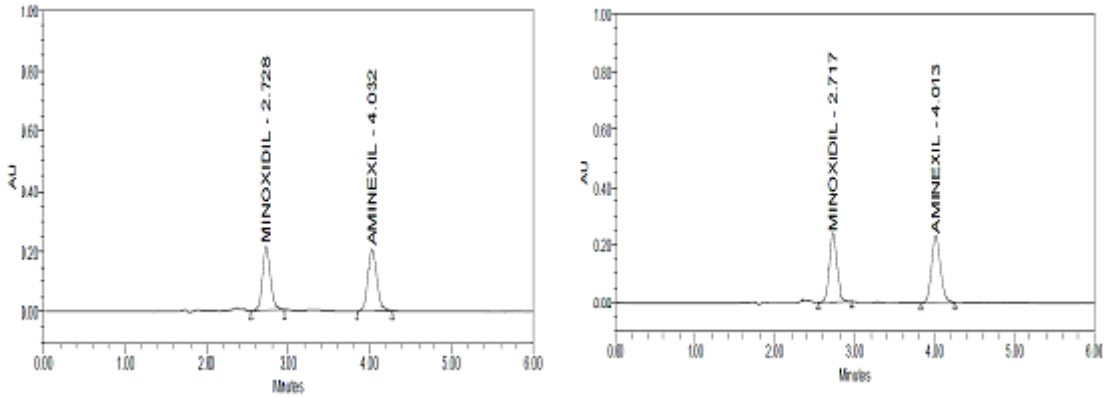

Fig. 12: Chromatograms of minoxidil and aminexil at LOD and LOQ levels

CONCLUSION

The proposed HPLC method can easily and conveniently adopted for routine quality control analysis of minoxidil and aminexil in pure and its pharmaceutical dosage forms.

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REFERENCES

1. Sica, D.A. *J. Clin. Hypertens (Greenwich).* **2004**, *6*, 283-287.
2. Olsen, E.A.; Dunlap, F.E.; Funicella, T.; Koperski, J.A.; Swinehart, J.M.; Tschen, E. H.; Trancik, R.J. *J. Am. Acad. Dermatol,* **2002***, 47,* 377–385.
3. Price, V.H.; Menefee, E.; Strauss, P.C. J. Am. Acad. Dermatol. **1999**, *41*, 717–721.
4. The United States Pharmacopeia, United States Pharmacopeia Convention, USP 23ed., Rockville Pais. 1995.
5. Asmus, O.; Landis, J.B.; Grant, M.E.; Havel, H.A. *J. Pharm. Sci.,* **1984**, *73*, 1290-1293.
6. Zarghi, A.; Shafaati, A.; Foroutan, S.M.; Khoddam, A. *J. Pharm. Biomed. Anal.,* **2004**, *36*, 377-379.
7. Carrum, G..; Abernethy, D.R.; Sadhukhan, M.; Wrigt, C.E. *J. Chromatogr., B: Biomed Sci. Appl.,* **1986**, *381*, 127-133.
8. Golden, M.H.; Zoutendam, P.H. *J. Pharm. Biomed. Anal.,* **1987**, *5*, 543-546.
9. Kamiewaska, T.; Kublin, E.; Weclawowicz, E.; Kwiatkowska, B. *Pharm. Pol.,* **1987**, *43*, 508-511.
10. Royer, M.E.; Cao, H.; Gillberson, T.J.; MacCall, J.M.; Jonston, K.T.; Stryd, R. *J. Pharm. Sci.* **1977**, *66,* 1266-1269.
11. Trüeb, R.M.; de Viragh, P.A. *Praxis.* **2001**, *90*, 241-248.