

Development and validation of a new RP-HPLC method for the estimation of dutasteride in bulk and pharmaceutical formulations

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ABSTRACT

A simple, specific, accurate and stability indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of dutasteride in pharmaceutical formulations and bulk drugs using C-18 column (150 mm × 4.6 mm, 5.0 μm). The mobile phase composed of methanol: phosphate buffer saline (PBS) (80:20, v/v), pH 6.8 adjusted with *O*-phosphoric acid and the flow rate was maintained at 1ml/min at 30°C temperature. The retention time of dutasteride was found to be 4.1 min. Linearity was established in the range of 1-15 μg/ml with a coefficient correlation of 0.999 and the eluents were monitored at 230 nm. The percentage recoveries were found to be in the range of 99.06-100.60%. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to limit of detection, limit of quantification, precision, accuracy, Linearity and system suitability. This method can be successfully employed for simultaneous quantitative analysis of dutasteride in pharmaceutical formulations and bulk drugs as well.

INTRODUCTION

Dutasteride is a selective inhibitor of both type 1 and 2 isoforms of enzyme, 5 α-reductase which is responsible for the conversion of testosterone to 5 α-dihydrotestosterone (DHT). DHT is an androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland (Drug bank, 2015). Thus dutasteride inhibits the conversion of testosterone to DHT. Currently, the use of dutasteride in the treatment of benign prostatic hyperplasia has increased due to its less side effects compared to the existing drug available for the treatment. It reduces the risk of acute urinary retention as well.

Dutasteride is a white powder freely soluble in acetonitrile, ethanol, methanol, and insoluble in water. Chemical name and empirical formula of dutasteride are: (5 α, 17 β)-N-{2, 5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide and C₂₇H₃₀F₆N₂O₂ respectively, has the molecular weight of 528.5g/mol (Budavari *et al.*, 2001; Drug bank, 2015).

HPLC, LC-MS, HPTLC, and TLC methods for development and validation were reported for the estimation and determination of the dutasteride in the pharmaceutical forms alone or with the combination of other drugs.

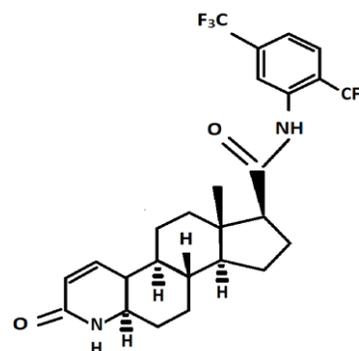


Fig1: Chemical structure of Dutasteride.

Mohammed Ishaq *et al.* (2014) determined the combination of dutasteride and tamsulosin by RP-HPLC method. Dipti *et al.* (2010) and Amritha *et al.* (2012) developed HPLC methods to use in analysis of bulk & pharmaceutical dosage form.

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Dipti *et al.* (2010) also developed and compared HPLC and HPTLC analysis for tamsulosin and dutasteride. Stability indicating TLC methods; LC-MS methods for dutasteride in tablets dosage form were also validated (Vishnu *et al.* 2009; Ramakrishna *et al.* 2004). Priyadarshani *et al.* (2015) developed a HPLC method for the combination of dutasteride and tamsulosin in the capsule dosage form.

Kazusaki *et al.* (2012) have explained the importance of the methods of validation approaches on different types of instruments including HPLC. Furthermore, their studies discussed about the validation characteristics to analyse bulk active pharmaceutical ingredients by assay methods and for trace compounds by impurity methods. The tests includes specificity, linearity, accuracy, precision and range for assay method and specificity, detection limit, quantitation limit, linearity, accuracy, precision and range for impurity method.

RP-HPLC has a non-polar stationary phase and an aqueous / moderately polar mobile phase. In the process of method development, the parameters varies with the size of the column, specification of the instruments and wavelength at which asymmetric peak can be obtained and the mobile phase. Hence it is very important to develop a method that is validated for the specific instrument and column. To demonstrate that the research data obtained from the instrument is accurate to evaluate the product, a method should be developed and validated. RP-HPLC method plays an important role in the discovery development and manufacture of pharmaceuticals. Formulations in liquid dosage forms have more advantages compared to other dosage forms. There are many studies conducted for the determination of tablet dosage forms of dutasteride and also with the combination of other drugs. But determination of soft gelatin capsules of dutasteride is quite uncommon. So, this study aims at the development of a simple, sensitive and reproducible RP-HPLC method for the estimation of dutasteride in capsule form by validating the method according to ICH guidelines (ICH 2005).

MATERIALS AND METHODS

Chemicals and solutions

Dutasteride was kindly donated by RA Pharm. Chem Ltd, Methanol (HPLC grade) was purchased from Merck, Millipore water obtained from Sartorius bio lab products water purification system, Germany. Phosphate Buffered Saline (Dulbecco A) was purchased from Chem solution (SIME scientific). Marketed dutasteride (Avodart (0.5mg)) capsule was purchased from a local pharmacy. The mobile was freshly prepared and filtered through a 0.45µm Millipore filter made of polyamide (250xx) and degassed in an ultrasonic bath. All the chemicals used for mobile phase were of HPLC grade.

HPLC instrumentation

PerkinElmer Life and analytical sciences, Shelton, USA controlled by Chromera software was used in the current analysis.

Chromatographic conditions

Brownlee HPLC column was used with the following descriptions.

Packing material

Brownlee Analytical C18 5µm; Column length: 150mm; Inside diameter: 4.6mm; Particle size: 5 µm.

Detector

PDA detector (Photo-diode –array).

Mobile Phase

An isocratic mobile phase consisting of a mixture of methanol and PBS pH.6.8 in a ratio of 80:20 was used throughout the analysis.

Flow rate

The flow rate of the mobile phase was 1.0mL/min.

Detector wavelength

Detector signal was monitored at a wavelength of 230nm. The column temperature was kept at 30°C and injection volume was 10µL.

Preparation of solutions

Mobile phase preparation

80 percent of methanol was taken in container A and 20 percent of PBS buffer pH 6.8 in container B and filtered individually, by using filtration assembly containing 0.45µm pore filter of polyamide to remove particle impurities. The mobile phase was degassed by for 20 min to remove the gases impurity.

Preparation of diluent

A mixture of methanol and PBS buffer pH 6.8 was prepared in the ratio of 80: 20 respectively was used as diluents for dilution of standard stock solution. PBS was prepared according to the instruction provided in the product label. PBS was prepared by dissolving 1 tablet in 100ml of double distilled water.

Stock Solution of Dutasteride

An accurately weighed quantity of dutasteride working standard 50mg was taken in a 50mL volumetric flask and dissolved in diluents and diluted up to mark with diluent to give a stock solution having strength of 1mg/mL \approx 1000µg/mL.

Working standard solution of dutasteride

Working standard solution is obtained by, accurately pipetting 1ml of dutasteride stock solution and transferred into 10ml volumetric flask and diluted up to the mark with the diluent. The solution was shaken well to obtain the concentration of 0.1mg/mL \approx 100µg/mL. Further dilution was done to obtain the concentrations of 1 to 15µg/mL. The resultant solution to be analysed have to be filtered through 0.45micron filters.

Sample preparation (Assay)

Accurately 10 capsules were weighed and the contents from the capsules were drawn out and the content of all 10 capsules are mixed up in order to ensure the content uniformity of capsules. Weight of content equivalent to 1mg of dutasteride was taken into 10ml of volumetric flask and diluted up to the mark. The solution was shaken well to obtain the concentration of 0.1mg/mL \approx 100 μ g/mL. The solution was further diluted obtain 10 μ g/mL. The resultant solution to be analysed have to be filtered through 0.45micron filters.

RESULTS AND DISCUSSIONS**Method development and optimization**

The optimization of the chromatographic conditions have to done to obtain symmetric peaks with better resolution and system suitability. Dutasteride was freely soluble in acetonitrile and methanol. So combination of different ratios of acetonitrile and water, acetonitrile and buffer 6.8 were studied. As well as combination of methanol and water; methanol and buffer were run to get a better peak resolution and symmetry. Methanol was used to optimize the retention time of eluting drug. Various concentration of combination of methanol and PBS buffer 6.8 with different wavelengths, and flowrates were run to obtain a good symmetric peak with better area. Finally the mobile phase with the combination of methanol and PBS buffer 6.8 in the ratio of 80:20 v/v gave a sharp symmetric peak shape and the mobile phase was finalized. The detection wavelength was selected were the drug showed maximum absorbance at 230nm based on the peak area.

The column temperature was maintained at 30°C and finalised the flow rate is 1mL/min. The retention time was found to be 4.134 \pm 0.010 (Fig.2).

Method Validation

The method was validated according to the ICH guidelines (ICH-Q2 (R1)) 2005. The developed method was validated for system suitability, linearity, sensitivity, precision and accuracy.

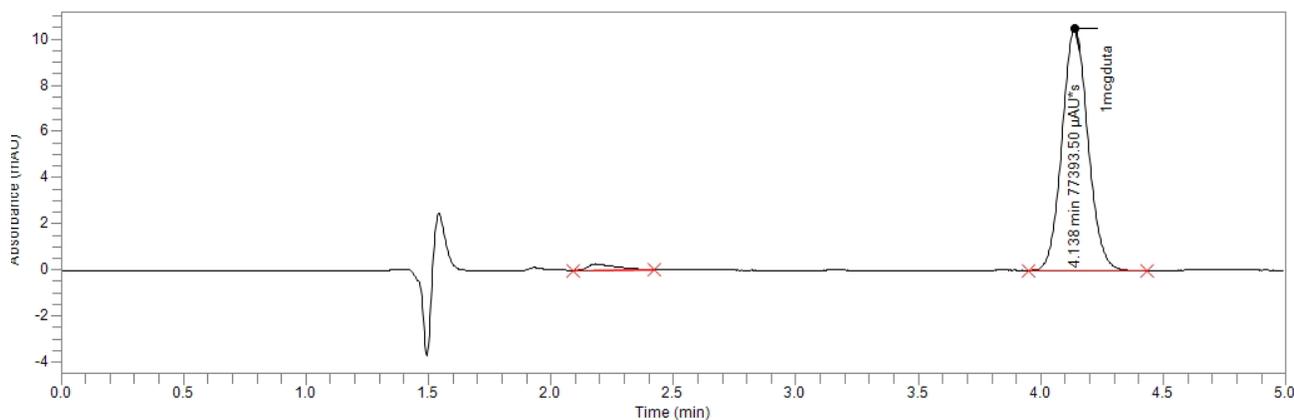
System suitability

System suitability tests are an integral part of chromatographic method which are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution for six times, at the concentration level 7.5 μ g/ml to check the reproducibility of the system. The system suitability was studied on the freshly prepared solution of dutasteride containing 7.5 μ g/ml and the parameters were reported.

The percent relative standard deviations and average of the peaks were measured. Based on the observation that the column efficiency as determined for dutasteride peaks is not less than 2000USP plate count and the tailing factor was not more than 2 shows that the good column efficiency and good peak symmetry respectively. The % RSDof the peak area is not more than 1 as shown in Table 1. The Fig 2 and 3 represent the chromatogram of standard and blank, respectively.

Table 1: System suitability (n=6).

Area	Retention time	Theoretical plates	Asymmetry
77356.47 \pm 343.03	4.11 \pm 0.01	7178.20 \pm 48.29	1.118 \pm 0.04
% RSD0.0044			



	Peak Name	RT	Area	Theoretical Plates	Tailing Factor
1	dutasteride	4.138	77393.5	7125	1.12

Fig. 2: Chromatogram of standard for system suitability.

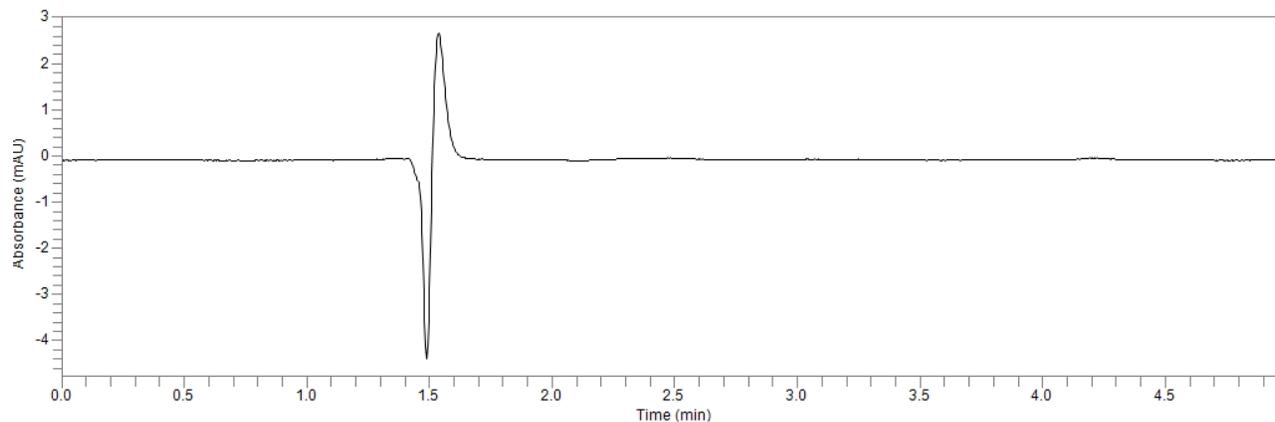


Fig.3: Chromatogram of blank.

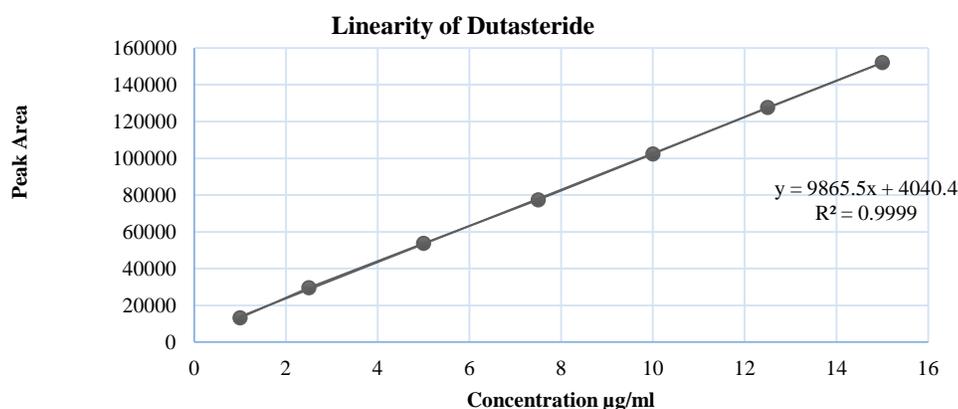


Fig. 4: Linearity of Dutasteride.

Table 2: Statistical fit of linear regression.

ANOVA	df	SS	MS	F	Significance F
Regression	1	15655854068	15655854068	39021.08956	6.30865E-11
Residual	5	2006075.976	401215.1952		
Total	6	15657860143			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept X	4040.417	450.57	8.967	0.0003	2882.191	5198.642	2882.191	5198.64
Variable 1	9865.48	49.942	197.538	6.3086E-11	9737.099	9993.86	9737.099	9993.86

Linearity and range

The calibration curves were plotted over the concentration range of 1- 15 µg/mL. Accurately measured standard solutions (1, 2.5, 5, 7.5, 10, 12.5, and 15 mL) of dutasteride was diluted with the diluent to obtain the concentration from 1, 2.5, 5, 7.5, 10, 12.5 to 15 µg/mL. The absorbance was measured at 230nm against mobile phase. The calibration curve was constructed by plotting absorbance against concentration and the correlation co-efficient and regression line equation was calculated and the results show a good correlation between peak area of the drug and their concentrations. The correlation co-efficient (r^2) value was found to be 0.9999 (Fig 4). ANOVA results showed that the r^2 value as 1. Regression fit of the correlation co-efficient was found to be fit, p value >0.05 (0.0003) (Table 2).

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

LOD is ability of an analytical method to detect the lowest concentration of the analyte. A signal-to-noise ratio should be 3 is generally considered acceptable for estimating the detection limit. LOQ is the lowest concentration of the analyte which can be quantitatively analysed with acceptable precision and accuracy. The LOD and LOQ were calculated using following formulae: $LOD = 3.3(SD)/S$ and $LOQ = 10 (SD)/S$, where SD =standard deviation of response (peak area) and S = average of the slope of the calibration curve.

The limit of detection (Fig. 5) and limit of quantification (Fig 6) were obtained by scanning the solutions of dutasteride in various lower concentration found to be 0.5 and 1 µg/ml respectively, which indicates that the method is sensitive.

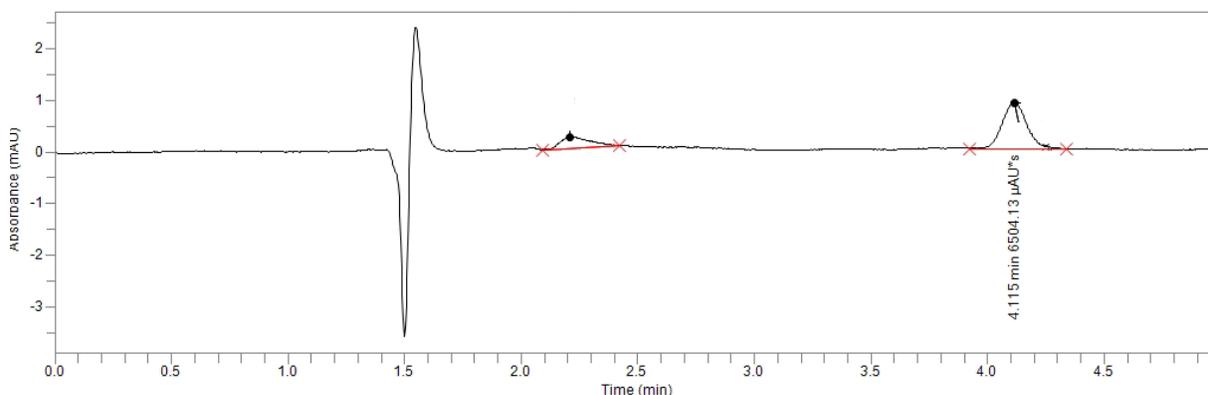


Fig .5: Chromatogram of Limit of detection

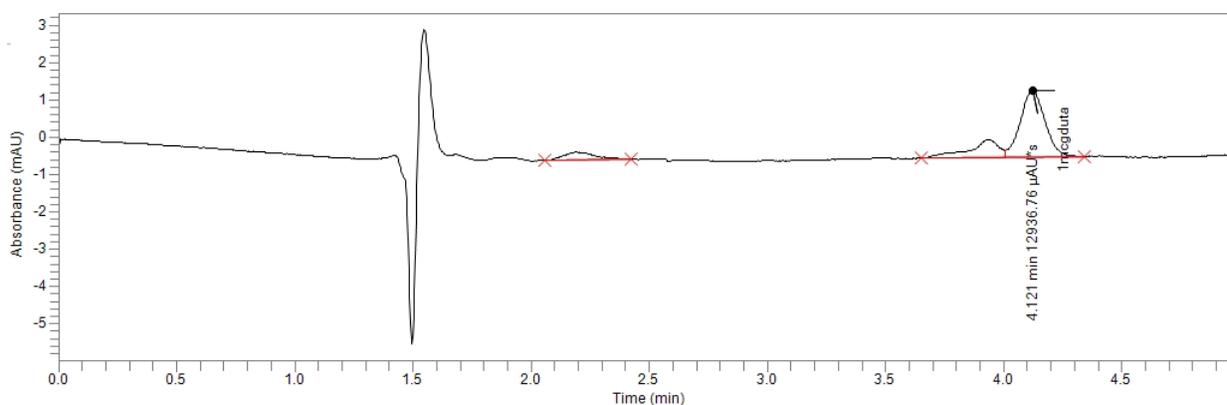


Fig.6: Chromatogram of Limit of Quantification

Table 3: Regression characteristics and validation parameters.

Parameters	Dutasteride
Linearity range (µg/ml)	1-15
Correlation coefficient (r ²)	0.9999
Regression equation (y=mx+c)	
Slope (m)	9865.5
Intercept (c)	4040.4
LOD1 (µg/ml)	0.5
LOQ2 (µg/ml)	1
Accuracy (%)	99.42- 100.35%
Repeatability(RSD3,%,n=6)	0.29%
Precision (RSD%)	
Interday (n=3)	0.0340- 1.0846 %
Intraday (n=3)	0.0875- 1.0891%

1-Limit of detection; 2 – Limit of Quantification; 3 –Relative Standard Deviation.

Accuracy

QC concentrations are defined as Low Quality Concentration (LQC), Medium Quality Concentration (MQC), and High Quality Concentration (HQC) with LQC being three times higher than Lower Limit of Quantification (LLOQ) which is 3µg/mL. MQC is calculated as 50% from the highest concentration of calibration curve while HQC is 75% of it, which gives 7.5µg/mL and 11.25µg/mL respectively. Accuracy of the method was determined by standard addition method in which standard addition of pure drug are at three different concentration levels

of 3 (20%), 7.5 (50%), 11.25 (75%) µg/ml. Accuracy of the method was determined by calculating percentage recovery of three concentrations on a single assay day to determine intra-day precision and accuracy. Analysis of six samples of three concentrations on 3 consecutive days were used to determine inter-day precision and accuracy. Accuracy of the method was evaluated using concentrations of 3, 7.5 and 11.25 µg/ml and the result found to be in the range of 99.4 to 100.3%. Hence the % accuracy was within the limit (98.0 -102.0%) and the method is accurate (Table 4). All the experiments are performed in triplicate (n=3)

Table 4: Recovery% in accuracy studies.

Concentration ($\mu\text{g/ml}$)	Area	Amount recovered (μg)	Recovery (%)
3.0	33539.10 \pm 208.14	2.9900 \pm 0.012	99.66955 \pm 0.261
7.5	77607.57 \pm 342.61	7.457013 \pm 0.011	99.42685 \pm 0.121
11.25	115413.80 \pm 289.52	11.28917 \pm 0.081	100.34826 \pm 0.162

Table 5: Intraday precision.

Time (hrs)	Concentration ($\mu\text{g/ml}$)	Mean of area	SD	RSD %
0	3.0	30205.13	328.9814	1.0891
4	3.0	30276.93	83.9335	0.2772
0	7.5	70867.96	62.0612	0.0874
4	7.5	70837.25	189.3702	0.2673
0	11.25	114628.83	313.3826	0.2733
4	11.25	114053.80	285.6216	0.2504

Table 6: Inter-day precision.

Day	Concentration ($\mu\text{g/ml}$)	Mean of Area	SD	RSD %
1	3.0	33801.12	227.0002	0.6715
3	3.0	30276.93	83.93351	0.2772
1	7.5	77215.84	553.9828	0.7174
3	7.5	71479.02	81.43041	0.1139
1	11.25	114535.38	1242.266	1.0846
3	11.25	108596.83	36.96041	0.0340

Table 7: Precision (repeatability).

S.no	Retention time (min)	Area	Amount recovered (μg)	Recovery %
1	4.10	76796.77	7.3748	98.3310
2	4.13	77607.57	7.4570	99.4268
3	4.11	77279.53	7.4237	98.9835
4	4.12	77633.26	7.4596	99.4615
5	4.11	77465.25	7.4425	99.2345
6	4.12	77325.59	7.4284	99.0457
% RSD	0.002	0.0039698	0.0041	0.0041
Average	4.11	77351.328	7.4310	99.0805

Precision

Precision of the method was determined by evaluating intraday and inter-day precision. Intra-day and Inter-day variation was analysed by selecting three concentrations which were 3, 7.5, 11.25 $\mu\text{g/ml}$ from linearity range. Intraday analysis was carried on same day whereas Inter-day analysis was carried on three different consecutive days.

The intra and inter day precision were determined by analysing standard solution of dutasteride at three different concentrations (3, 7.5, 11.25 $\mu\text{g/ml}$).

The % RSD was found to be 0.08- 1.08% and 0.034- 1.08 % for intra and inter day precision respectively (Table 3, 5 & 6). The RSD values were below 3% indicates the precision is good.

Repeatability (Precision)

Repeatability expresses precision under the same operating conditions over a short interval of time. Repeatability is also termed as intra-assay precision.

Repeatability of the method for 7.5 $\mu\text{g/ml}$ of dutasteride solution for six times and peak area was measured and %RSD of assay was found to be 0.39% RSD of the sample preparations was 0.29% which is within the normal range (Table 7 and Table 3).

Robustness

Robustness was determined by changing the chromatographic conditions. The flow rate was changed and observed for 0.8 and 1.2 ml/min. The pH of the buffer was observed for 6.8 and 1.2. Mobile phase variation of methanol and buffer in the ratio of 90:10 and 70:30 were studied. From the observation (Table 8), it is found that the parameters such as tailing factor, theoretical plates and retention time were not affected. This shows that the method is robust.

Stability Studies

Stock solution stability in refrigerated condition

The stability of dutasteride was investigated at a temperature of 4° C. 10 $\mu\text{g/mL}$ in three replicates were analysed after 24 hours of storage at 4° C (Fig.7 and table 9). The concentration of dutasteride was compared with the initial concentration of the sample. The % recovery was calculated using the formula,

Stress degradation studies

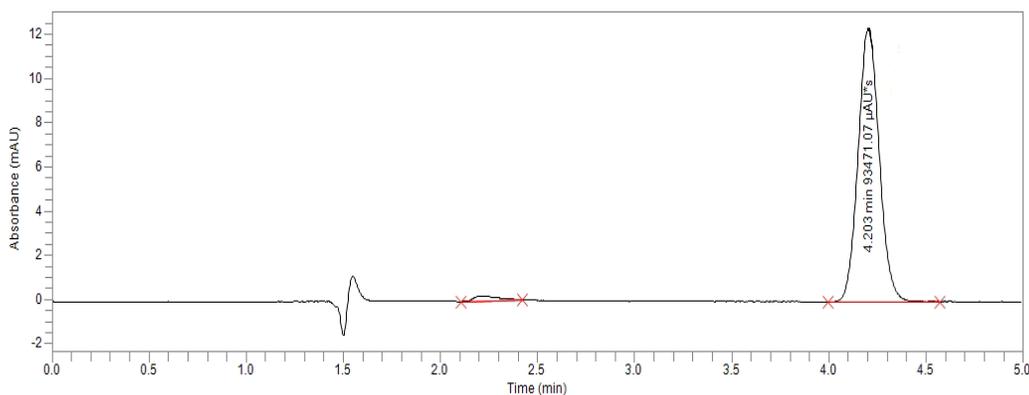
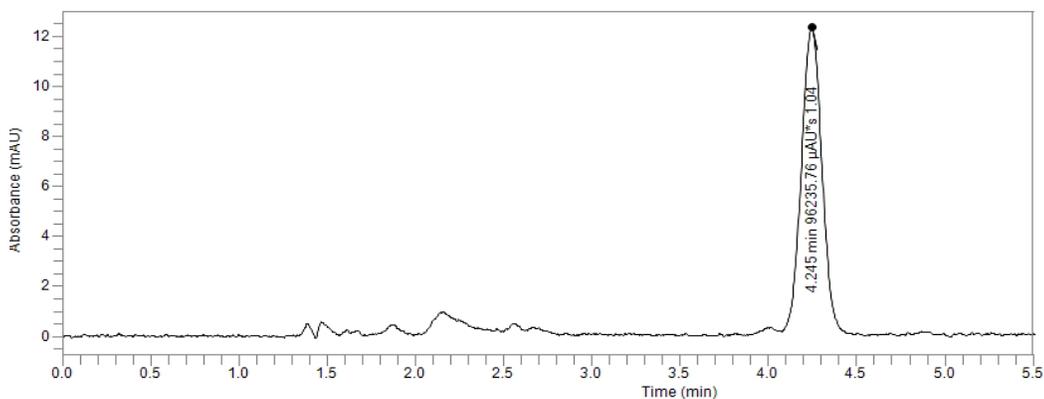
Stress degradation study was performed according to the ICH guidelines Q1A (R2) stability testing of new drug substances and products, using the proposed validated method.

Table 8: Robustness.

Standard Parameter	Variation in flow (n=3)		Variation in mobile phase (n=3)		Variation in pH (n=3)	
	Flow rate (Mean±SD) (0.8ml/min)	Flow rate (Mean±SD) (1.2ml/min)	Methanol and buffer (90:10) (Mean±SD)	Methanol and buffer (70:30) (Mean±SD)	6.8 (Mean±SD)	1.2 (Mean±SD)
Retention time (min)	4.7 ±0.15	3.47±0.014	2.39±0.14	5.78±0.056	4.139±0.009	4.36±0.006
Tailing factor	1.30±0.31	1.23±0.073	1.2±0.09	1.6±0.04	1.13±0.006	1.05±0.11
Theoretical plates	7726.2± 56.5	7193.21 ±25.12	8323.21±111.2	6476.2 ±63.5	7164.4±48.99	6447.2±24.32

Table 9: Stability studies.

Condition	Area obtained	% Assay (n=3)	% Degradation(n=3)
4°C	93471.07±307.21	90.65±0.560	9.045±0.42
0.1N HCl	96235.76±230.42	93.45±0.40	6.233±0.21
0.1N NaCl	95665.63±524.81	92.87±0.51	6.813±0.05

**Fig.7:** Chromatogram of temperature stability study.**Fig.7:** Chromatogram of 0.1N HCl degradation study.

Acid hydrolysis

From the prepared stock solution, 10 µg/ml of the solution was taken and then 1 ml of 0.1N HCl was added. The volumetric flask was kept at normal condition for 1hr and further it was neutralized with 0.1 N NaOH. The resultant solution to be analysed for the stability have to be filtered through 0.45micron filters.

Alkaline degradation

From the prepared stock solution, 10 µg/ml of the solution was taken and then 1 ml of 0.1N NaOH was added. The volumetric flask was kept at normal condition for 1hr and further it was neutralized with 0.1 N HCl.

The resultant solution was analysed. The resultant solution to be analysed for the stability have to be filtered through 0.45micron filters. The % degradation was calculated by using the following formula,

$$\frac{\text{Response of the unstressed sample} - \text{response of the stressed sample}}{\text{Response of the unstressed sample}} \times 100$$

Acceptable stability limits is of 90%, and approximately 10% degradation is optimal for use in analytical validation (Saimalakondaiah et.al, 2014). Based on the results the table 9 and Fig 7 & 8, the stability studies are in the acceptable limits.

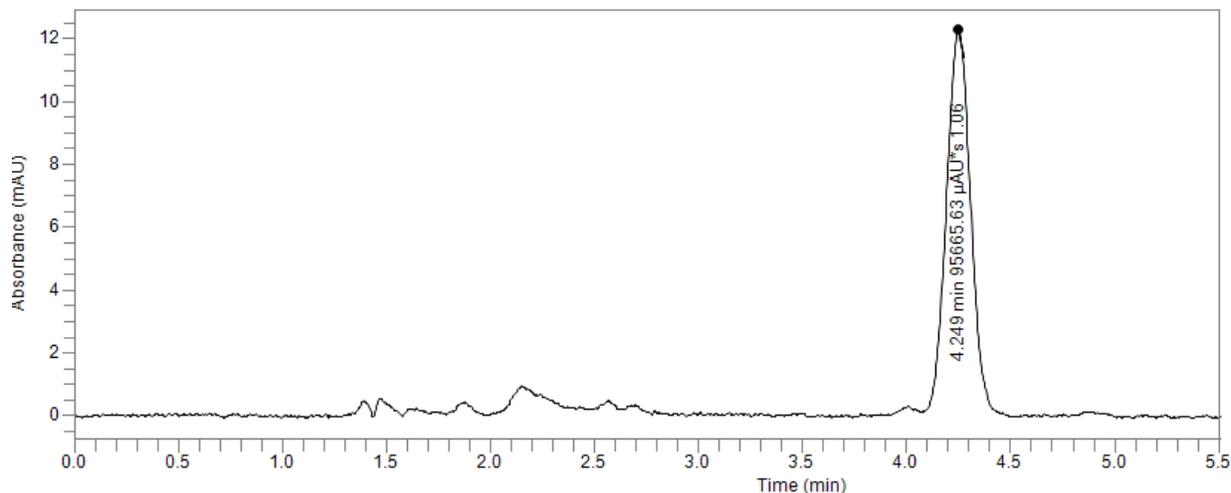


Fig.8: Chromatogram of 0.1N NaOH degradation study.

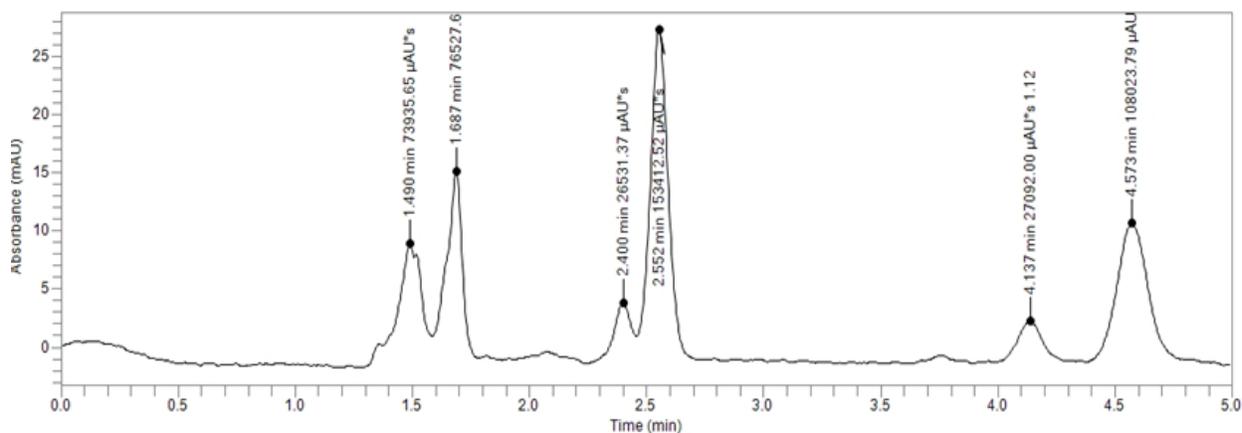


Fig.9: Chromatogram of marketed product.

Table10: Assay of marketed product.

Formulation	Label claim (mg/cap)	% of label claim (n=3) SD
Capsule	0.5	100.197± 0.50

Assay

The proposed method was applied to determine the dutasteride in capsule form and the amount present was found to be 100.2% (Table 10) and a representative graph is provided as fig 9.

CONCLUSION

A simple, precise and sensitive RP-HPLC method was developed and validated for linearity and range, accuracy, precision, repeatability and system suitability of the drug dutasteride. The amount of dutasteride in the soft gelatin capsule formulation was estimated. It was found that the degradation product of the stressed sample and capsule dosage forms didn't

interfere in the estimation of the drug. The proposed, validated method can be successfully applied to determine the dutasteride in liquid formulation and also in a routine laboratory analysis.

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Conflict of Interests: There are no conflicts of interest.

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