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Stability Indicating Method Development and Validation of Finasteride by High-Performance Thin-Layer Chromatography Studies

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The present research work determines the stability of finasteride by forced degradation studies. Finasteride was treated with hydrolytic stressors such as acid, alkali and neutral stress, further finasteride was also treated with oxidative, photolytic and thermal stress conditions. Method development, validation and separation of the degradation products for the drug were carried out by using high-performance thin-layer chromatographic system (HPTLC). The drug was found to degrade under basic and oxidative stress conditions. The mobile phase composition for HPTLC analysis consisted of chloroform-methanol (8:2 % v/v). The UV spectrophotometric spectra of finasteride showed λ_{max} at 210 nm; hence this wavelength was chosen for densitometric analysis. The R_F value for finasteride was found to be 0.57 with a compact spot. The method was validated as per ICH guidelines Q2(R1) and good linear relationship was observed in the concentration range of 200-1400 ng band^{-1}. Percent recovery for the drug was within limit and was found to be in the range of 98.52–99.66 %. For precision studies the % relative standard deviation (% RSD) was < 1.5 %. The developed HPTLC method has been successfully applied for the analysis of finasteride in tablet dosage form.

Keywords: Finasteride, Stability indicating method, HPTLC.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common disorder of the male urogenital tract frequently taking place in men over the age of 50 years [1]. Two main pharmacological classes of drugs are available; the selective α_1-adrenoreceptor blockers and the 5α-reductase inhibitors. 5α-reductase (5-AR) enzyme acts by converting testosterone to 5α-dihydrotestosterone (DHT). This enzyme is responsible for enlargement of prostate and hence 5α-reductase inhibitors are used in treatment of benign prostatic hyperplasia [2].

Finasteride is a 4-aza-3-oxosteroidal inhibitor of human 5α-reductase. Chemically it is [*N*-(1,1-dimethylethyl)-3-oxo-4-aza-5a-androst-1-ene-17b-carboxamide (Fig. 1). It belongs to the family of compounds referred to as 4-azasteroids. The 4-azasteriods act by blocking the intracellular metabolism of testosterone and thereby the more potent androgen dihydrotestosterone shows it action [3,4].

The drug finasteride is official in IP, BP and USP. Liquid chromatography method for estimation of finasteride is mentioned in IP [5], BP [6] and USP [7]. According to literature review UV spectrophotometric methods [8-10], gas chromatography [11], RP-HPLC, RP-HPLC-PDA method, stability indicating LC, LC-MS, UPLC [12-18] are reported for the estimation of finasteride alone and HPTLC method in combination with other drugs [19-21]. Alone as a single drug no stability indicating HPTLC method has been reported for finasteride.

Hence the aim of the present work was to develop and validate stability indicating HPTLC method which is simple, rapid, selective and cost effective for determination of finasteride in pharmaceutical formulation.

EXPERIMENTAL

Finasteride was kindly gifted from Cipla Lab limited, Mumbai (India). All other chemicals and reagents of analytical grade were purchased from Merck fine chemicals, Mumbai (Maharashtra, India) and used without purification.

HPTLC analysis: HPTLC method development and separation of drugs was performed on TLC plates pre-coated with silica gel 60 F254 (10 cm × 10 cm with 250 mm layer thickness) from E. Merck, Germany. Spotting of the sample was performed in the form of bands with a Camag 25 microlitre sample syringe (Hamilton, Bonaduz, Switzerland). The width of the spotted band was 6 mm. Application of sample was performed using Camag Linomat 5 (Switzerland) sample applicator. The distance between applications of two bands was 8 mm. Scanning speed employed was 20 mm/s. The dimension of

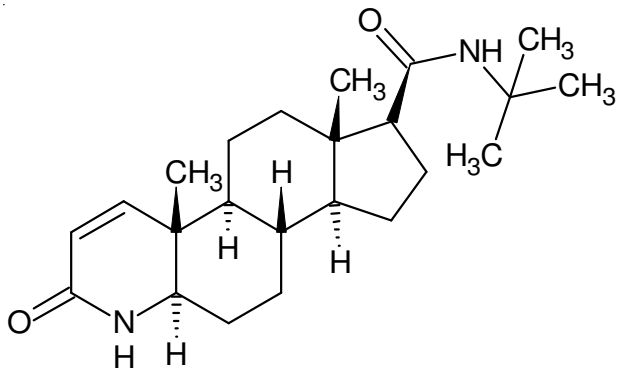

Fig. 1. Structure of finasteride

the slit was 6 mm × 0.45 mm micron. The composition of mobile phase was chloroform: methanol (8:2 v/v). Twin trough glass chamber was used. It was saturated with mobile phase. Development was carried out in linear ascending manner. The chamber was saturated with mobile phase for 20 min. Chamber saturation was carried at room temperature and 20 min was the optimized time. Approximately 70 mm was the chromatogram run length. Following the development TLC plate was dried. Drying was carried out with the help of an air dryer. Camag TLC scanner 3 was used for densitometric scanning. Software used was winCATS software (V 1.4.2, CAMAG). Deuterium lamp was used as the source of radiation.

Preparation of finasteride standard stock solution: Stock solution was prepared by weighing 10 mg of the finas-teride and transferred to a 10 mL volumetric flask and dissolved in methanol. The volume was made upto the mark to obtain 1 mg/mL (1000 µg/mL) as standard stock solution.

Mobile phase selection: Development of plates was carried out using mobile phase of composition chloroform: methanol (8:2 v/v).

Migration: Twin trough glass chamber was used for migration. The mobile phase of 20 mL was used for saturation and plate development. Migration was carried out till 70 mm length.

Calibration curve (linearity) of finasteride: Standard stock solution of finasteride (1000 µg/mL) prepared in methanol was spotted on TLC plate to obtain concentration of 200, 400, 800, 1000, 1200 and 1400 ng per spot respectively. The plot of peak area *versus* drug concentration was obtained. It was statistically analyzed by linear least square regression.

Precision: Precision studies were carried out for intra-day and inter-day variation using six replicates of the 400 ng per spot of finasteride.

Robustness of method: Robustness studies were carried by introducing small changes in the mobile phase composition. The effects of these variations on the results were examined. Different mobile phase compositions of chloroform:methanol (7.9:2.1 and 8.1:1.9 v/v) were tried and chromatogram run were studied.

Variation in time from spotting to chromatograph development was also carried out in robustness studies.

Limit of detection (LOD) and limit of quantitation (LOQ): Determination of LOD and LOQ were carried out from the calibration (linearity) curve plotted in concentration range of 200-1400 ng/mL for finasteride. The calculation of LOD and LOQ was carried out using formulae LOD = 3.3 σ/S, LOQ = 10 σ/S, where σ = standard deviation of response and S = slope of calibration curve.

Specificity: Analysis of standard drug and sample was carried out to ascertain the specificity of the method. The R_f value of spot for finasteride in sample was compared with the R_f value of the spot of standard drug. To assess the peak purity of finasteride the comparison of the spectra at three different levels was carried out. The spectra at peak start (S), peak apex (M) and peak end (E) were compared.

Recovery studies: Accuracy was determined by using pre-analyzed sample which was spiked with 80, 100 and 120 % of the standard finasteride. Three determinations were performed at each level. For the accuracy studies 400 ng of sample solution was taken as constant and it was spiked with 320, 400 and 480 ng of standard.

Analysis of finasteride in formulation: To determine the concentration of drug in tablets (Finast 5mg; Dr Reddy Laboratories), 20 tablets were weighed and their average weight determined. The powder equivalent to 10 mg of finasteride was weighed and dissolved in methanol. It was sonicated for 20 min, so that the complete extraction of the drug can be carried out. Volume was made up to 10 mL (1000 µg/mL solution). The above prepared solution was applied on TLC plate to obtain concentration of 400 ng/spot followed by development and scanning.

This analysis of tablets was repeated six times. The interferences of excipient in the analysis were also studied.

Forced degradation of finasteride: Forced degradation studies of bulk drug in solid and solution state stress were carried out. The conditions for degradation studies and the stressors that were selected were based on published guidelines. The concentration and preparation of samples were carried out as per specifications. The stock solution of 2 mg/mL (2000 µg/mL) of the drug was prepared in methanol. For the hydrolytic stress studies; the stock solution of 2000 µg/mL was diluted 50:50 (v/v) with HCl, NaOH and water. The hydrolytic studies were conducted at 60 °C. For the oxidative stress 30 % (v/v) H_2O_2 at room temperature was used. For thermal stress testing, finasteride drug sample was placed in a thermostatic oven at 60 °C for 21 days. Photolytic stability studies were carried out by exposure of the drug in the solid state to sunlight. Samples were withdrawn at proper time interval after subjecting to stress. The data for optimized stressed conditions is given in Table-1.

RESULTS AND DISCUSSION

Development of optimum mobile phase: Optimization of the HPTLC method was carried with a view to developing a stability-indicating assay method. Initially, various trials of mobile phases were carried out. Combinations of chloroform: methanol (5:5, 6:4 v/v) were tried. Good resolution with an R_f value of 0.3 for finasteride was obtained. To increase the R_f for better resolution of degradants the mobile phase consisting of chloroform: methanol (8:2 v/v) was finally optimized. It gave a sharp and well-defined peak at an R_f value of 0.57 (Fig. 2). When the chamber was saturated with the mobile phase for 20 min, well-defined spots were obtained.

TABLE-1
OPTIMIZED CONDITION OF STRESSORS

Stressors	Hydrolysis at 80 °C			Oxidative at room temperature	Photolytic degradation	Thermal at 60 °C
	Acid	Neutral	Base		Solid	
Concentration of stressor	2 N HCl	H_2O	0.1 N NaOH	30 % H_2O_2	–	–
Duration	4 days	4 days	24 h	24 h	12 days	21 days

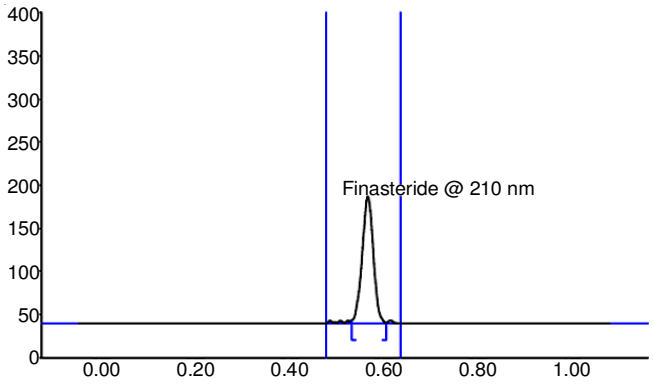

Fig. 2. Typical Densitogram obtained for finasteride hydrochloride

Specificity: The specificity was observed by comparing the spectra of finasteride in sample and standard. The method was found to be specific as no interference from the excipients in the formulation was observed. Peak purity was 99.99 %.

Linearity: Finasteride showed good linearity over the concentration range 200-1400 ng per spot with respect to peak area. The calibration curve is shown in Fig. 2 and the linear regression data for the calibration curve is shown in Table-2.

Precision: The inter-day and intra-day precision results were in the range of 97 to 102.19 % and % RSD for inter-day and intra-day was 1.17 and 1.31 respectively, which was less than 2 % indicating that the method was precise and results for precision studies are given in Table-3.

Recovery studies: The % recovery of finasteride was in the range of 98.21-99.66 % for 80, 100 and 120 % level. Results for the recovery studies are given in Table-4.

TABLE-2
LINEARITY FOR FINASTERIDE

Concentration (ng/band)	Area
200	1150
400	1722
800	2855
1000	3542
1200	4147
1400	4683

LOD and LOQ: The limit of detection and limit of quantitation were calculated from linearity data and found to be 41.89 and 126.92 ng, respectively.

Robustness of method: There was no major change in the R_f value with small variations in mobile phase composition and sample application time. The standard deviation and % RSD was less than 2 % indicating that the method was robust.

Assay: Finasteride in marketed formulation with label claim of 5 mg was analyzed. The percentage amount of drug found in tablets was in the range of 99.83 ± 0.266; which was within the limit of standard monographs and there is no interference from excipient(s). The results are given in Table-5.

TABLE-5
ASSAY OF FINASTERIDE

Drug name	Sample conc. (ng/band)	Mean amount found (%)* ± SD
Finasteride	400	99.83 ± 0.266

*Average of six determinations

TABLE-3
PRECISION STUDIES FOR FINASTERIDE

S. No.	Concentration (ng/band)	Peak area		Amount of drug found (ng/band)		Drug found (%)	
		Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day
1	400	1722	1750	399.33	407.85	99.83	101.96
2	400	1744	1741	406.74	404.82	101.66	101.20
3	400	1680	1685	385.19	386.01	96.30	96.50
4	400	1695	1691	390.24	388.02	97.56	97.00
5	400	1739	1725	405.05	399.45	101.26	99.86
6	400	1750	1750	408.76	407.85	102.19	101.96
Mean						99.80	99.75
SD						1.1704	1.319
RSD (%)						1.1702	1.318

TABLE-4
ACCURACY STUDIES FOR FINASTERIDE

Recovery level (%)	Concentration of drug (ng/band)		Total amount of drug found (%)*	SD	RSD (%)
	Drug taken	Standard drug added			
80	400	320	98.52	0.514	0.521
100	400	400	99.66	0.674	0.676
120	400	480	99.21	0.473	0.476

*Average of three determinations

Stability-indicating method: Under basic and oxidative stress finasteride degraded into degradation products. In acidic, neutral and photolytic degradation studies it was observed that there was insignificant degradation. For basic medium the extent of degradation for the drug was 26.86 %. The chromatogram of the drug with the degradation product in basic stress is shown in Fig. 3. Finasteride degraded to 30 % in oxidative stress after exposing the drug to 30 % H_2O_2 at room temperature. The chromatogram for basic degradation is shown in Fig. 3. The drug spot was well resolved from the spots of degraded product. The summary of developed and validated method for finasteride is given in Table-6.

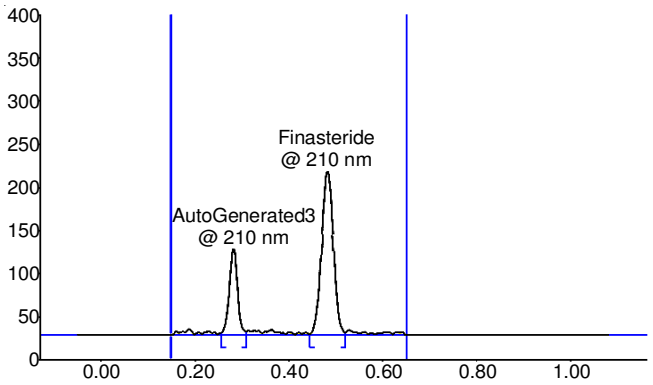

Fig. 3. Densitogram of finasteride hydrochloride in basic stress

TABLE-6
SUMMARY OF DEVELOPED AND VALIDATED METHOD FOR FINASTERIDE

Parameters	HPTLC
Method details	Stationary phase: Silica gel 60 F_{254} Mobile phase: Chloroform : Methanol (8:2 v/v) UV Maxima: 210 nm
Specificity	Specific
Linearity	Linearity: 200-1400 ng/band Line equation y = 2.9766x + 535.99; r^2 = 0.9993
Precision	Inter-day – 1.1702 Intra-day – 1.318
Limit of detection	41.89 ng/band
Limit of quantitation	126.92 ng/band
Accuracy	99.13 ± 0.5576
Robustness	< 2 %
Application	Tablet formulation
SIAM	HPTLC
Acid (2 N HCl, Kept for 4 days)	No degradation
Base (0.1 N NaOH, kept for 24 h)	% Degradation = 26.86 % No. of Degradants = 01
Neutral (kept for 4 days)	No degradation
H_2O_2, 30% (kept for 24 h)	% Degradation = 30 % No. of Degradants – 2
Dry Heat (60 °C for 21 days)	No degradation
Photo stability	No degradation

Conclusion

Stability indicating HPTLC method has been developed for finasteride. The developed method is precise, specific, accurate and has been validated as per ICH guidelines. Low values of standard deviation and % RSD indicate that the method is precise, selective, accurate and specific for the analysis of finasteride in bulk drug and in pharmaceutical formulations. The drug degraded in basic and oxidative mediums with well resolved peaks. The developed method can also be extended to study the degradation kinetics. Bioanaly-tical studies for estimation of finasteride in biological samples and plasma could also be carried out by this method.

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