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A Stability Indicating UPLC Method for Dutasteride and Its Related Impurities

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ABSTRACT

The objective of the present research work is to develop a gradient, reversed-phase liquid chromatographic (RP-UPLC) method for the determination of Dutasteride in pharmaceutical bulk drugs for assay and its related impurities. The chromatographic separation was achieved on a Waters ACQUITY ™ UPLC C8 Column (100mm×2.1mm, 1.7µm),. The isocratic LC method employs mixture of buffer and Acetonitrile in the ratio of (50:50 v/v) solutions as mobile phase. The buffer solution contains 1.0mM potassium di hydrogen orthophosphate pH adjusted to 5.0 with dil.Potassium hydroxide solution (Buffer) .The flow rate was 0.4 ml/min and the detection wavelength was 210 nm. In the developed UPLC method, the resolution between Dutasteride and its potential impurities, namely Imp-1, Imp-2 and Imp-3 was found to be greater than 4.0. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in Acidic medium and mild degradation observed in base hydrolysis stress conditions. Degradation product formed during acidic hydrolysis was found to be Unknown impurity. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed RP-UPLC method was validated with respect to linearity, accuracy, precision and robustness. The developed method was found to be linear in the range of 2.5-15µg/mL with correlation coefficient of 0.999 for assay procedures and found to be linear in the range of 0.05-3µg/mL with correlation coefficient of 0.999 for related impurities

Keywords: RP-UPLC; Forced degradation; Validation; Dutasteride, Method development

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INTRODUCTION

Dutasteride, chemically known as (5α, 17β)-N-{2,5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-l-ene-17-carboxamide (Figure 1), is a synthetic 4-azasteroid compound with antiandrogenic activity. Dutasteride is used to treat benign prostatic hyperplasia in men having an enlarged prostate gland and in the treatment of male pattern baldness. It belongs to a class of drugs called 5α-reductase inhibitors, which competitively and specifically inhibits type 1 (active in the sebaceous glands of most regions of skin and liver) and type 2 (primarily active in the reproductive tissues like prostate, seminal vesicles, epididymides, hair follicles and liver) isoforms of 5α-reductase, an intracellular enzyme that converts testosterone to 5α-dihydrotestosterone. The decrease in dihydrotestosterone levels may mitigate or prevent enlargement of the prostate gland. Dutasteride does not bind to the human androgen receptor [1-8].

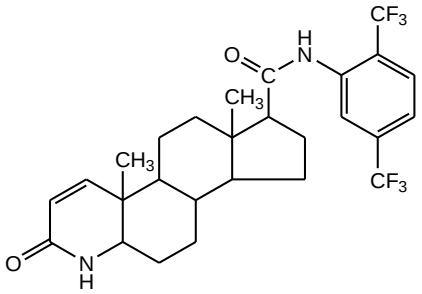


Figure 1: Chemical structure of Dutasteride

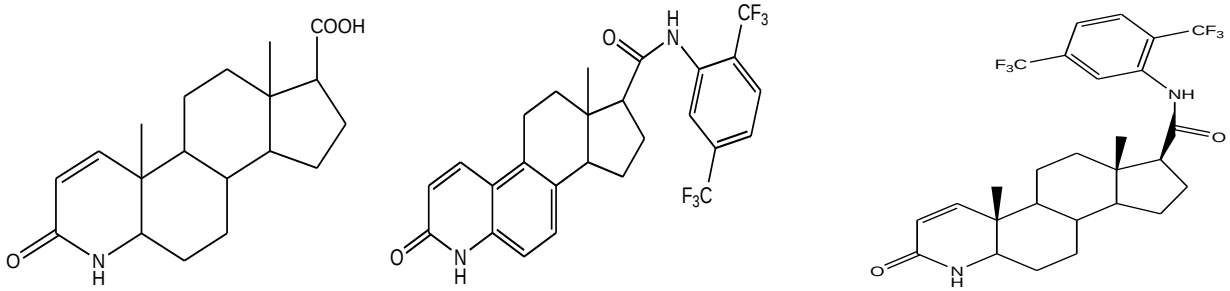
A limited number of analytical techniques have been reported for the quantitative determination of Dutasteride in pharmaceutical preparations and human plasma when present alone or in combination with other drugs (Alfuzosin and Tamsulosin). These techniques are LC–MS[9-11], HPTLC[12], Enzyme-linked immunosorbent assay[13], HPLC and stability-indicating RP-HPLC[14-18].In order to commercialize an active pharmaceutical ingredient, it is mandatory requirement from regulatory authorities to show the proper qualification of its impurities identification and characterization of known impurities that are present. Organic impurities can arise during the manufacturing process and storage of the drug substances and their acceptance upto certain limits are based on pharmaceutical studies or known safety data. In the present study we describe a simple, economic and time efficient reversed phase liquid chromatography (RP-UPLC) method for the separation and quantification of process related impurities of Dutasteride. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and Linearity of the method was determined in accordance with ICH guidelines[19] and found to be suitable for quality assurance of Dutasteride. The paper provides the validated stability indicated RP-UPLC method which separates potential impurities for first time. To the best of our knowledge, there was no

method application by UPLC presented for the related impurities. Here we are presenting first time the use of UPLC method for quantification of Dutasteride and its impurities with a run time of 16 min by separating the related four impurities with a resolution more than 4. The limit of quantification of Imp-1, Imp-2 and Imp-4 were 0.021, 0.024 and 0.039% (of analyte concentration, i.e. 0.5 mg/ml) with 1µl injection volume.

MATERIALS AND METHODS

Chemicals

Samples of Dutasteride and its related impurities were obtained as gift samples from Emmanr industries (Hyderabad, India) (Figure 1 & Figure 2). HPLC grade acetonitrile, analytical reagent grade potassium dihydrogen orthophosphate and potassium hydroxide were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurities used in this study were of greater than 95.0% purity.



Impurity-1 Impurity-2 Impurity-3

Figure 2a: Chemical structures of Impurity-1, Impurity-2 and Impurity-3

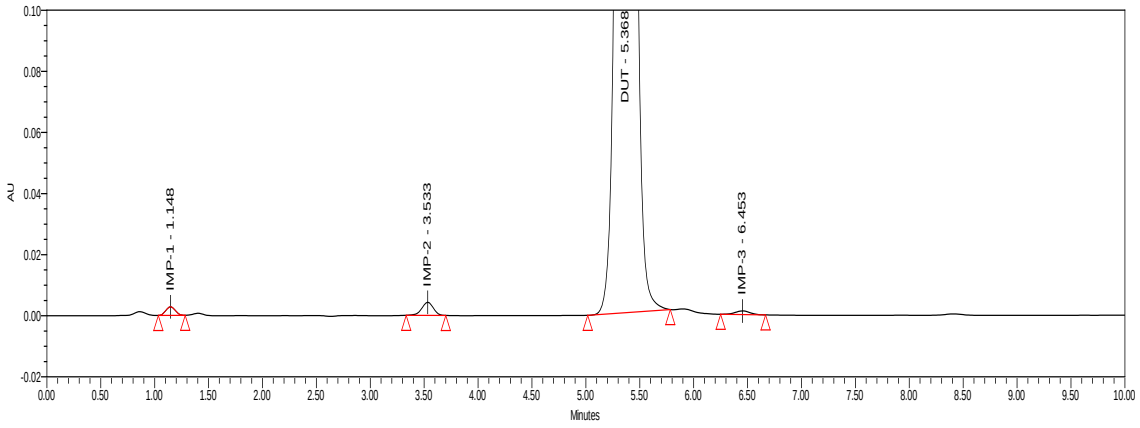


Figure:-2b Typical chromatogram of Impurities blend solution

Equipment

The UPLC system, used for method development, forced degradation studies and method validation was waters ACQUITY ™ UPLC system equipped with a diode array detector , from

Waters Corp. (Milford, MA, USA). The output signal was monitored and processes using Empower software (Waters) Water bath equipped with temperature controller was used to carry out degradation studies for all solution. Photo stability studies were carried out in a photo stability chamber (Mack Pharmatech, Hyderabad, India). Thermal stability studies were performed in a dry air oven (Mack Pharmatech, Hyderabad, India).

Chromatographic Conditions

The chromatographic column used was a waters ACQUITY ^{TM} UPLC BEH C8 Column 100mm×2.1mm, 1.7µm , all obtained from Waters Corp. (Milford, MA, USA). The isocratic LC method consists buffer and Acetonitrile in the ratio of (50:50 v/v) as mobile phase. The buffer solution contains 1.0 mM potassium dihydrogen orthophosphate pH adjusted to 5.0 with potassium hydroxide solution (Buffer).The flow rate of the mobile phase was 0.4 ml/min. The column temperature was maintained 40°C and the detection was monitored at a wavelength of 210nm. The injection volume was 1µl. Mixture water: Acetonitrile (2:8) was used as a diluent. The concentration is 0.5mg·mL–1 for related impurities method and 0.01 mg·mL–1 for Assay method.

Preparation of Solutions

A stock solution of Dutasteride (0.5 mg·mL–1) was prepared by dissolving appropriate amount in the diluent. Working solutions were prepared from above stock solution for related impurities determination and assay determination, respectively. A stock solution of impurities (mixture of imp-1, imp-2 and imp-3) at a concentration of 0.5 mg·mL–1 was also prepared in diluent.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities[24]. Stress testing of the drug impurities can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the developed LC method for Dutasteride was determined in the presence of its impurities, namely Imp-1, Imp-2, Imp-3 and degradation products. Forced degradation studies were also performed on Dutasteride to provide an indication of the stability indicating property and specificity of the proposed method. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60 ◦C), acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH), water hydrolysis and oxidation (3% H2O2). For heat and light studies, study period was 10 days whereas for acid, base, water hydrolysis and oxidation, it was 24 h.

Peak purity of stressed samples of Dutasteride was checked by using Photo diode array detector (PDA). The purity factor is within the threshold limit obtained in all stressed samples demonstrates the analyte peak homogeneity. Assay studies were carried out for stress samples against qualified reference standard and the mass balance (%assay + %impurities + %degradation products) was calculated. Specificity of the Dutasteride was shown by spiking all three impurities (Imp-1, Imp-2 and Imp-3) at the specification level (i.e. 0.15% of analyte concentration which is 0.5mg/ml).

ANALYTICAL METHOD VALIDATION

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and system suitability.

Precision

The precision of the related impurities method was checked by injecting six individual preparations of (0.5 mg·mL–1) Dutasteride spiked with 0.15% each imp-1, imp-2and imp-3. The %RSD area of each imp-1, imp-2 and imp-3 was calculated.

Assay method precision was evaluated by carrying out six independent assays of test sample of Dutasteride against qualified reference standard. The percentage of R.S.D. of six assay values obtained was calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for imp-1, imp-2, imp-3, imp-4 and imp-5 estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of imp-1, imp-2 and imp-3, calculated the %RSD for the areas of each impurity.

Linearity and Range

A linearity test solution for related impurities method was prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels. From 0.05 to 0.3% of the permitted maximum level of the impurity (*i.e.* 0.05, 0.1%, 0.15%, 0.2%, 0.25% and 0.3% was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses.

Linearity test solutions for assay method has prepared from stock solution at five concentration levels from 25 to 150% of assay analyte concentration (2.5, 5, 7.5, 10, 12.5 and 15 µg·mL–1).

Accuracy

The accuracy of the related impurities method was evaluated in triplicate at 0.05%, 0.1%, 0.15%, 0.2%,0.25% and 0.3% of the analyte concentration (500 µg·mL–1). The percentage of recoveries for imp-1, imp-2 and imp-3 were calculated. UPLC chromatograms of blank, spiked sample with all three impurities in Dutasteride bulk drug sample are shown in **Figure 3 & Figure 4**.

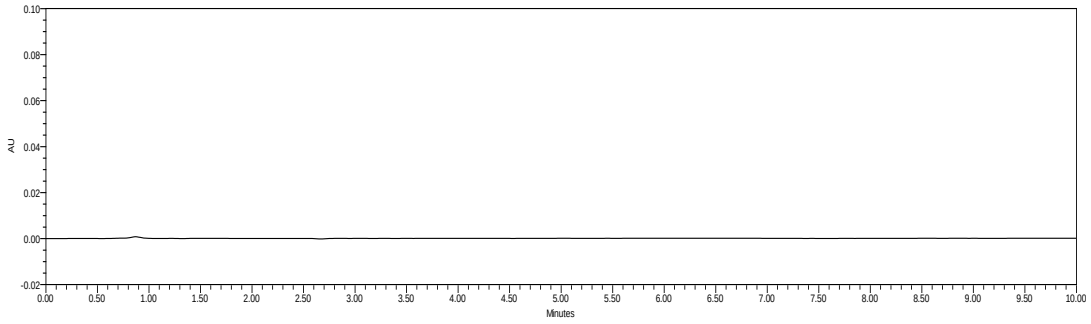


Figure:-3 Typical chromatogram of Diluent

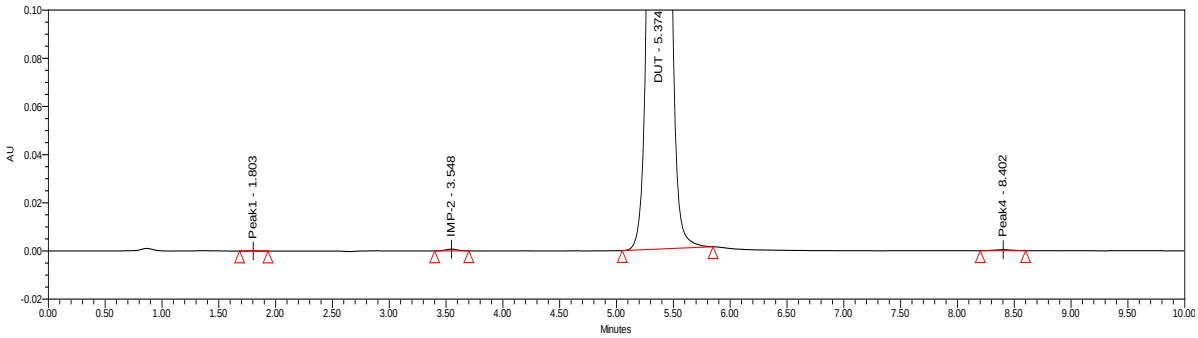


Figure:-4 Typical chromatogram of Sample

The accuracy of the assay method was evaluated in triplicate at three concentration levels, *i.e.* 50, 100 and 150 µg·mL–1 in bulk drugs. At each concentration, three sets were prepared and injected in triplicate. The percentage of recovery was calculated at each level.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (*Rs*) between Dutasteride, imp-1, imp-2 and imp-3 was evaluated. The flow rate of the mobile phase was 0.4 mL·min–1. To study the effect of flow rate on the developed method, 0.02 units of flow was changed (*i.e.* 0.38 and 0.42 mL·min–1). The effect of column temperature on the developed method was studied at 35°C and 45°C instead of 40°C. In the all above varied conditions, the components of the mobile phase were held constant.

Solution Stability and Mobile Phase Stability

The solution stability of Dutasteride and its related impurities were carried out by leaving both spiked sample and unspiked sample solution in tightly capped volumetric flask at room

temperature for 48 h. Content of imp-1, imp-2and imp-3 was determined at every 6 h interval, up to the study period. Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions, for every 6 h interval. Content of imp-1, imp-2 and imp-3 was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

The solution stability of Dutasteride in the assay method was carried out by leaving the test solutions of samples in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed at 6 h intervals up to the study period against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 6 h intervals up to 48 h. Mobile phase prepared was kept constant during the study period. The %RSD of assay of Dutasteride was calculated for the study period during mobile phase and solution stability experiments.

RESULTS AND DISCUSSION

Method Development and Optimization

The main target of the chromatographic method is to get the separation of critical closely eluting peaks, namely Imp-2, Dutasteride and Imp-3. Impurities were co-eluted by using different stationary phases like C18, Phenyl and cyano and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2–8) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase.

The chromatographic separation was achieved on a Waters ACQUITY ^{TM} UPLC BEH C8 Column 100mm×2.1 mm, 1.7µm column, The isocratic LC method consists buffer and Acetonitrile in the ratio of (50:50 v/v) as mobile phase. The buffer solution contains 1.0mM potassium dihydrogen orthophosphate pH adjusted to 5.0 with Potassium hydroxide solution (Buffer).The flow rate of the mobile phase was 0.4 ml/min. The column temperature was maintained 40°C and the detection was monitored at a wavelength of 210nm. The injection volume was 1µl. Mixture water: Acetonitrile (2:8) was used as a diluent. The concentration is 0.5mg·mL–1 for related impurities method and 0.01 mg·mL–1 for Assay method. The peak shape of Dutasteride was found symmetrical. In the optimized conditions Dutasteride, Imp- 1, Imp-2 and Imp-3 were well separated with a resolution of greater than 4.0 and the typical retention times of Imp- 1, Imp-2, Dutasteride and Imp-3 were about 1.15, 3.53, 5.36 and 6.45 min respectively. The system suitability results are given in Table 1 and the developed UPLC method was found to be specific for Dutasteride and its three impurities, namely Imp-1, Imp-2

and Imp-3.

After many logical trials, chromatographic condition was established such that which could be suitable for separation of drug-degradation products and drug-three known impurities. Using the optimized conditions Dutasteride and its known impurities were well separated with a resolution of greater than 4.0 The system suitability results are given in Table 1.

Table-1: System suitability Report

Compound	RRT	USP Resolution (Rs)	USP Tailing factor(T)	No.of theoretical plates
Impurity-1	0.21		1.03	4280
Impurity-2	0.66	12.89	0.96	4970
Dutasteride	1.00	8.34	1.03	8439
Impurity-3	1.20	4.39	1.07	10553

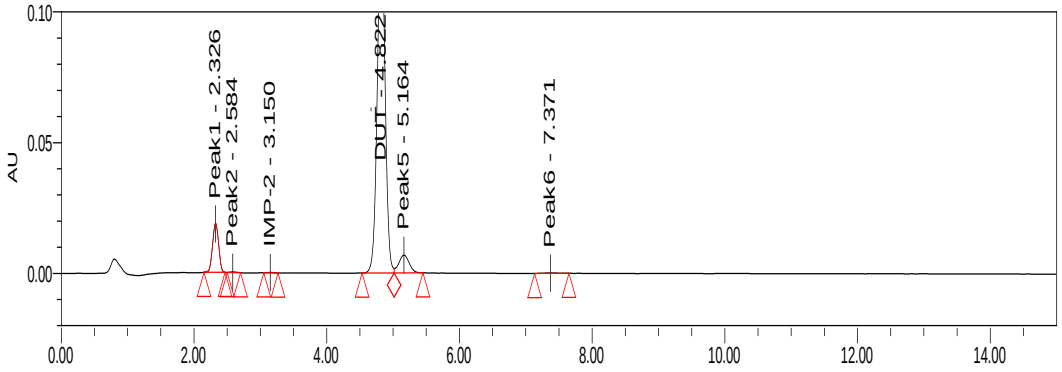


Figure 5: Typical chromatogram of Acid degradation

Results of Forced Degradation Studies

The drug was exposed to 0.1N HCl at 70°C for 24 h. DUT has shown significant sensitivity towards the treatment of 0.1N HCl. The drug gradually undergone degradation with time in 0.1N HCl and prominent degradation was observed (~15%). The representative chromatogram present in **Figure 5.**

Degradation in Basic Solution

The drug was exposed to 0.1N NaOH at 70°C for 24h. Dutasteride has shown mild sensitivity towards the treatment of 0.1N NaOH. The drug gradually undergone degradation with time in 0.1N NaOH and degradation was observed (~2%). The chromatogram present in Figure 6.

Oxidative Conditions

The drug was exposed to 3% hydrogen peroxide at room temperature for 24 hours. Dutasteride has shown no significant sensitivity towards the treatment of 3% hydrogen peroxide and the drug stable under oxidative conditions.

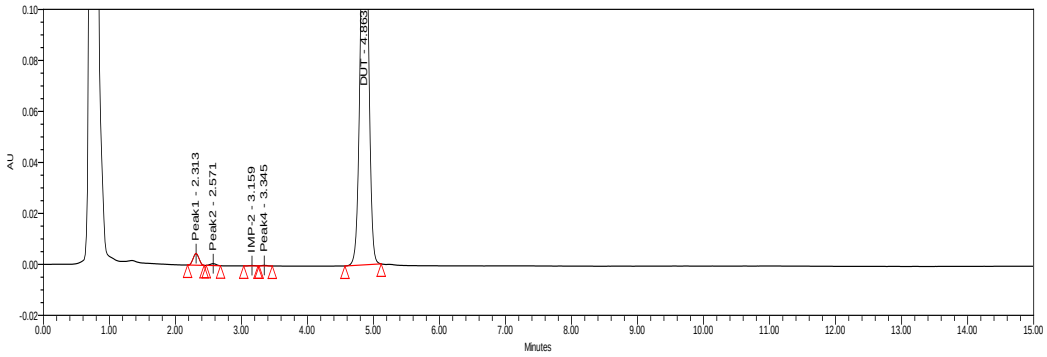


Figure 6: Typical chromatogram of Base degradation

Table-2: Summary of Forced degradation study report

Stress conditions	Time	Purity %	% of Degradation	Remarks
As such sample		99.82	-	
Acid Hydrolysis (0.1N Hcl)	24 hrs at 70°C	84.57	15.25%	Unknown degradation peaks
Base Hydrolysis (0.1 N NaoH)	24 hrs at 70°C	97.12	2.70%	Unknown degradation peaks
Oxidation (3%H2O2)	24hrs at RT	99.78	-	No degradation observed
Water Hydrolysis	24 hrs at 60°C	99.79	-	No degradation observed
Thermal Degradation	10 days	99.8	-	No degradation observed
Photolytic degradation	10 days	99.77	-	No degradation observed

DUT was stable under forced photo and thermal degradation. From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the DUT peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.5%. No degradants were observed after 10 min in the extended runtime of 15 min of all the DUT samples. The developed UPLC method was found to be specific in the presence of imp-1, imp-2, imp-3 and its degradation products confirm the stability indicating power of the developed method. The forced degradation study results are given in Table 2. The representative chromatogram present in Figure 7.& 8

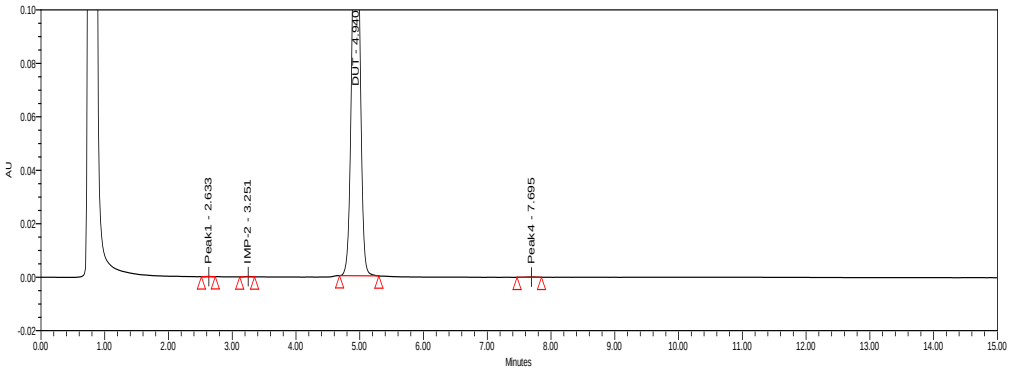


Figure 7: Typical chromatogram of oxidation degradation

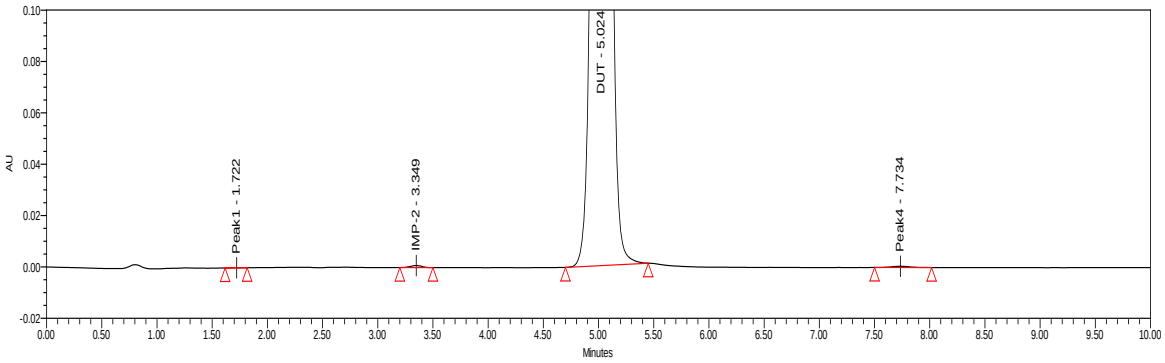


Figure 8: Typical chromatogram of Thermal degradation

Method validation

Precision

The %RSD of area of imp-1, imp-2 and imp-3 in related impurities method precision study were 1.6.The %RSD of assay of Dutasteride during assay method precision study and intermediate precision study was 0.21 which confirms the good precision of the developed analytical method for both assay and related impurities.

Sensitivity

The limit of detection of Imp-1, Imp-2 and Imp-3 were 0.0077, 0.0081 and 0.013% (of analyte concentration, i.e. 0.5 mg/ml) for 1µl injection volume. The limit of quantification of Imp-1, Imp-2, Imp-3 and Imp-4 were 0.023, 0.024 and 0.039% (of analyte concentration, i.e. 0.5 mg/ml) for 1µl injection volume. The precision at LOQ concentration for Imp-1, Imp-2 and Imp-3 were below 2 %.Experimental data shown in Table 4.

Table 4: LOD , LOQ and Linearity Experimental Data

Conc	Impurity-1	Impurity-2	Impurity-3
0.05	3000	3259	2478
0.1	6001	6506	4906
0.15	9198	10156	7730
0.2	12096	13073	10628
0.25	15612	16669	12744
0.3	18546	20069	15710
Correlation	**0.9998**	**0.9997**	**0.9993**
Slope	**62549.14286**	**67117.71429**	**52898.28571**
STEYX	**145.705**	**163.817**	**208.167**
LOD	**0.0077**	**0.0081**	**0.013**
LOQ	**0.023**	**0.024**	**0.039**
Intercept	**-203.93**	**-123.60**	**-224.53**
% Y-INTER	**-2.22**	**-1.22**	**-2.90**

Linearity and Range

Linear calibration plot for related impurities method was obtained over the calibration ranges

tested, *i.e.* 0.05 to 0.3% for imp-1, imp-2 and imp-3. The correlation coefficient obtained was greater than 0.999 for all three impurities. The result shows an excellent cor-relation existed between the peak area and concentration of imp-1, imp-2, and imp-3.

Linear calibration plot for assay method was obtained over the calibration ranges tested, *i.e.* 2.5 - 15 µg/mL and the correlation coefficient obtained was greater than 0.999. The result shows an excellent correlation existed between the peak area and concentration of the analyte.

Accuracy

The percentage recovery of Dutasteride in bulk drug samples ranged from 99.4% - 101.8%. The percentage recovery of imp-1, imp-2 and imp-3 in bulk drug samples ranged from 95.3% to 97.2% (Table 3).

Table 3: Precision and Accuracy Data

Accuracy level(n=3)	Impurity-1	Impurity-2	Impurity-3
Accuracy at 0.05%	97.3	99.6	99.8
Accuracy at 0.10%	97.8	94.9	95.6
Accuracy at 0.15%	95.3	100	96.9
Accuracy at 0.20%	95.6	94.1	94.4
Accuracy at 0.25%	92.4	94.6	96.5
Accuracy at 0.30%	95.5	95.4	97.3

n=3 number of determination

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, pH and column temperature) revealed that the resolution between closely eluting peaks, namely Dutasteride and imp-3 was always greater than 4.0, illustrating the robustness of the method.

Solution Stability and Mobile Phase Stability

No significant changes were observed in the content of imp-1, imp-2 and imp-3 during solution stability and mobile phase stability experiments for related impurities. The %RSD of assay of Dutasteride during solution stability and mobile phase stability experiments was within 1.0.The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related impurities determination were stable up to the study period of 48 h.

Assay Analysis

Analysis was performed for different batches of Dutasteride in bulk drug samples (n = 3) ranged from 99.95% - 99.96%.

CONCLUSION

The RP-UPLC method developed for both assay and related impurities was linear, precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested for both assay and related impurities as per ICH guidelines. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Dutasteride samples same procedures on a different day (interday precision). To the best of our knowledge the specified method presented in the article successfully measures Dutasteride and its related impurities by UPLC.

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