Simultaneous Estimation of Minoxidil and Finasteride by RP-HPLC in Presence of Soy Lecithin Excipient in Lotion Dosage Form

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Abstract: **A simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the simultaneous determination of Minoxidil and Finasteride from bulk and liquid formulations in the presence of soy lecithin excipient. Chromatographic separation was achieved isocratically on a Phenomenex C18 column (250×4.6 mm, 5 µ particle size) using a mobile phase, Phosphate buffer: acetonitrile: methanol (50:50 v/v) (adjusted to pH 5.8 with NaOH) in the ratio of 30:70. The flow rate was 1.2 ml/min and effluent was detected at 211nm. The retention time of Minoxidil and Finasteride were 3.16, and 8.23 min respectively. Linearity was observed in the concentration range of 50 to 250 µg/ml, 1 to 5 µg/ml for Minoxidil and finasteride with correlation coefficient 0.999 for both the drugs. Percent recoveries obtained for both the drugs were 99.275-99.72 and 98.54-99.3% w/w. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed can be used for the routine analysis of Minoxidil and Finasteride in combined dosage form.**

Key words: **RP-HPLC Method; Phenomenex; Minoxidil; Finasteride; soy lecithin**

I. INTRODUCTION

Finasteride,N-(1,1-dimethylethyl)-3-oxo-(5α,17β)-4-azaandrost-1-ene-17-carboxamide., a type II 5 alpha reductase inhibitor, The mechanism of action of Finasteride is based on its preferential inhibition of 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 (approximately 20-30% after 6-24 months of continued therapy). In men with androgenic alopecia, the mechanism of action has not been fully determined, but 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, Minoxidil , 6-piperidin-1-ylpyrimidine-2,4-diamine 3-oxide. The mechanism by which minoxidil promotes hair growth is not fully understood. Minoxidil contains the nitric oxide chemical moiety and may act as a nitric oxide agonist. Similarly, minoxidil is a potassium channel opener, causing hyper polarization of cell membranes. Minoxidil is less effective when there is a large area of hair loss. In addition, its effectiveness has largely been demonstrated in younger men who have experienced hair loss for less than 5 years. Minoxidil use is indicated for central (vertex) hair loss only. Minoxidil is also a vasodilator. Hypothetically, by widening blood vessels and opening potassium channels, it allows more oxygen, blood, and nutrients to the follicle. This may cause follicles in the telogen phase to shed, which are then replaced by thicker hairs in a new anagen phase.

Literature review reveals various works on UV[1-4], HPLC[4-7], of Minoxidil , UV[8], HPLC[9-12], LC MS/MS[13], UPLC[14], UPLC MS/MS[15] of Finasteride. To the author's best knowledge, no method has been reported for simultaneous estimation of Minoxidil and Finasteride in combined lotion dosage form. Hence, it was thought to be of interest to develop RP-HPLC methods for simultaneous estimation of Minoxidil and Finasteride in bulk as well as in combined lotion dosage form.

II. EXPERIMENTAL

Chemicals, Reagents and Solutions

Minoxidil (>98%) was purchased from Cipla R&D Mumbai, Finasteride purchased from Dr.Reddys Hyderabad. Acetonitrile and methanol (HPLC-grade) was purchased from Sigma Aldrich. Analytical grade potassium dihydrogen phosphate was obtained from SD Fine chemicals LTD Mumbai. The other chemicals were of analytical-reagent grade. Morr F (Batch No. TIM 12002) were manufactured by Intas Pharmaceutical Industry Co., Ltd, Ahmedabad. An amount of minoxidil and finasteride was weighed accurately, dissolved in methanol and then diluted to 1mg/ml. Working standard solutions were prepared by diluting the stock solution with the mobile phase. All standard solutions were stored at 20°C. Before RP-HPLC analysis, the working solution was filtered through a 0.22-µm membrane prior to injection into the HPLC system.

Sample Preparation

One gram liquid was extracted with 10ml of methanol and filtered through whatmann filter paper No: 41. The final mixed sample solution was prepared, corresponding to 150ug/ml of Minoxidil and 3µg/ml of finasteride. After filtration through a 0.22-µm membrane filter, the chromatographic analysis was carried out.

Chromatography

Chromatographic separation and quantitative determination were performed using a high-performance liquid chromatography containing a Shimadzu pump (model LC-20AT), a PDA detector (***SPD-6AV***, Shimadzu), a Rheodyne 7120 with a 20-µL loop. As the stationary phase, a Phenomenex RP-18 column, 5 µm particle size, 250 × 4 mm (***Merck, Darmstadt, Germany***), was used. The mobile phase consisted of 70 vol of acetonitrile: methanol and 30 vol of phosphate buffer (pH 5.8). The flow rate of the mobile phase was 1.2 ml/min. The wavelength of the PDA detector was set at 211nm.

Method Validation

A linear calibration curve was generated by plotting the peak area versus minoxidil and finasteride concentrations. The slope, intercept, and correlation coefficient values were estimated by using a least squares regression analysis. The limit of detection (LOD) was defined as 3 times the signal-to-noise ratio (S/N). The limit of quantification (LOQ) was defined as the lowest concentration in the linear calibration curve. Validation of HPLC method for minoxidil and finasteride was then carried out. Quality control samples containing 150ug/ml of minoxidil and 3ug/ml of finasteride were used to evaluate the precision and accuracy of the proposed method. Intraday variability and precision were determined by analyzing the quality control sample in duplicate on the same day. Interday variability and precision were evaluated by injecting duplicate processed samples at each control concentration for 5 days. The assay precision was reflected by the relative standard deviation (RSD). The accuracy of the method was assessed by the use of the standard addition technique. Known amounts of pure minoxidil and finasteride were added to the liquid dosage form in which the sample concentration was determined. The amounts of analytes recovered were estimated by use of the regression equation of the calibration plots. The relative recovery in the liquid dosage form was evaluated at 50ug/ml of minoxidil and 1ug/ml of finasteride. The accuracy study was performed three times.

III. RESULTS AND DISCUSSION

Sample Analysis

The developed RP-HPLC method was applied to analyze Minoxidil and finasteride in Morr F liquid dosage form. The chromatogram of a sample is shown in Fig. 3. Based on the peak area of Fig. 3, the content of Minoxidil and finasteride in Morr F liquid dosage form is about 50mg/ml and 1mg/ml with an RSD of 0.91% (n= 6) according to the previously established linear regression equation.

Linearity

Under the above described experimental conditions, a linear relationship was established by plotting the peak area against the drug concentration. The concentration ranges were found to be 50–250ug/ml and 1–5ug/ml for minoxidil and finasteride respectively. Linear regression analysis of the data gave the following equations:

Write y=mx+c equation of both drugs

Statistical analysis of the data gave a high value of the correlation coefficient (r) of the regression equation; small values of the intercept (Sa) and of slope (Sb) and small values of the percentage relative standard deviation (Table No 1). These data proved the linearity of the calibration graph.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ and LOD were calculated according to ICH Q2R1 recommendations using the following equation [26]:

LOQ= 10XSD/Slope and LOD= 3.3 XSD/Slope

Where SD is the standard deviation of the intercept of the calibration curve

LOQ values were found to be 18.11ug/ml and 2.54ug/ml, while LOD values were found to be 5.97ug/ml and 0.83ug/ml for minoxidil and finasteride respectively.

Accuracy and Precision

In order to further evaluate the validity of the proposed method for the assay of minoxidil and finasteride in real samples, a recovery experiment was carried out. The Sample was spiked with a known amount of pure drug and the recovery was determined. The satisfactory recovery of 99.10-99.72for minoxidil and 98.54-99.34 for finasteride in the Morr F liquid dosage form indicates that the proposed method is reliable for the quantification of the Minoxidil and finasteride content in the sample.

Intraday and interday precisions were assessed using single concentration and six replicates. The relative standard deviations were found to be very small, indicating reasonable repeatability and intermediate precision of the proposed method **(Table no 2)**

Precision data for the determination of Minoxidil and finasteride by the proposed method

Robustness of the Method

The stability of the peak area with deliberate changes in the experimental parameters indicates the robustness of the proposed method. These parameters include methanol: ACN concentration (70 ± 1% (v/v)) and the ionic strength of buffer (***0.04 M ± 0.005***). These minor changes did not greatly affect the peak area ratios of any of the drugs.

Selectivity

The selectivity of the method was investigated by observing any interference encountered from common tablet excipients. It was shown that these compounds did not interfere with the results of the proposed method.

IV. CONCLUSION

It was concluded that the developed RP-HPLC method was found to be very simple, reliable and selective for providing satisfactory accuracy and precision. The methods are suitable for routine quantitative analysis in pharmaceutical dosage forms. In this present study the retention time of minoxidil and finasteride was found to be 3.16min and 8.23min respectively. Furthermore the method has been shown to be specific and selective. From this current study concluded that lecithin in the formulation as the excipient used for permeation enhancer is which has strong absorbance in the UV region it is minimized by this extraction method methanol and the mobile phase used for the RP-HPLC method developed for drugs.

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Table No 1: Analytical performance data

Parameter	Minoxidil	Finasteride
Linearity range (μg mL^{-1})	50-250	1-5
Intercept (a)	95871	14750
Slope (b)	43911	38369
Correlation coefficient (r)	0.999	0.999
Percentage relative standard deviation, %RSD	1.303	0.816
Limit of detection, LOD (μg/ml)	5.97	0.83
Limit of quantitation, LOQ (μg/ml)	18.11	2.54

Table No 2: Assay of the drugs in pure form

Compound	**Amount taken**	**Amount recovered**	**%recovery**
Minoxidil	80	79.42	99.275±0.43
	100	99.1	99.10 ± 0.40
	120	119.67	99.72±0.27
Mean±SD		99.39+20.19	
Finasteride	80	78.83	98.54 ± 0.45
	100	99.10	99.10 ± 0.40
	120	119.21	99.34±0.37
Mean±SD		99.04+20.1	

Table No 3: Inter day studies

Day	**Conc. of MINOX (μg/ml)**	**Peak Area**	**% RSD**	**Conc. of FNS (μg/ml)**	**Peak Area**	**% RSD**
Day 1	150	6480024 6553981 6467521 6538754 6512345 6577874	0.65	3	1285736 1279543 1288757 1268774 1254718 1287169	1.04
Day 2	150	6478956 6533789 6536578 6578893 6576892 6437789	0.85	3	1276548 1286738 1297893 1287945 1250870 1278754	1.25
Day 3	150	6475022 6556979 6499739 6566179 6489911 6498986	0.57	3	1277691 1285367 1274705 1293903 1265241 1291547	0.87

Table No 4: Intraday studies

No of Injection	Conc. Of MINOX (µg/ml)	Peak Area	% RSD	Conc. of FNS (µg/ml)	Peak Area	% RSD
6	150	6549350 6481452 6479201 6538641 6474394 6357835	1.05132	3	1201452 1216530 1194672 1214525 1214525 1201962	1.3937

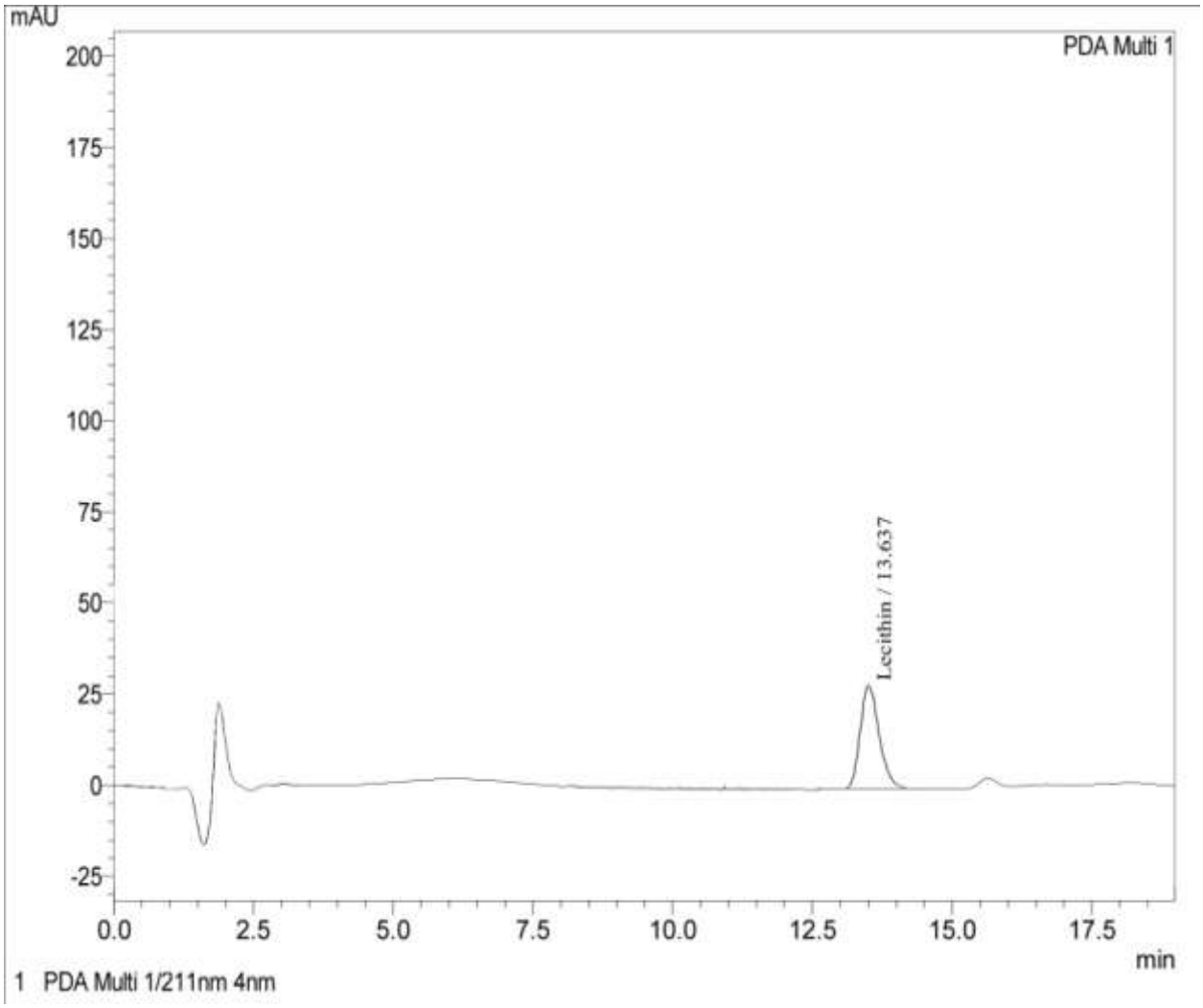

Figure 1.Standard chromatogram of Lecithin

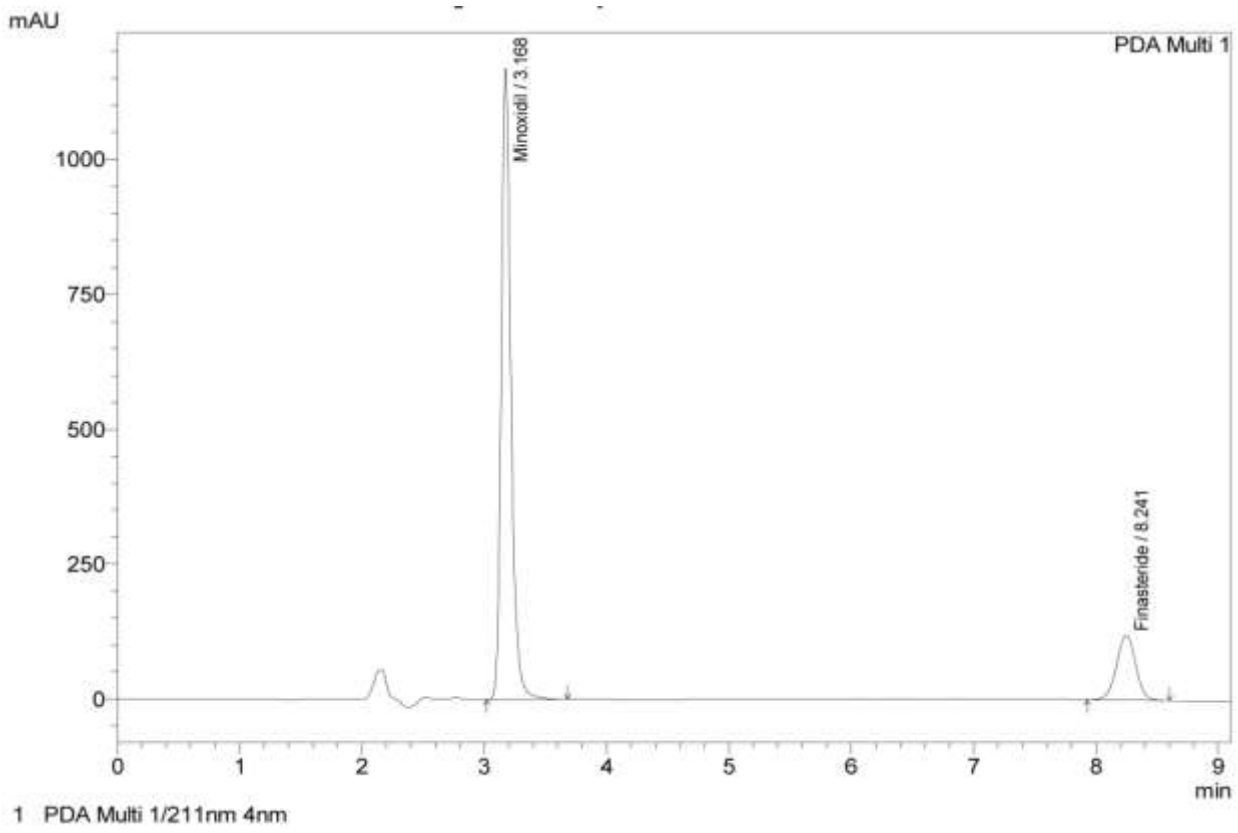

Fig 2.Standard chromatogram of Minoxidil and Finasteride

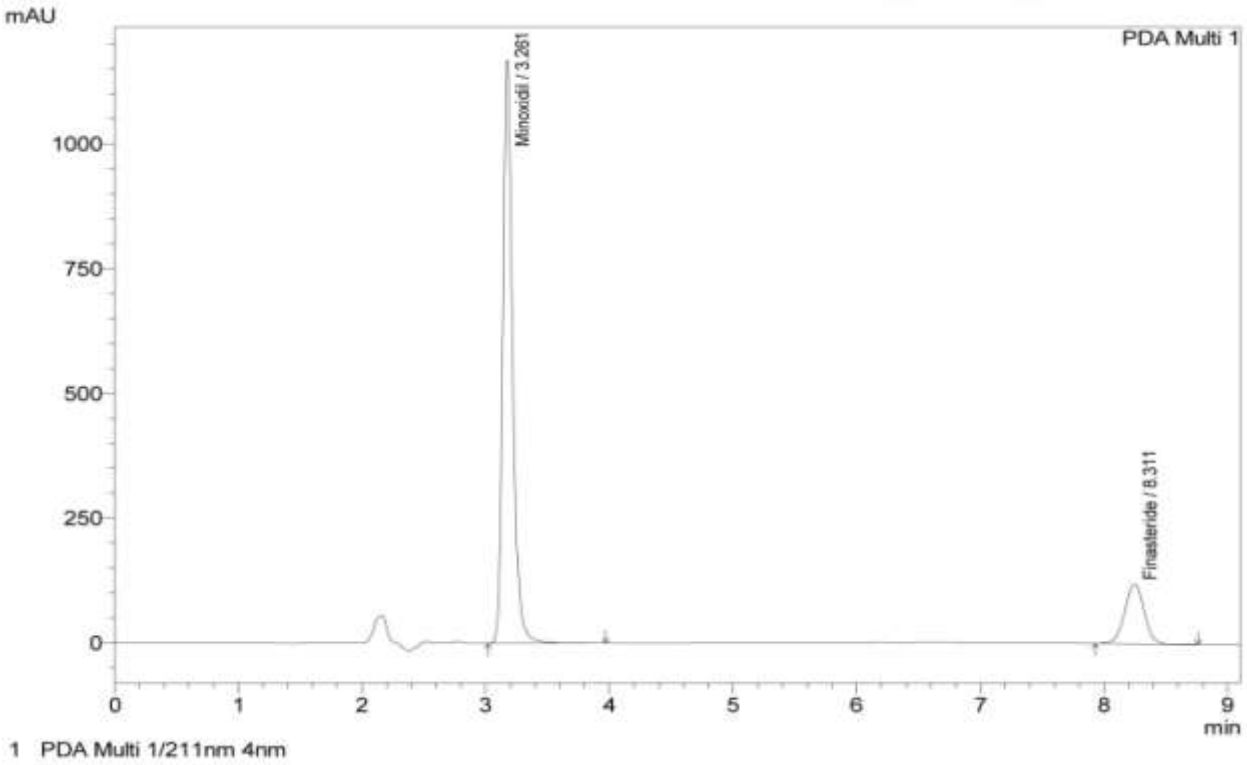

Figure 3 Sample chromatogram