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New analytical method development for the estimation of minoxidil by rp hplc method

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ABSTRACT

In the present work RP-HPLC method for estimation Minoxidil has been developed. The proposed methods are precise, accurate and do not suffer from any interference due to common excipient. The validation parameters according to I.C.H Q2B guidelines were studied. The accuracy of the methods was proved by performing recovery studies in newly developed formulations. Values greater than 98% indicate that the proposed method is accurate for the analysis of drug. A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Minoxidil API. The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of Minoxidil different formulations. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

Keywords: RP-HPLC, Minoxidil, Validation parameters, Estimations

INTRODUCTION [12,3,4,5,6]

Quality is important and essential in every product or service but it is more vital in medicines as it is related to life. A Compromise in pharmaceutical quality is nothing but playing with the life of consumer. Pharmaceutical analysis plays a key role here. The terms quality, quality control, quality assurance, total quality management are correlated. The ultimate goal of all these is to provide a product with good quality, safety, efficiency, purity, strength and identity[1]. High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids.

Solvent delivery system

Types of pumps in HPLC

Syringe pump (screw driven) 1. Reciprocating pump 2.Single piston reciprocating pump- Dual piston reciprocating pump- Reciprocating diaphragm pump Pneumatic pump- Direct pressure pump Amplifier pump

System Suitability Parameters

The parameters that are affected by the changes in chromatographic conditions are Retention time(t_R), Resolution (R_S), Capacity factor ($k`$), Selectivity(α), Number of Theoretical plates (N), HETP, Asymmetry factor, Tailing factor.

HPLC method development

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling. During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separations on a C_{18} column with UV detection.

Sample and Analyte Information

This information is useful for the selection of appropriate sample preparation procedures as well as the initial detection and chromatographic modes. The chemical structure of the

analyte furnishes data on molecular weight and the nature of the functional groups. Particular attention should be directed to acidic, basic, aromatic, or reactive functional groups from which estimates of pKa, solubility, chromophoric, or stability data can be inferred. If sufficient purified reference material is available, solubility studies of the analyte in common solvents such as water, alcohol, ether, and hexane should be conducted.

Selection of chromatographic mode[7]

Table 1: Choice of chromatographic mode

Sample type	Analytes type	Common mode
Macromolecules	Organic polymers	GPC
(MW > 2,000)	Biomolecules	SEC, RPC, IEC, HILIC, HIC
Organics	Polar	RPC, NP, HILIC
(MW < 2,000)	Medium polarity	RPC
	Nonpolar	RPC, NARP, NP
	Ions, ionizable compounds	RPC (ion suppression), RPC-IP,
		IEC, HILIC
Preparative	All	NP, RPC, GPC, IEC

Selection of Flow Rate

Generally flow rate shall not be more than 2.0 ml/min. The flow rate shall be selected based on the following data. The flow rate which gives least retention times, good peak symmetries, least back pressures and better separation will be selected.

Column Efficiency& Plate Number

The number of theoretical plates or plate number is a measure of column efficiency. An efficient column produces sharp peaks and can separate many sample components in a relatively short time. Theoretical plates (N) are defined as the square of the ratio of the retention time divided by the standard deviation of the peak (σ).

$$N = \left(\frac{t_R}{\sigma}\right)^2 = \left(\frac{4t_R}{w_b}\right)^2 = 16\left(\frac{t_R}{w_b}\right)^2.$$

Resolution

The goal of most HPLC analyses is the separation of one or more analytes in the sample from all other components present. Resolution (Rs) is a measure of the degree of separation of two adjacent analytes. Rs is defined as the difference in retention times of the two peaks divided by the average peak width. The resolution of two adjacent peaks can be calculated by using the formula

$$R_s = \frac{t_{R2} - t_{R1}}{\left(\frac{w_{b1} + w_{b2}}{2}\right)} = \frac{\Delta t_R}{w_b}$$

ANALYTICAL METHOD VALIDATION

Method validation[9] can be defined as per ICH as, "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Specificity/Selectivity[9]

Linearity, Range accuracy precision repeatability intermediate precision reproducibility limit of detection based on visual evaluation based on signal-to-noise based on the standard deviation of the response and the slope limit of quantification ruggedness [1]

METHOD VALIDATION

Accuracy

Recovery study

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Minoxidil were taken and added to the pre-analyzed formulation of concentration 30µg/ml. From that percentage recovery values were calculated. The results were shown in Table-2.

Table 2: Accuracy Readings

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Injected	Conc. Recovered	Peak Area		
S_1 : 80 %	24	24.022	472546	101.916	Mean= 101.3317
S_2 : 80 %	24	23.937	471121	101.736	S.D. = 0.860928
S_3 : 80 %	24	24.206	475612	100.343	% R.S.D.= 0.849614

S_4 : 100 %	30	30.103	574216	100.113	Mean= 101.018
S_5 : 100 %	30	30.521	581211	101.394	S.D. = 0.787478
S_6 : 100 %	30	30.575	582121	101.547	% R.S.D.= 0.779542
S_7 : 120 %	36	36.041	673514	100.858	Mean= 100.2287
S_8 : 120 %	36	36.502	681214	99.737	S.D. = 0.57304
S_9 : 120 %	36	36.557	682132	100.091	% R.S.D.= 0.571732

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Minoxidil (API) the percent relative standard deviations were calculated for Minoxidil is presented in the Table-3.

Table 3: Repeatability Results of Precision

HPLC Injection Replicates of Minoxidil	Retention Time (Minutes)	Peak Area
Replicate – 1	3.816	598647
Replicate – 2	3.815	586484
Replicate – 3	3.799	584624
Replicate – 4	3.797	598642
Replicate – 5	3.815	584213
Replicate -6	3.816	579874
Average	**3.809667**	**588747.3**
Standard Deviation	**0.00907**	**7966.538**
% RSD	**0.238081**	**1.353134**

Intermediate Precision

Intra-assay & inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Minoxidil revealed that the proposed method is precise.

Table 4: Results of intra-assay & inter-assay

Conc. Of Minoxidil(API) (µg/ml)	Observed Conc. Of Minoxidil (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
24	23.98	1.03	24.02	1.02
30	30.06	0.97	30.09	0.97
36	36.03	0.95	35.93	0.65

Linearity & Range

The calibration curve showed good linearity in the range of 10-50µg/ml, for Minoxidil (API) with correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of y = 16721x + 70860 for Minoxidil. To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 10, 20, 30, 40 and 50µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed[28] under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). The results which are given in Table below were within acceptable limits.

Preparations

10µg/ml: 0.1ml of Minoxidil stock solution in 10ml of volumetric flask diluted up to the mark with mobile phase.
20µg/ml: 0.2ml of Minoxidil stock solution in 10ml of volumetric flask diluted up to the mark with mobile phase.
30µg/ml: 0.3ml of Minoxidil stock solution in 10ml of volumetric flask diluted up to the mark with mobile phase.
40µg/ml: 0.4ml of Minoxidil stock solution in 10ml of volumetric flask diluted up to the mark with mobile phase.
50µg/ml: 0.5ml of Minoxidil stock solution in 10ml of volumetric flask diluted up to the mark with mobile phase.
Acceptance criteria: correlation coefficient should not be less than 0.990.

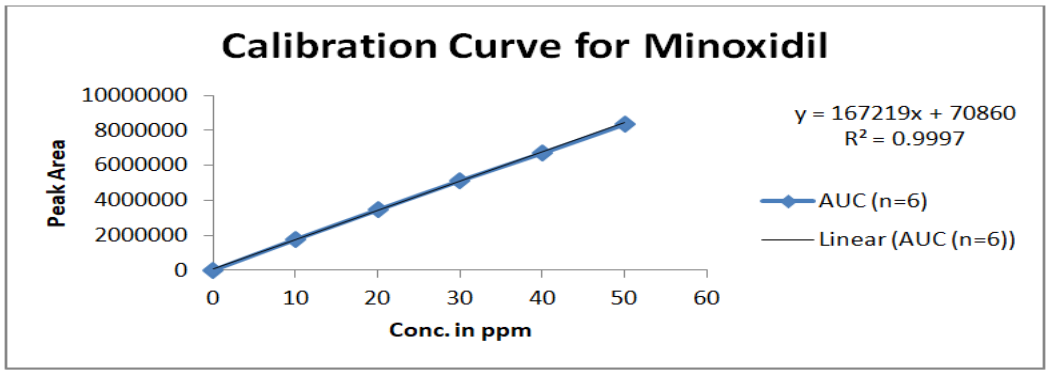

Fig 1: Calibration curve of Minoxidil (API).

Table 5: Linearity results of Minoxidil

CONC.	AUC (n=6)
0	0
10	1768452
20	3468421
30	5146243
40	6735124
50	8389756

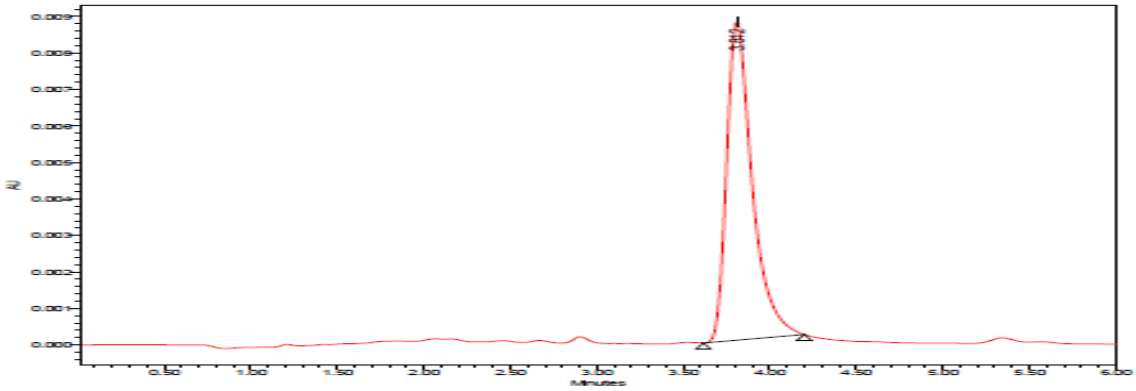

Fig 2: Chromatogram for 10 ppm

Table 6: Results of 10 ppm

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Minoxidil	3.812	1768452	4120	1.06

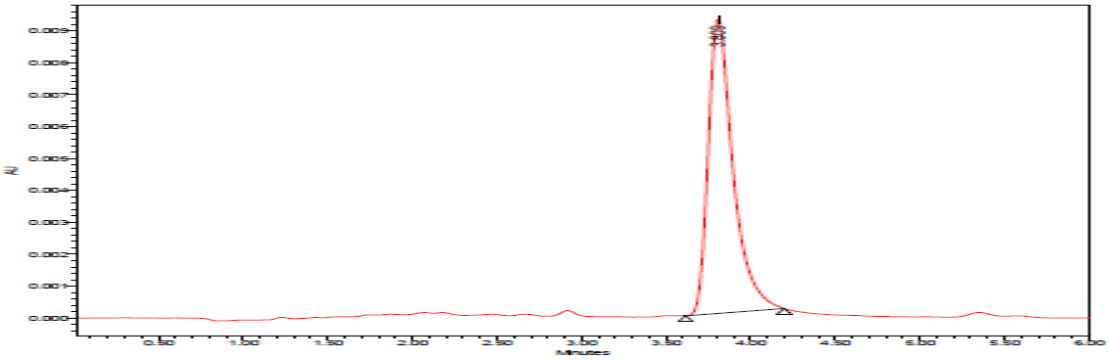

Fig 3: Chromatogram for 20 ppm

Table 7: Results of 20 ppm

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Minoxidil	3.809	3468421	4142	1.10

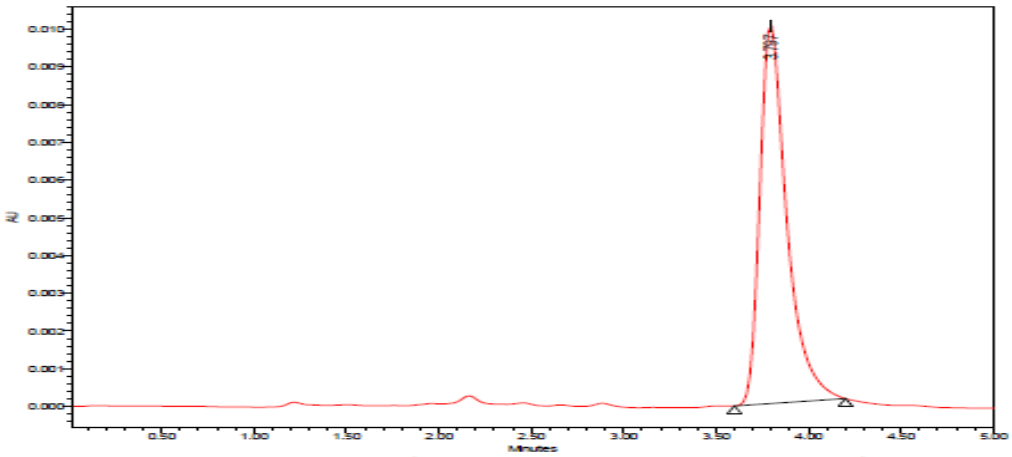

Fig 4: Chromatogram for 30 ppm

Table 8: Results of 30 ppm

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Minoxidil	3.797	5146243	4140	1.21

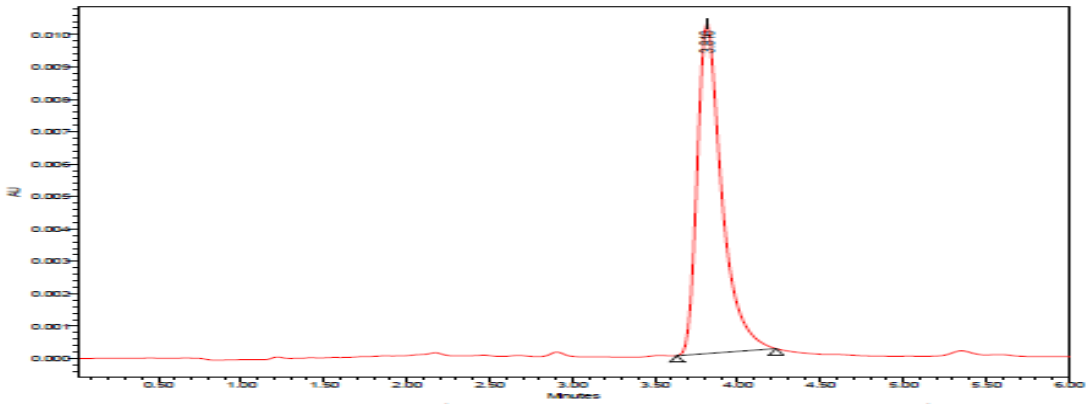

Fig 5: Chromatogram for 40 ppm

Table 9: Results of 40 ppm

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Minoxidil	3.816	6735124	3987	1.29

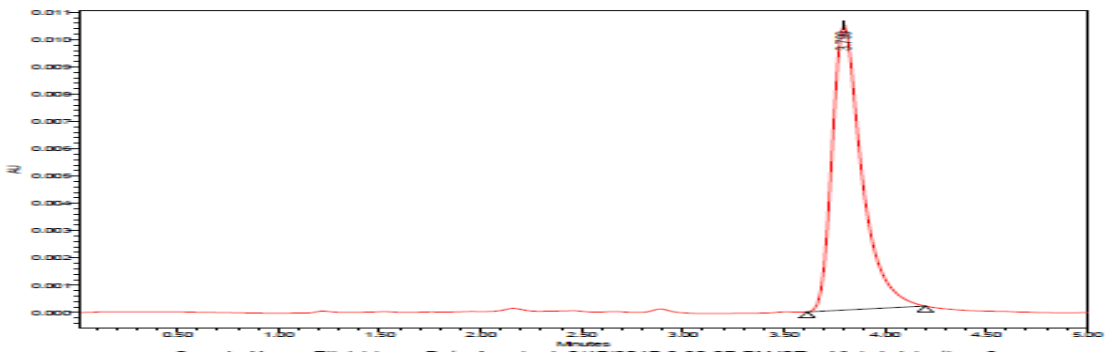

Fig 6: Chromatogram for 50 ppm

Table 10: Results of 50 ppm

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Minoxidil	3.799	8389756	4325	1.19

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-41, % RSD < 2%) the developed RP-HPLC method for the analysis of Minoxidil (API).

Table 11: Results of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.26
Flow (0.9 ml/min)	0.09
Wavelength of Detection (244 nm)	0.13
Wavelength of detection (240 nm)	0.18

LOD & LOQ

Limit of detection is the lowest concentration of analyte in a sample which can be detected, but not necessarily quantities, as an exact value under the stated experimental conditions. Limit of quantification is the lowest concentration of analyte in a sample which can be quantitatively determined with acceptable precision and accuracy under the stated experimental conditions.

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S

where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27µg/ml respectively.

ASSAY OF MINOXIDIL IN DOSAGE FORM

Estimation of MINOXIDIL in TABLET Dosage Form

MINOXIDIL 10 mg

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of HPLC grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table- 12

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \quad \text{Avg. Wt} \quad = \text{mg/tab}$$

Where: AT = Peak Area of drug obtained with test preparation, AS = Peak Area of drug obtained with standard preparation, WS = Weight of working standard taken in mg, WT = Weight of sample taken in mg, DS = Dilution of Standard solution, DT = Dilution of sample solution, P = Percentage purity of working standard

Assay was performed as described in previous chapter. Results obtained are tabulated below:

Table 12: Assay of MINOXIDIL Tablets

Brand name of Tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
LONITAB-10 (Cipla Pharmaceuticals Limited)	10	9.9 (±0.65)	99(±0.57)

The assay of LONITAB-10 Tablets containing Minoxidil was found to be 99(±0.57) %.

CONCLUSION

In the present work RP-HPLC method for estimation Minoxidil has been developed. The proposed methods are precise, accurate and do not suffer from any interference due to common excipients. The validation parameters according to I.C.H Q2B guidelines were studied. The accuracy of the

methods was proved by performing recovery studies in newly developed formulations. Values greater than 98% indicate that the proposed method is accurate for the analysis of drug. A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Minoxidil API. The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of Minoxidil different formulations. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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