

Research Article

Validated Stability Indicating HPTLC Method for the Determination of Dutasteride in Pharmaceutical Dosage Forms

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This paper describes simple, sensitive, precise, specific, and stability-indicating high-performance thin-layer chromatographic method for the determination of dutasteride (DUTA) in bulk and tablet formulation. Validation was carried out in compliance with International Conference on Harmonization guidelines. The thin-layer chromatographic method employed aluminium plates precoated with silica gel G60F₂₅₄ as stationary phase. The solvent system consisted of toluene/methanol/triethylamine (9:2:1, v/v/v). This solvent system was found to give compact spots for dutasteride with R_f value 0.71 ± 0.01 . Densitometric analysis of DUTA was carried out in the absorbance mode at 274 nm. Linear regression analysis showed good linearity ($r^2 = 0.9989$) with respect to peak area in the concentration range of 200–3000 ng per spot. The method was validated for precision, accuracy, specificity, and robustness. Pure drug was subjected to acid and alkali hydrolysis, oxidation, photo degradation, dry heat and wet heat treatment. The drug underwent degradation under acidic, basic, oxidative, and wet heat conditions. The degraded products were well separated from the pure drug. Statistical analysis proved that the method is reproducible and selective for estimation of DUTA in bulk and tablets. As the method could effectively separate the drugs from their degradation products, it can be employed as a stability indicating method.

1. Introduction

Dutasteride, $5\alpha,17\beta$ -N- $\{2,5$ bis(trifluoromethyl)phenyl $\}$ -3-oxo-4-azaandrost-1-ene-17-carboxamide [1], is a selective inhibitor of 5α -reductase, an intracellular enzyme that converts testosterone to DHT and is used for the treatment of patients with symptomatic benign prostatic hyperplasia [2–4]. Several bioanalytical and analytical methods have been reported for the analysis of DUTA. These include LC-MS method for determination of DUTA in human plasma [5], HPLC and HPTLC methods in pure powder and formulations [6, 7], and stability-indicating LC methods in pharmaceutical dosage form [8]. Recently, liquid chromatography coupled to mass spectrometry (LC-MS-MS) [9] and UV-visible spectrophotometric [10] methods have been reported for the simultaneous estimation of tamsulosin and dutasteride in plasma and formulations, respectively.

The present work deals with development and validation of stability indicating HPTLC method for the quantitative

analysis of DUTA and its stress degradation products. The aim of the present work was to develop an economic, accurate, specific, reproducible, and stability-indicating HPTLC method using densitometric detection for the determination of DUTA in the presence of its degradation products, either in bulk form or in tablets.

2. Experimental

2.1. Instruments and Apparatus. A Camag HPTLC instrument consisting of Linomat V automatic spotter equipped with a 100 μ L syringe, Scanner-III, flat-bottom twin-trough developing chambers, and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland) were used. HPTLC plates used were of aluminium backed silica gel G60F₂₅₄ with 0.2 mm thickness, 20 \times 10 cm (E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai). Sartorius CP224S analytical balance (Gottingen,

Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

2.2. Reagents and Materials. Dutasteride pure powder with 99.98% purity was kindly gifted by Intas pharmaceutical Ltd. All the reagents used during the study were procured from S. D. Fine Chemical and were of analytical grade. Tablets were purchased from the local pharmacy.

2.3. Chromatographic Conditions. Before analysis, HPTLC plates were cleaned by predevelopment with methanol and activated at 110°C for 5 min for solvent removal. Solutions of DUTA were applied to plates (10 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 µL syringe and operated with settings of band length, 6 mm; distance between bands, 10 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin-trough chamber previously saturated for 30 min with the mobile phase, toluene/methanol/triethylamine (9:2:1, v/v/v) to 8.5 cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 274 nm for all measurements and operated by the CATS software.

2.4. Preparation of Standard Solution. Accurately weighed 10 mg of DUTA was transferred to a 10 mL volumetric flask and dissolved in and diluted up to the mark with methanol to obtain a standard solution of DUTA (1000 µg/mL). From this solution, 2 mL was further diluted to 10 mL with methanol to obtain a working standard solution of DUTA (200 ng/µL).

2.5. Method Validation. The HPTLC method was validated as per ICH guidelines [11].

2.5.1. Linearity. Accurate quantities from working standard solutions (1, 2, 4, 6, 8, 10, and 15 µL) were applied to the TLC plate to give bands containing 200–3000 ng of DUTA per spot, and the plate was developed, using the previously described optimized mobile phase, and scanned. The experiment was repeated for five times. The calibration curves were constructed by plotting peak areas versus concentrations.

2.5.2. Accuracy (% Recovery). Accuracy of the method was determined by standard addition method in which the known amount of standard DUTA solutions were added to preanalyzed tablet solution. These amounts corresponded to 50, 100, and 150% of the amounts claimed on the label. The amounts of DUTA were estimated by applying these values to the regression equation of the calibration curve. Accuracy study was performed for five times, and % recovery of DUTA was calculated.

2.5.3. Method Precision (Repeatability). The precision of the instruments was checked by repeated spotting of same standard solution of DUTA and repeated scanning of the same spot ($n = 6$) of DUTA without changing the position of plate for the HPTLC method. Repeatability is reported in terms of relative standard deviation (% RSD).

2.5.4. Intermediate Precision (Reproducibility). The intraday and interday precisions of the proposed methods were

determined by estimating the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of DUTA (800, 1200, and 1600 ng/spot). The results are reported in terms of relative standard deviation (% RSD).

2.5.5. Limit of Detection and Limit of Quantification. The limit of detection (LOD) and the limit of quantification (LOQ) of the both drugs were found by visual inspection.

2.5.6. Robustness of the Method. Robustness of the proposed method was estimated by changing different conditions like mobile phase composition (± 0.2 mL for each component), mobile phase volume (varied $\pm 3\%$), scanning wavelength ± 1 nm, peak areas were measured after development of plate, and % RSD was calculated. A concentration level of 1000 ng per band was employed.

2.5.7. Solution Stability. The stability of standard solutions was tested after 0, 6, 12, 24, 48, and 72 h of storage. The stability of the solutions was determined by comparing peak area percentage and peak purity at 1000 ng per band.

2.6. Analysis of DUTA in Tablets. To determine the content of DUTA in conventional tablets, 30 tablets were weighed, their mean weight determined and finely powdered. Powder equivalent to 10 mg of DUTA was transferred into a 100 mL volumetric flask containing 20 mL methanol, sonicated for 30 min, and diluted to the mark with methanol. The resulting solution was centrifuged for 10 min, and the supernatant was diluted with methanol to obtain 100 ng/µL of the drug. 10 µL of this solution was applied to the HPTLC plate at 1000 ng per spot and followed by development. Analysis was carried out in triplicate, peak areas were measured at 274 nm, and sample concentrations calculated. The potential interference from excipients was also examined.

2.7. Forced Degradation of Dutasteride. Decomposition studies were performed in solutions containing DUTA at a concentration of 1 mg/mL. Samples were withdrawn at suitable time intervals and subjected to HPTLC analysis. The drug was subjected under different stress conditions as follows.

2.7.1. Acid- and Base-Induced Degradation. 1 mL of the methanolic stock solution was diluted with each of 1 N HCl and 1 N NaOH to 25 mL separately and was refluxed at 50°C.

2.7.2. Oxidative Degradation. 1 mL of the methanolic stock solution was diluted with 30% (v/v) of hydrogen peroxide to 25 mL. The solution was refluxed at 50°C.

2.7.3. Wet Heat Degradation. 1 mL of the methanolic stock solution was diluted with distilled water 25 mL and was refluxed in a boiling water bath to assess wet heat degradation.

2.7.4. Dry Heat Degradation. For dry heat degradation, the standard drug was placed in an oven at 100°C for 24 h.

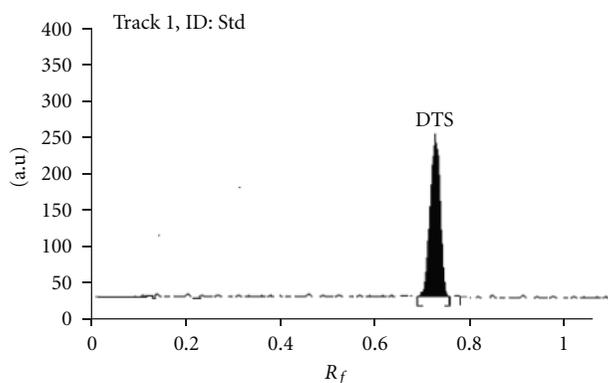


FIGURE 1: A typical densitogram of standard dutasteride with R_f value 0.71 ± 0.01 .

2.7.5. Photostability. The photochemical stability of the drug was studied by exposing the solution to direct sunlight for 48 h kept on a terrace. For UV degradation analysis, the solution was exposed to UV radiation for 48 h.

3. Results and Discussion

3.1. Optimization of the Chromatographic Conditions. The TLC procedure was optimized with a view to develop a stability indicating assay method used for the quantification of the DUTA in pharmaceutical tablets. Both the pure drug and the degraded products were spotted on the TLC plate and run in different solvent systems. Initially, different solvent systems were tried but Toluene/methanol/triethylamine (9:0.5:0.1, v/v/v) gave good resolution with the value of 0.32 for DUTA, but typical peak nature was missing; moreover, it was felt necessary to increase better resolution of impurities. Finally, this mobile phase was optimized to achieve good peak symmetry and better separation of DUTA from degradation peaks obtained under different stressed conditions. The spot appeared to be more compact with a more symmetrical peak shape when TLC plates were pretreated with methanol and activated at 110°C for 5 min. Well-defined standard spots along with its degradation products were obtained when the chamber saturation time was optimized to 30 min at 25°C temperature.

Good resolution and considerable high R_f value (0.71 ± 0.01) were obtained for DUTA using optimized mobile phase toluene/methanol/triethylamine (9:2:1, v/v/v) with densitometric scanning at 274 nm (Figure 1).

3.2. Validation of the Method. The calibration plot was linear over a concentration range of 200–3000 ng per spot for DUTA. A good linear relationship observed over this range ($r^2 = 0.9989 \pm 0.0069$, slope = 1.753 ± 0.05 , intercept = 600.38 ± 0.242) indicated that the method is linear. Repeatability of sample application and sample measurement was expressed as % RSD and was found to be 0.53% and 0.39% for six replicate determinations. The low values of % RSD indicate that the proposed method is repeatable. The % RSD value obtained for intraday and interday variation

TABLE 1: Summary of validation parameters of proposed HPTLC method.

Parameters	Value
Linearity and range	200–3000 ng/spot
Correlation coefficient (r^2)	0.9989
Limit of detection	10 ng/spot
Limit of quantification	50 ng/spot
% accuracy \pm SD ^a ($n = 3$)	$99.81\% \pm 0.697$
Precision (% RSD ^b)	
Repeatability of sample application ($n = 6$)	0.53%
Repeatability of sample measurement ($n = 6$)	0.39%
Intraday ($n = 3$)	0.684%–1.048%
Interday ($n = 3$)	1.012%–1.224%
Robustness	Robust
Specificity	Specific
% assay \pm SD ^a	$99.49\% \pm 0.56$

^aStandard deviation; ^brelative standard deviation.

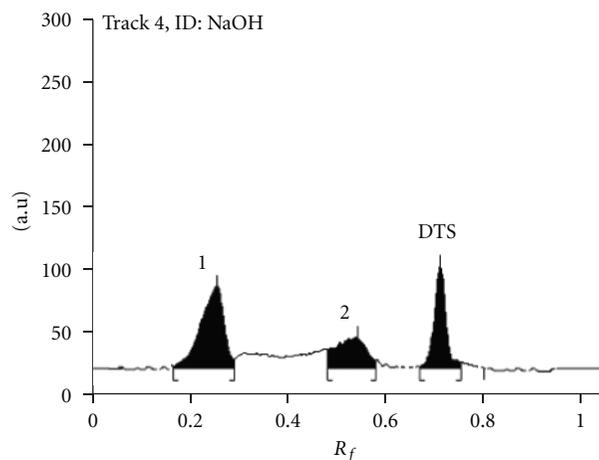


FIGURE 2: Chromatogram obtained after alkaline degradation of dutasteride, 1 N NaOH refluxed for 4 h at 50°C , Peak 1 = Degradant ($R_f = 0.26$), Peak 2 = Degradant ($R_f = 0.55$), Peak 3 = dutasteride ($R_f = 0.72$).

were 0.684–1.048% and 1.012–1.224%, respectively, which indicates that proposed method is precise. RSD of peak areas during robustness studies were calculated for changes in parameters and were less than 2% which indicates that this method is robust and reproducible. LOD and LOQ values were found to be 10 and 50 ng per spot, respectively, and pointed towards adequate sensitivity of the method. Peak purity for DUTA was assessed by comparing spectra acquired at the start (S), apex (M), and end (E) of the peak obtained from the scanning of spot, that is, $r(S,M) = 0.9996$ and $r(M,E) = 0.9994$. The high value of r^2 indicates specificity of the method. Accuracy was determined on previously analysed formulations after spiking with 50, 100, and 150% of the additional drug. Mean recovery obtained is $99.81\% \pm 0.697$. Validation parameters are summarized in Table 1.

3.3. Solution Stability. There was no indication of degradation in sample solutions of DUTA as revealed by peak purity

TABLE 2: Summary of degradation products of DUTA under different stress conditions.

Stress conditions	R_f values of degradation products	Peak purity data $r(S,M)$, $r(M,E)$
Acid hydrolysis, 0.1 N HCl, refluxed for 8 h at 50°C	0.55	0.9991, 0.9996
Alkaline hydrolysis, 1 N NaOH, refluxed for 4 h at 50°C	0.26, 0.55	0.9998, 0.9994
Oxidation (H_2O_2 , 30% v/v), refluxed for 3 h at 50°C	0.39, 0.55	0.9996, 0.9995
Wet heat, refluxed for 10 h at 100°C	0.55	0.9993, 0.9996
Dry heat, heated in oven at 100°C for 24 h	Not detected	0.9990, 0.9994
Day light, exposed to sunlight for 48 h	Not detected	0.9993, 0.9996
UV light, exposed to UV light (254 nm) for 48 h	Not detected	0.9995, 0.9997

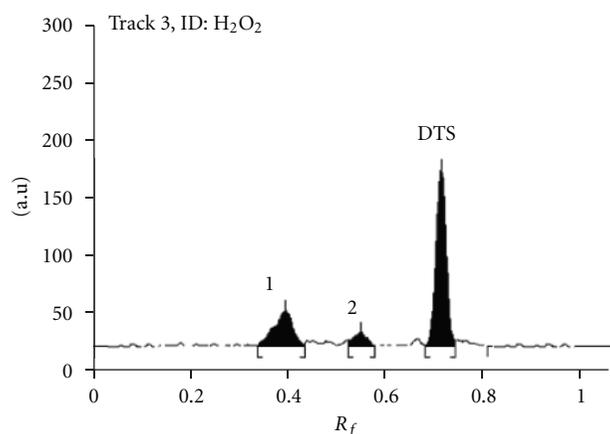


FIGURE 3: Chromatogram obtained after oxidative degradation of dutasteride. H_2O_2 , 30% v/v, refluxed for 3 h at 50°C, Peak 1 = Degradant ($R_f = 0.39$), Peak 2 = Degradant ($R_f = 0.55$), Peak 3 = dutasteride ($R_f = 0.71$).

data and from the value of RSD (<2%) for peak areas of bands of solution stored at different times. The solution was found to be stable at ambient temperature for 72 h, and no unknown peaks were observed.

3.4. Analysis of Formulated Tablets. A single band was observed in samples extracted from tablets, and there was no interference from the excipients which might have been present in the tablets. The amount of DUTA obtained in tablets is in good agreement with label claim. The content of DUTA in tablets was found to be 99.49% with standard deviation 0.56. It was therefore inferred that there is no interference of excipients during the analysis of DUTA normally present in tablets. Thus the method can be applied for the routine analysis of DUTA in pharmaceutical formulations.

3.5. Stability Indicating Properties. The chromatograms obtained after exposure of solution of DUTA (2000 ng/spot) to acidic and alkaline conditions (Figure 2), oxidative conditions (Figure 3), and wet heat degradation showed well separated bands of DUTA from additional bands of degradation impurities at different R_f values. For samples of same concentration that were subjected to sun light, ultraviolet (UV) light at 254 nm, and dry heat, no additional

band was detected which indicated stability of DUTA under these conditions. Spots obtained from degradation products were well resolved from the active ingredient. Peak purity of these spots of DUTA resolved during stability study was assessed by comparing the respective spectra at peak start, peak middle, and peak end, that is, $r(S,M)$ and $r(M,E)$. The peak purity data indicated that peaks of DUTA resolved after application of stress conditions were pure. Degradation products obtained under different stress conditions are summarized in Table 2.

4. Conclusion

The developed HPTLC procedure was simple, precise, specific and accurate. Separation of DUTA from degradation products confirmed stability indicating properties of this method. Statistical analysis indicated that the method was reproducible and selective for the analysis of DUTA in bulk drug and in tablets without interference from excipients. This methodology may also be applied to the study of degradation kinetics and for its determination in plasma and other biological fluids.

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