IJPSR (2015), Vol. 6, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 May, 2015; received in revised form, 12 June, 2015; accepted, 10 September, 2015; published 01 November, 2015

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS DETERMINATION OF MINOXIDIL AND FINASTERIDE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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Keywords:

Finasteride, Minoxidil, RP-HPLC, Validation

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ABSTRACT: A simple, precise, rapid, accurate RP-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 (25 cm × 4.6 mm, 5 µ particle size) using a mobile phase comprising methanol: water along with 0.5 % triethyl amine (TEA), pH 6.38 adjusted with ortho phosphoric acid (OPA) in a ratio of 70:30 v/v. The flow rate was 1ml/min and eluents were detected by UV detector at 210 nm. Retention times were found to be 4.661 min and 10.005 min of Finasteride and Minoxidil respectively. The calibration curve was linear over the range of 12-24 µg/ml of Minoxidil and 0.4-0.8 µg/ml of Finasteride. The results of all the validation parameters were well within their acceptance values. 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 the two drugs. The proposed method is suitable for routine quality control analysis.

INTRODUCTION: Chemically, Minoxidil (MINO) is 2, 4-diamino-6-piperidinopyrimidine 3-oxidemainly 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-tert-butylcarbamoyl)-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 [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(11).4882-85

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(11).4882-85

The literature survey revealed methods for estimation of MINO by spectrophotometry [3], RP-HPLC [4], stability indicating HPLC [5], electrochemical determination [6] and voltammetric method [7] and methods for estimation of FINA by spectrophotometry [8], RP-HPLC [9-10] and stability indicating HPLC method [11]. Recently one RP-HPLC method [12] has been published for simultaneous determination of both the drugs in their pharmaceutical formulation.

MATERIALS AND METHODS:

Preparation of standard stock solution:

An accurately weighed 300 mg of MINO and 10 mg of FINA were transferred to 100 ml volumetric flasks, dissolved in a HPLC grade methanol and diluted up to 100 ml with HPLC grade methanol to give solution of concentration of a 3000 µg/ml of MINO and 100 µg/ml of FINA.

Preparation of working standard solution: 300 µg/ml of MINO and 10 µg/ml of FINA solution

were prepared by diluting 10 ml of MINO and FINA stock solution with HPLC grade methanol in 100 ml volumetric flasks.

Preparation of sample solution:

1 ml of formulation equivalent to 30 mg of MINO and 1 mg of FINA was taken in 100 ml volumetric flask, 60 ml of HPLC grade methanol was added, sonicated for 30-35 min and diluted to 100 ml with HPLC grade methanol. This solution was filtered through Whatmann filter paper No.41 (300 µg/ml of MINO and 10 µg/ml of FINA). From this solution 0.5 ml was transferred to 10 ml volumetric flask and volume made up with methanol to achieve a concentration of 15 µg/ml of MINO and 0.5 µg/ml of FINA.

Chromatographic conditions

Chromatographic separation was performed on C_{18} (Shim pack xR ODS II 25 cm × 4.6 mm, 5 µ particle size) column. The composition of mobile phase was methanol: water along with 0.5 % TEA, pH 6.38 adjusted with OPA (70:30 v/v). The mobile phase was delivered at a flow rate of 1 ml/min. Analysis was performed at ambient temperature. Injection volume was 20 µl and detection was carried out at 210 nm.

RESULTS:

Optimization of chromatographic conditions:

Chromatographic conditions were optimized by changing the mobile phase composition. Different experiments were performed to optimize the mobile phase and results are given in **Table 1**. Optimized mobile phase consists of a methanol: water along with 0.5 % TEA, pH 6.38 adjusted with OPA (70:30 v/v) with flow rate of 1 ml/min. FINA and MINO were eluted at 4.661 and 10.005 minutes respectively with a run time of 13 minutes. **Fig.1** depicts typical chromatogram for simultaneous determination of MINO and FINA.

TABLE 1: OPTIMIZATION OF MOBILE PHASE

Trial	Mobile phase	Flow rate (ml/min)	Ratio	Retention time (min)		Remark
				MINO	FINA	
1.	ACN: water	1	70:30	7.717	4.582	Peak was observed but height of Minoxidil peak was more as compare to Finasteride peak.
2.	ACN: water	1	36:46	15.457	4.652	Both broad peaks were observed with tailing.
3.	ACN: water with 0.1 % TEA, pH 6.92 adjusted with OPA	1	60:40	8.138	4.038	Peak was observed with tailing, splitting and less theoretical plates.
4.	Methanol: water	1	70:30	10.225	4.680	Peaks were observed with tailing.
5.	Methanol: water with 0.5 % TEA, pH 6.38 adjusted with OPA	1	70:30	10.005	4.661	Sharp peaks with good resolution.

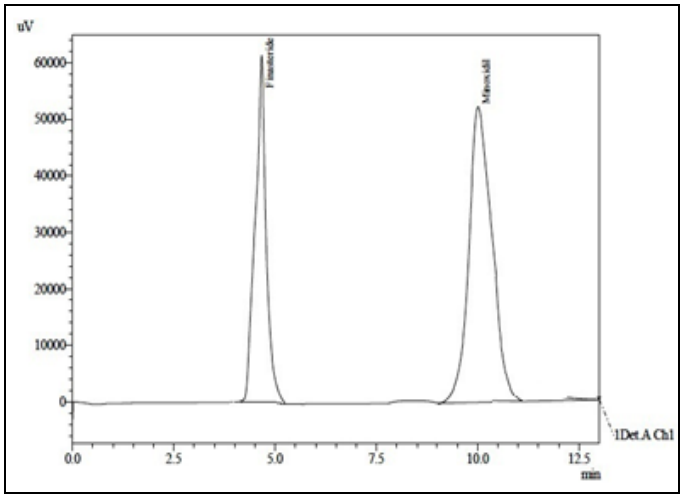

FIG. 1: TYPICAL CHROMATOGRAM OF MINO AND FINA UNDER

Optimized Chromatographic Conditions:

Analysis of marketed formulation:

Sample solution containing of 15 µg/ml of MINO and 0.5 µg/ml of FINA was injected into the chromatographic system and peak area were measured for MINO and FINA. The percentage of label claim of MINO and FINA were calculated and was found to be 99.616 % and 99.04 % respectively.

System suitability test:

Working standards were injected to the HPLC system in 6 replicates and retention time, theoretical plates, tailing factor, capacity factor and resolution were observed for system suitability of

proposed method. The results of system suitability test are given in **Table 2**.

TABLE 2: RESULTS OF SYSTEM SUITABILITY TEST

Parameters	Data obtained	
	MINO	FINA
Retention time	10.005 min	4.661 min
Repeatability (n=6)	3009280	1326542
Theoretical plates per column	7525.465	8106.656
Symmetry factor/Tailing factor	1.077	1.019
Resolution	-	6.262
Capacity factor	1.167	

Method validation:

Linearity: Response of detector in terms of peak area to the concentration of analytes in solution was assessed by injecting triplicate injection of solutions containing MINO in concentration range of 12-24 µg/ml and 0.4-0.8 µg/ml for FINA. Peak area vs. concentration graph was plotted and correlation co-efficient values of MINO and FINA were found to be 0.9923 and 0.9939 respectively.

Precision and accuracy:

The system precision of proposed method was determined by injecting solution having a concentration of 18 µg/ml of MINO and 0.6 µg/ml of FINA for six times and peak area was measured. The method precision of proposed method was determined by injecting solution having a concentration of 15, 18 and 21 µg/ml of MINO and 0.5, 0.6 and 0.7 µg/ml of FINA for three times and % RSD of peak area was calculated. The accuracy of proposed method was determined by calculating recoveries of MINO and FINA at 80%, 100% and 120% of test concentration. To the fixed amount of pre-analysed solution (9 µg/ml of MINO and 0.3 µg/ml of FINA) an increasing aliquots from working standard solution of MINO and FINA were added. The amounts recovered were calculated. The results of precision and accuracy are given in **Table 3**.

TABLE 3: RESULTS OF PRECISION AND ACCURACY

Parameters		MINO	FINA
Precision (%RSD)			
Repeatability (n=6)		0.0361	0.1085
Intraday (n=3)		0.0114-0.0133	0.0215-0.0275
Interday (n=3)		0.0243-0.0329	0.0405-0.0472
% Recovery ± S.D (n=3)	80%	100.73 ± 0.2616	100.27 ± 0.3889
	100%	100.66 ± 0.3889	100.83 ± 1.1737
	120%	99.95 ± 0.2757	100.83± 1.1737

Limit of Detection and Limit of Quantitation:

LOD was found to be 1.589 µg/ml and 0.047 µg/ml of MINO and FINA respectively using the formula:

$$LOD = 3.3 \times \frac{\sigma}{s}$$

LOQ was found to be 4.816 µg/ml and 0.142 µg/ml of MINO and FINA respectively using the formula

$$LOQ = 10 \times \frac{\sigma}{S}$$

Robustness:

Robustness, a deliberate change in the flow rate and mobile phase composition was made to evaluate the impact on the method. The results reveal that the method is robust enough. The results of robustness are summarized in **Table 4**.

TABLE 4: ROBUSTNESS STUDY OF MINO AND FINA

Sr. No.	Parameters	Variation	Assay ± S.D (n=3)		% RSD	
			MINO	FINA	MINO	FINA
1.	Flow rate (1 ± 0.2 ml/min)	0.8 ml/min	98.87 ± 0.0507	99.23 ± 0.0453	0.0512	0.0456
		1.0 ml/min	99.61 ± 0.3329	99.04 ± 1.4859	0.3342	1.5003

		1.2 ml/min	99.56 ± 0.9565	99.39 ± 0.5678	0.9607	0.5712
2.	Mobile phase (70:30 ± 2 v/v)	68:32	99.78 ± 1.2458	98.93 ± 0.9827	1.2485	0.9933
		70:30	99.61 ± 0.3329	99.04 ± 1.4859	0.3342	1.5003
		72:28	98.67 ± 0.3897	99.16 ± 0.7829	0.3949	0.7895

DISCUSSION: A new RP-HPLC method was developed and validated for simultaneous determination of MINO and FINA in combined pharmaceutical dosage form and assured the satisfactory precision and accuracy. The developed method is accurate enough to determine lower concentration of each drug in its pharmaceutical dosage form. The method has been found to be better because of use of a more economical and readily available mobile phase and lack of extraction procedures. The proposed method is more advantageous as compared to the reported method [12].

Incase of the reported method the chromatograms obtained are wide and have more tailing factor as compared to the proposed method. Further the peak shape and size in the reported method are poor as compared to the method proposed in this paper. Therefore it can be concluded that the proposed method is more reliable and can be used in laboratories with very high accuracy and a wide linear range.

CONCLUSION: The RP-HPLC method proposed in this paper is simple, accurate and precise for simultaneous determination of Minoxidil and Finasteride from its pharmaceutical dosage forms. The excipients in the formulation do not interfere with determination of the two drugs. Thus the method is specific enough to identify the two drugs from their formulation.

ACKNOWLEDGEMENT: The authors are heartily indebted to Shri D. D. Patel, President Om Gayatri Education and Charitable Trust and the Management of Pioneer Pharmacy Degree College for providing necessary research facilities.

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How to cite this article:
Patel N and Meshram D: Development and Validation of Analytical Method for Simultaneous Determination of Minoxidil and Finasteride in Pharmaceutical Dosage form by RP-HPLC Method. Int J Pharm Sci Res 2015; 6(11): 4882-85.doi: 10.13040/IJPSR.0975-8232.6(11).4882-85.