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Development and validation of stability indicating RP-HPLC with UV detection method: Analysis of Dutasteride in pharmaceutical dosage forms

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ABSTRACT

A simple and rapid stability indicating liquid chromatographic method with UV detection was developed and validated for the determination of dutasteride in bulk drug and pharmaceutical dosage form. Chromatographic separation has been achieved within 5 minutes by using an Agilent Zorebax SB Phenyl analytical column (250 mm × 4.6 mm, 5 µm) as the stationary phase with a mobile phase consisted of orthophosphoric and methanol (60:40 v/v) at a flow rate of 1.0 ml/min. Using an UV detector, detection was performed at 248 nm. The method was validated in accordance with International Conference on Harmonization guidelines with respect to linearity, sensitivity, selectivity, accuracy, precision, specificity and robustness. Regression analysis showed good correlations (R^2=0.9994) for dutasteride in the concentration range of 4-60 µg/ml. The dutasteride was exposed to acidic, basic, oxidation, photo degradation and dry heat stress conditions. The developed HPLC method can efficiently separate the dutasteride from its degradation products. Therefore, it can be employed as stability-indicating method. The percentage recovery was in the range of 99.84–100.13% with relative standard deviation in the range of 0.046–0.076 %, for dutasteride from the pharmaceutical dosage form. The proposed method is suitable for determination of dutasteride in bulk drug and in its pharmaceutical dosage form.

Keywords: Dutasteride, Agilent Zorebax SB Phenyl column, HPLC analysis, pharmaceutical dosage form.

INTRODUCTION

Dutasteride (DST) is a 5-α-reductase inhibitor. DST inhibits the conversion of testosterone to dihydrotestosterone by blocking the action of the 5-α- reductase enzyme. DST is approved for the treatment of benign prostatic hyperplasia by the food and drug administration [1-3]. Chemically, DST is known as (5 alpha, 17 beta)-N-{2, 5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-l-ene-17-carboxamide (Fig. 1).

Various techniques have been reported for the quantification of DST. Dutasteride in pharmaceutical dosage forms or biological fluids either present alone or in combination with other drug was quantitated by many techniques, such as UV spectrophotometry [4,5], visible spectrophotometry [6,7], LC–MS [8,9,10], HPTLC [11], enzyme-linked immunosorbent assay [12], HPLC [13,14,15] and stability indicating RP-HPLC [16,17].

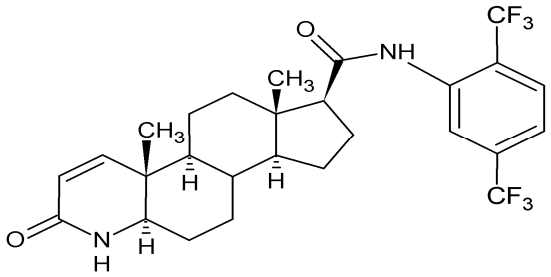

Figure 1: Structure of dutasteride

The LC–MS [8,9,10], HPTLC [11] and enzyme-linked immunosorbent assay [12] techniques may have the highest sensitivity, but the determination process is complicated, costly and time consuming. In addition enzyme immune assay [12], LC–MS [8,9,10] methods were not applied to pharmaceutical formulations. Although the UV spectrophotometric methods [4,5] are simple and easy to perform, they suffers from decreased selectivity due to measurement in ultraviolet region. The visible spectrophometric methods [6,7] suffers from disadvantages such as use of costly reagent, extraction of colored complex, use of more volume of organic solvents and narrow range of linearity. Most of the reported HPLC [14,15] are utilized for the determination of dutasteride in combination with other drugs (tamsulosin hydrochloride/alfuzosin hydrochloride). Only a few stability indicating RP-HPLC with UV detection methods [16,17] have been reported for the quantification of dutasteride alone in bulk and pharmaceutical dosage forms. However the reported stability indicating RP-HPLC with UV detection methods [16,17] suffer from the lack of broad linearity range, sensitivity, precision and accuracy. In addition, the retention time of DST in the reported stability indicating RP-HPLC methods [16,17] is greater than four minutes which in turn increases the time of analysis.

By considering into the above disadvantages of the reported methods, the aim of the present work was to develop and validate a rapid, sensitive, precise and accurate stability indicating RP-HPLC with UV detection method, without the use of internal standard, for determination of DST in bulk and in pharmaceutical dosage forms.

EXPERIMENTAL SECTION

Chemicals and Reagents:
All chemicals and solvents were analytical and HPLC grade, respectively and used as received. All solutions were prepared in Milli-Q water from Merck Specialties Private Ltd, Hyderabad, India. Pharmaceutical grade DST was obtained as gift sample from the Hetero Drugs Limited, Hyderabad, India. Tablet dosage forms of DSE such as Duprost (labeled to contain 0.5 mg/tablet, manufactured by Dr. Reddy's Lab. Ltd., H. P., India), Dutas (labeled to contain 0.5 mg/tablet, manufactured by Dr. Reddy's Lab. Ltd., H. P., India) and Sterdu (labeled to contain 0.5 mg/tablet, manufactured by Mercury, Lab. Ltd., H. P., India) were purchased from local pharmacy market. Orthophosphoric acid and methanol were obtained from Sd fine-chem Ltd, Mumbai, India.

Apparatus and Chromatographic Conditions:
Samples were analyzed on a Waters Alliance HPLC system equipped with 2695 separation modules having 2996 photodiode array detector. The detector wavelength was fixed at 248 nm and peak areas were integrated automatically using the Millennium 32 software program. Other apparatus included a Shimazdu (Tokyo, Japan) electronic weighing balance model BL 220 H for weighing the samples and an Elico pH meter (Hyderabad, India) LI 120 model.

Agilent Zorebax SB Phenyl (250 mm × 4.6 mm I.D., 5 µm particle size) was maintained at 30^0C temperature. The mobile phase was composed of a mixture of orthophosphoric acid and methanol in the ratio of 60:40 *v/v*. The pH of the mobile phase was adjusted to 2.5 with 85% orthophosphohric acid. Prior to use, the mobile phase was filtered and degassed via 0.45 µm membrane filter. The flow rate of the mobile phase was set at 1.0 ml/min. Measurements were made with 10 µl of injection volume. The total run time was about 5 minutes

Preparation of Standard Solutions:
Standard stock solution of DST (1 mg/ml) was prepared in mobile phase. Series of working standard solutions were diluted with the same solvent to the desired concentration for linearity (4, 8, 15, 20, 30, 40, 50 and 60 µg/ml), system suitability studies (40 µg/ml), sensitivity (4 µg/ml), selectivity (40 µg/ml), accuracy (4, 40, 60 µg/ml), precision (4, 40, 60 µg/ml) and robustness (4, 60 µg/ml).

Preparation of tablet sample solution:
To determine the concentration of DST in tablet dosage forms (label claim: 0.5 mg per tablet), 100 tablets were weighed, their mean weight was determined and they are finely powdered. A precisely weighed powder sample equivalent to 25 mg of DST was transferred into a 25 ml volumetric flask containing 15 ml mobile phase. The content of the flask was sonicated for 15 min and the resulting solution was filtered through 0.45 µm membrane filter. The volume was completed with mobile phase and the solution reached 1 mg/ml (stock solution). An appropriate aliquot of the stock solution was transferred into a volumetric flask and diluted with the mobile phase to obtain concentration equal to 40 µg/ml of DST. The solution was filtered through 0.45 µm membrane filter before analysis.

Preparation Placebo blank solution:
A placebo blank containing starch (10 mg), acacia (10 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (10 mg), magnesium stearate (10 mg), lactose (10 mg), glucose (10 mg) and sodium alginate (10 mg) was prepared and extracted with 20 ml mobile phase. The placebo blank solution was prepared as described under section "Preparation of tablet sample solution".

Preparation of Solutions for degradation studies:
Acid degradation:
For acid degradation study, 50 mg of DST was taken in 50 ml volumetric flask. 5 ml of 0.1 N HCl was added in the flask and heated on water bath at 80ºC for 2 hours. After completion of the stress the solution was neutralized by using 0.1 N NaOH and completed up to the mark with mobile phase.

Alkali degradation:
For alkali degradation study, 50 mg of DST was taken in 50 ml volumetric flask. Five ml of 0.1 N NaOH was added in the flask and heated on water bath at 80ºC for 2 hours. After completion of the stress, the solution was neutralized by using 0.1 N HCl and completed up to the mark with mobile phase.

Oxidative degradation:
For oxidative degradation study, 50 mg of DST was taken in 50 ml volumetric flask. Ten ml of 3% H_2O_2 was added in the flask and sonicated for 30 minutes. After completion of the stress, the 50 ml flask was completed up to the mark with mobile phase.

Dry heat degradation:
For dry heat degradation study, 50 mg of DST was taken in glass petri dish and placed in hot air oven at 105°C for 2 hours. After 2 hours, the sample was cooled and transferred to a 50 ml volumetric flask containing 15 ml of mobile phase and mixed well. The volume of the flask was made up to mark with mobile phase.

Photolytic degradation:
For photolytic degradation study, 50 mg of DST was taken in a glass petri dish and placed in the sunlight for 24 hrs. After completion of the stress, the sample was transferred to a 50 ml volumetric flask containing 15 ml of mobile phase and mixed well. The volume of the flask was completed up to mark with mobile phase.

The above stress degraded sample solutions were appropriately diluted with mobile phase to get a final concentration of 40 µg/ml of DST. Ten µl of the degraded sample solutions were injected into the HPLC system. The respective chromatograms and peak area were recorded.

RESULTS AND DISCUSSION

Method Development:
To develop an efficient and simple stability indicating HPLC method for the assay of DST, preliminary tests were conducted to select the suitable and optimum conditions. HPLC parameters, such as detection wavelength, ideal mobile phase and their proportions, flow rate and column temperature were carefully studied. The HPLC parameters were finally chosen based on the criteria of peak properties like height, area, retention time and peak symmetry.

The ultraviolet spectra of DST dissolved in mobile phase showed the maximum absorption wavelength at 248 nm. Therefore, 248 nm was selected as detection wavelength. Different combinations of acetonitrile, methanol, propanol, dichloromethane, orthophosphoric, dipotassium hydrogen phosphate buffer & formate buffer were tested. The optimum condition at orthophosphoric-methanol (60:40, *v/v*), was reached. The mobile phase with different pH (2-5) was tried. The best peak shape and tailing factor with reasonable analysis time for DST was accomplished at pH 2.5. Therefore, mobile phase with pH 2.5 was chosen. Two different stationary phases (Agilent Zorebax RX C8, 150 mm x 4.6 mm, 5 µm particle size and Agilent Zorebax SB Phenyl, 250 mm x 4.6 mm, 5 µm particle size) were investigated and the peak properties were compared. The best peak properties were obtained by using Agilent Zorebax SB Phenyl Column (250 mm x 4.6 mm, 5 µm particle size). The mobile phase with flow rates in the range 0.8-1.6 ml/min was investigated. At the flow rate 1.0 ml/min, symmetric and well retained peak was obtained. Therefore, the flow rate 1.0 ml/min was selected. The effect of temperature on the column efficiency was studied. Different temperatures of 15°C-35°C with 5°C increments were evaluated. The chromatograms were recorded. It was found that the peak shape was good at 30^0C temperature, therefore 30^0C temperature was chosen. Under the described chromatographic conditions, the retention time of dutasteride was 3.172 minutes.

HPLC Method Validation:
After the successful optimization, the optimized HPLC method was validated in accordance to the International Conference on Harmonization guidelines [18,19]. Parameters such as system suitability, selectivity, sensitivity, linearity range, accuracy, precision, specificity and robustness were all validated.

System Suitability:
The system suitability was determined by injecting six replicates of the DST standard solutions (40 µg/ml) and analyzing for its retention time, peak area, theoretical plates, plates per meter, height equivalent to theoretical plate, and peak asymmetry. The system suitability results revealed %RSD of less than 1.0% for all the parameters. As shown in Table 1, the proposed method meets the accepted requirements.

Table 1: System suitability parameters

Parameter	Mean value*	% RSD
Retention Time (min)	3.172	0.945
Peak area	6453276.4	0.313
Theoretical Plates (n)	8166	0.823
Plates per Meter (N)	32764.8	0.774
Height equivalent to theoretical plate (HETP)(mm)	$3.061x10^{-5}$	0.836
Tailing factor	1.239	0.870

** Average of five determinations*

Selectivity:
Blank mobile phase (without drug), DST standard (40 µg/ml), placebo blank and tablet sample (40 µg/ml) solutions were all injected into the HPLC column to assure the selectivity of the optimized method. There were no peaks in mobile phase blank and placebo blank (Figures 2 & 3). A comparison of the retention time of DST in tablet sample solution and in the standard solution was exactly the same. Figures 4 and 5 showed that there were no interferences at the retention time for DST due to the excipients in tablet dosage forms. Hence, the proposed method was found to be selective and is suitable for the quantification of the DST in tablet dosage forms.

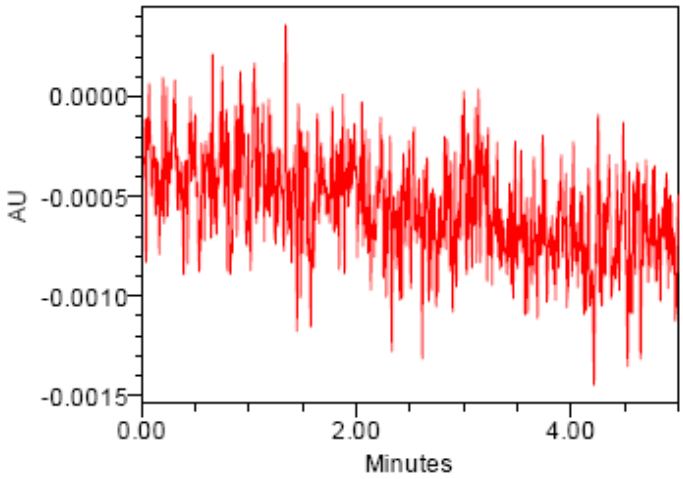

Figure 2: Chromatogram of mobile phase blank

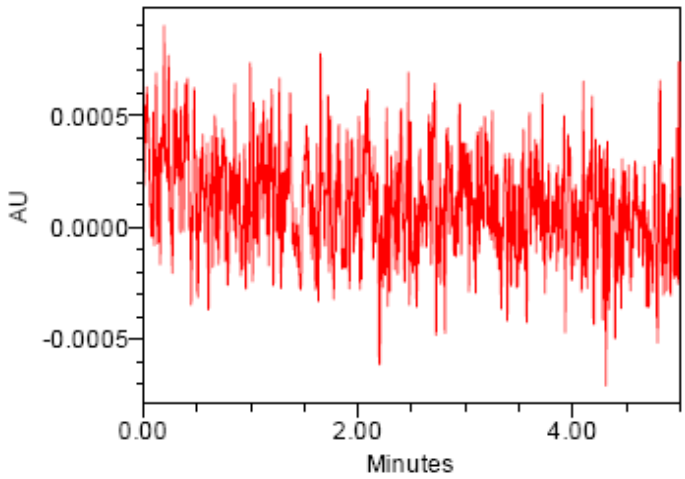

Figure 3: Chromatogram of placebo blank

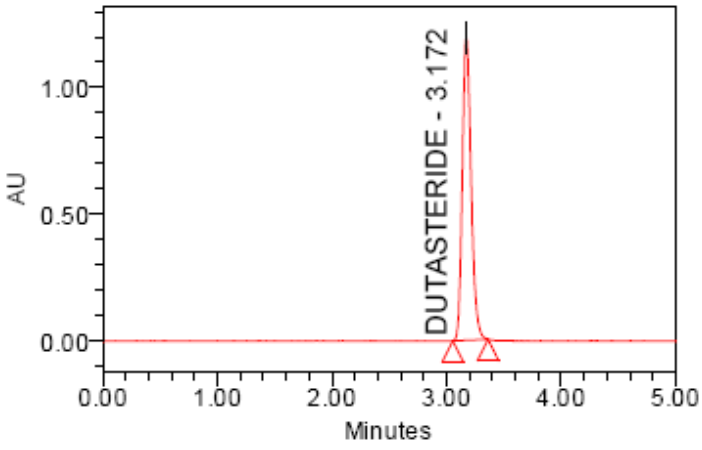

Figure 4: Chromatogram of tablet sample (40 µg/ml)

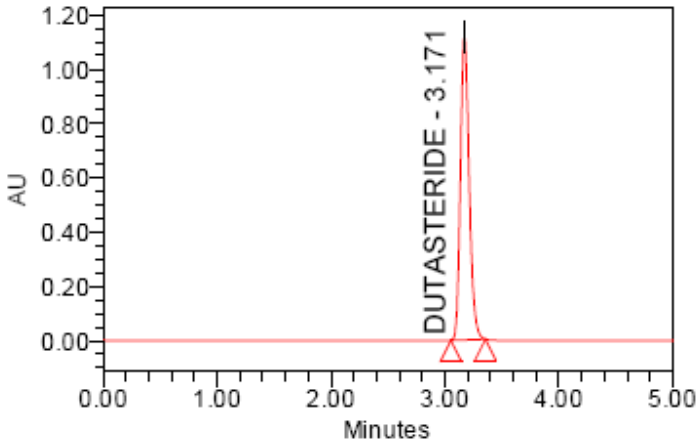

Figure 4: Chromatogram of standard dutasteride sample (40 µg/ml)

Sensitivity:

The sensitivity of the proposed method was investigated via measurement of the limit of detection (LOD) and limit of quantitation (LOQ) at a signal-to-noise ratio of 3 and 10, respectively. It was achieved by injecting working standard solution of DST with known concentration (4 µg/ml) into the HPLC column five times. The LOD and LOQ were found to be 0.103 µg/ml and 0.342 µg/ml, respectively. These values suggest that the developed method is sensitive to quantify DST.

Linearity:

An eight-point (4, 8, 15, 20, 30, 40, 50 and 60 µg/ml) calibration curve was prepared. The peak area for each concentration was obtained by injecting 10 µl of the each DST working standard solution into the column. Calibration curve was plotted by taking the peak area on the y-axis and the concentration of DST (µg/ml) on the x-axis (Figure 5). The linearity was assessed by the least square regression method. The regression line confirmed linearity in the tested range (4-60 µg/ml). The linear regression equation was y = 160402x + 55839 (y = peak area: x = concentration of DST in µg/ml). The regression line was linear with R^2 of 0.9994.

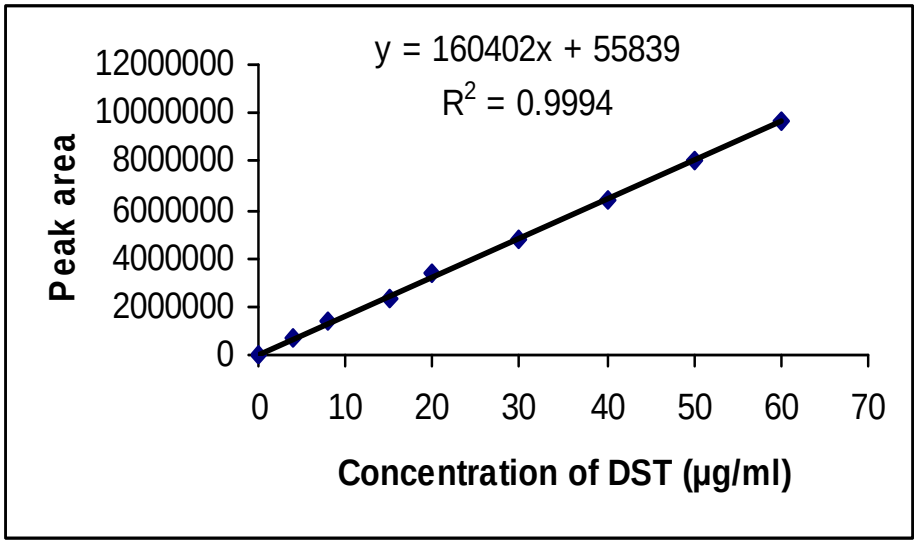

Figure 5: Linearity of dutasteride

Precision and Accuracy:

The precision and accuracy of the method was determined by intra-day (repeatability) & inter-day (intermediate) assay and was expressed as relative standard deviation and recovery percentage, respectively. Five replicate

injections of the standard solutions of DST at concentrations 4, 40 and 60 µg/ml prepared. The intra-day variation was assessed over one day, while inter-day assay was carried out over 3 days. Table 2 provides data obtained from the intra-day & inter-day experiments. The relative standard deviation values for intra-day and inter-day precision were in the range of 0.029-0.684% and 0.025-0.688% (acceptance criteria proposed: RSD <2.0%), respectively. The percent recovery values for intra-day and inter-day accuracy were in the range of 100.01- 100.02% and 100.01-100.20% (acceptance criteria proposed: %Recovery range - 80 to 120%), respectively. The results (Table 2) indicating that the method has sufficient precision and accuracy.

Table 2: Precision and accuracy

Concentration of DST(µg/ml)		%RSD	% Recovery	% Error
Taken	Found* ± SD			
Intra-day assay				
4	4.001±0.0274	0.684	100.02	0.020
40	40.010±0.023	0.058	100.02	0.02
60	60.010±0.0179	0.029	100.01	0.01
Inter-day assay				
4	4.008 ±0.0276	0.688	100.2	0.20
40	40.008±0.0113	0.0283	100.2	0.20
60	60.006±0.0153	0.0254	100.01	0.01

** Average of five determinations*

Recovery:

Accuracy was further determined by the recovery study of known concentration of DST standard added to a preanalysed tablet sample solution. The recovery study was performed five times. The average recovery data (Table 3) of DST showed results between 99.84% and 100.13% with relative standard deviation between 0.046% and 0.076%.

Table 3: Recovery study

Concentration of DST (mg)		% RSD	% Recovery
In tablet + Spiked	Found* ± SD		
0.5+0.25	0.751±0.000577	0.0768	100.133
0.5+0.5	0.999±0.000577	0.0582	99.90
0.5+0.75	1.248±0.000577	0.0462	99.84

** Average of five determinations*

Specificity:

In order to evaluate the specificity of the proposed method, stress degradation studies were performed using different ICH prescribed stress conditions such as acidic, alkali, oxidative, thermal and photolytic stresses.

Under acidic conditions DST was degraded up to 15.04%. Under alkali stress DST was degraded up to 10.71%. Under oxidative stress DST was degraded up to 9.01 %. Only a small percent of degradation occurred in dry heat and photolytic stresses. Under dry heat and photolytic stresses, the percent of DST degradation was 6.02% and 7.33%, respectively. From these stress studies it is therefore concluded that DST was degraded more in the applied acidic, alkali and oxidative stress conditions whereas least percent of degradation occurred in dry heat and photolytic stress conditions. The results are summarized in Table 4.

Table 4: Percentage of degradation

Type of stress	Peak area	% Recovery	% Degradation
Undegraded	6453276	100	0
Acid hydrolysis	5483253	84.96	15.04
Alkali hydrolysis	5762267	89.29	10.71
Oxidative stress	5869485	90.95	9.05
Dry heat	6065076	93.98	6.02
Photolytic	5980506	92.67	7.33

One degradation product was produced under acidic, alkali and oxidative stress conditions (Figures 6,7 & 8). Though degradation of DST occurred in dry heat and photolytic stress conditions (Figures 9 & 10), the degradation product was not detected by the proposed method due to its low concentration. The developed method successfully

separated the degradation product from analyte peak. As a result, the proposed method is considered specific for determination of DST in the presence of degradation products.

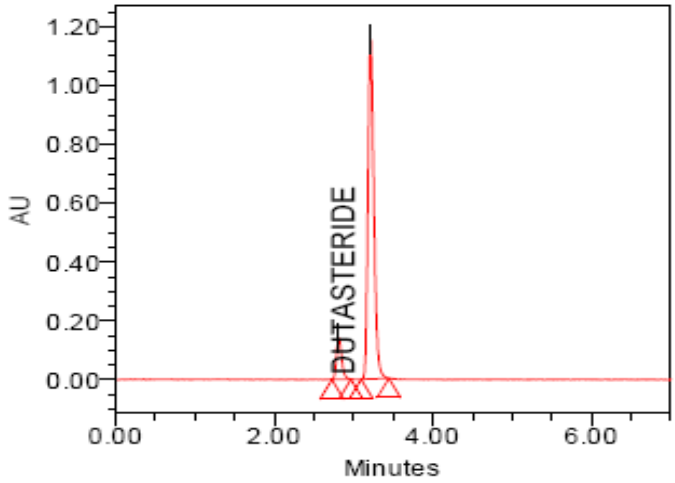

Figure 6: Chromatogram of dutasteride after acid degradation

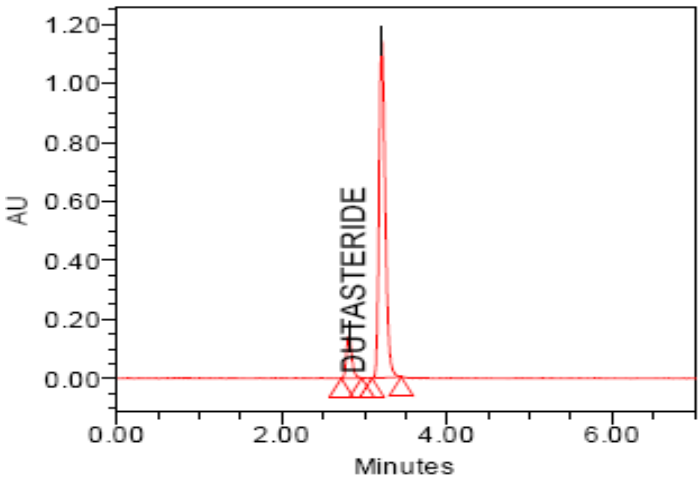

Figure 7: Chromatogram of dutasteride after alkali degradation

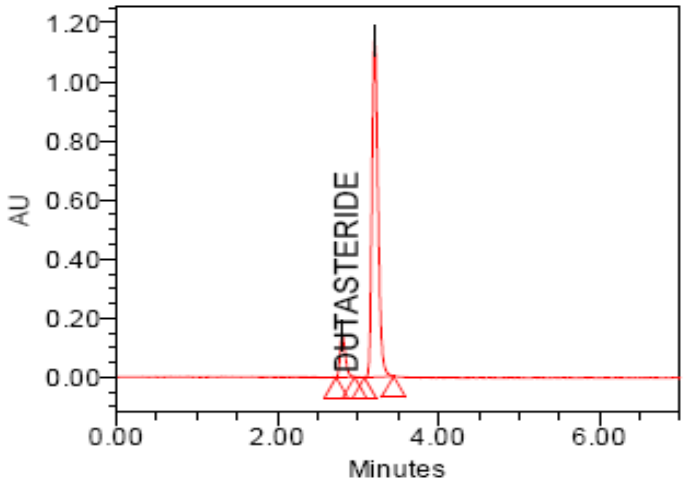

Figure 8: Chromatogram of dutasteride after oxidative degradation

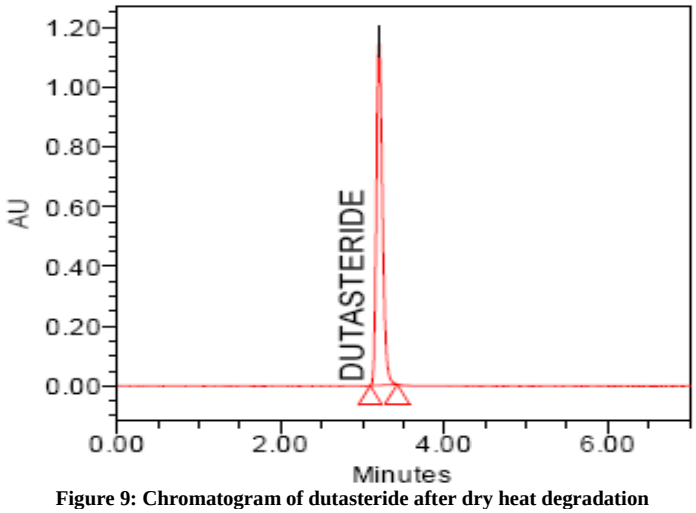

Figure 9: Chromatogram of dutasteride after dry heat degradation

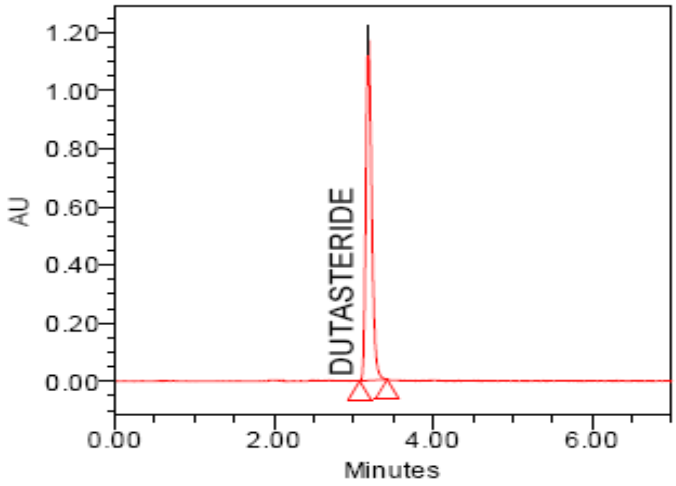

Figure 10: Chromatogram of dutasteride after photolytic degradation

Table 5: Method robustness

Experimental variable	Investigated range	4 µg/ml		60 µg/ml	
		Mean Peak area*	%RSD	Mean Peak area*	%RSD
Mobile phase ratio** (*v/v*)	58:42	646924	0.472	9455489	0.204
	60:40				
	62:38				
pH of mobile phase	2.3	645739	0.645	9676123	0.190
	2.4				
	2.5				
Temperature of the column (^{o}C)	28	645665	0.548	9676112	0.462
	30				
	32				
Flow rate of mobile phase (ml/min)	0.9	645171	0.679	9676109	0.357
	1.0				
	1.1				

** Average of three values*

*** mobile phase composition: orthophosphoric acid and methanol*

Robustness:

The robustness of the method was illustrated by assaying the DST standard solutions (4 and 60 µg/ml), when mobile phase flow rate (±0.1 ml/min), mobile phase composition ratio (±2%), column temperature (±2^{0}C) and mobile phase pH (±0.1) and were deliberately varied. The peak areas (Table 5) of the DST standard solutions were not affected by

the varying conditions. The relative standard deviation is in the range of 0.472%-0.679% at 4 µg/ml concentration level and in the range of 0.190%-0.462% at 60 µg/ml concentration level. The proposed method thus remained unaffected by slight but deliberate changes in the analytical conditions (Table 5).

Application of the proposed method to assay of DST in pharmaceutical dosage forms:

The above-mentioned validation results indicated that the proposed method gave satisfactory results with DST in bulk. Therefore its pharmaceutical dosage forms [Duprost (labeled to contain 0.5 mg/tablet, manufactured by Dr. Reddy's Lab. Ltd., H. P., India), Dutas (labeled to contain 0.5 mg/tablet, manufactured by Dr. Reddy's Lab. Ltd., H. P., India) and Sterdu (labeled to contain 0.5 mg/tablet, manufactured by Mercury, Lab. Ltd., H. P., India)] were subjected to the assay of their DST contents by the proposed method. The label claim percentages were in the range of 99.40 ± 0.673% to 101.60 ± 0.1.279% (Table 6). Good recoveries with low relative standard deviation values indicate the non interference of excipients commonly present in the pharmaceutical dosage form.

Comparison with the reported UV spectrophotometric method:

The results obtained above were compared with that obtained from the reported UV spectrophotometric method [4] by statistical analysis with respect to the accuracy (by student *t*-test) and precision (by variance *F*-test). At 95% confidence level, no significant differences were found between the calculated and theoretical values of *t*- and *F*-tests confirming similar accuracy and precision in the determination of DST by both methods (Table 6).

Table 6: Determination of dutasteride in dosage forms and statistical comparison with the reference method

Method	Dosage form	Declared value (mg)	Found value (mg) ± S.D*	RSD (%)	Recovery (%)	t Value**	F Value**
Reference	Duprost	0.5	0.502±0.0048	0.956	100.40	-	-
	Dutas	0.5	0.491±0.0064	1.303	98.20	-	-
	Sterdu	0.5	0.505±0.0071	1.405	101.00	-	-
Proposed	Duprost	0.5	0.508±0.0065	1.279	101.60	1.89	5.28
	Dutas	0.5	0.497±0.0052	1.046	99.40	1.79	4.59
	Sterdu	0.5	0.505±0.0034	0.673	101.00	1.58	4.34

**Average of five determinations*

*** Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39.*

CONCLUSION

A simple and rapid stability indicating HPLC with UV detection method equipped with UV detection at 248 nm has been developed for the quantification of DST. The results of validation undertaken according to the International Conference on Harmonization guidelines reveal that the method is linear, sensitive, selective, accurate, precise, specific and robust. The method is appropriate for the routine analysis of DST in either bulk or in pharmaceutical dosage forms.

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