Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **05 Year 2023** Page **244:261***

A New Method Development and Validation of 5α Reductase Inhibitor (Dutasteride) Using R-HPLC In Bulk and Pharmaceutical Dosage Form (Dexamethasone Use as An Internal Standard)

Mahtab Ali[1], D. K. Sharma[2]

[1]School of Pharmacy & Research Centre, Sanskriti University,28 km Stone, Chhata Mathura -281001 U.P. (India).

**Corresponding author's E-mail: mahtab.ali009@gmail.com*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 14 Dec 2023

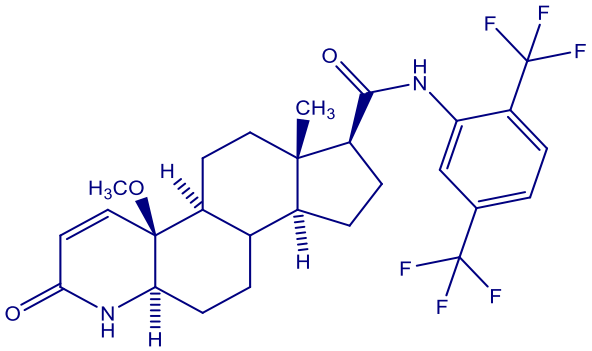
Abstract

The proposed HPLC Method was found to be simple,precise,accurate and economicalfor the estimation of dutasteride in Bulk and tablet dosage form. The present work, developed HPLC method was found suitable and validated. Luna – C-18 (250 mm x 4.6 mm, 20 µL) column was used for the assay. Mobile phase was Acetonitrile and Water in the ratio 80:20. Linearity was established in the range of 5-100 µg/ml with a coefficient correlation of 0.9998 and eluents were monitored at 242 nm and at 1 ml/min. flow rate. The Retention time was found to be 5.9 min. The efficiency of the column, expressed as the number of theoretical plates for six replicate injections was 11566 ± 119.695 (%CV1.03%) and USP tailing factor was 1.25 ± 0.012 (%CV 0.79). The regression equation for the calibration plot was Y = 0.0618 X – 0.008. Intra-day precision from 0.166 to 1.449 and Inter-day precision from 0.186 to 1.449. The% recovery of dutasteride from API and dosage form were 100.70% and 99.66%. The drug was subjected to stress conditions such as Oxidative and Photodegradation, Considerable degradation was found to occure in acidic and basic medium on heating at 100°C but no degradation was observed at room temperature. The drug was exposed to 10% H2O2{heating at 100°C forming degradation product at RT: 2.557}. The LOD and LOQ was 0.257µg and 0.779 µg respectively.When the flow rate was reduced, the retention time of dutasteride increased.The assay result with analyst #1 and Analyst #2 were % Assay = 98.40% (%RSD = 0.13%) and % Assay = 99.07% (%RSD =0.53) respectively. Robustness and ruggednesswasobserved that results were well within acceptance limits of 98–102%, with %RSD ±2.0%, indicating the method is rugged and provides consistent and reliable results which are not affected by small changes in experimental conditions. This method can be used for routine analysis of dutasteride. Running Title: A New Method Development and Validation of 5α-Reductase inhibitor using R-HPLC.

Keywords: *R-HPLC, Dutasteride, Validation, LOD, LOQ, ICH guidelines*

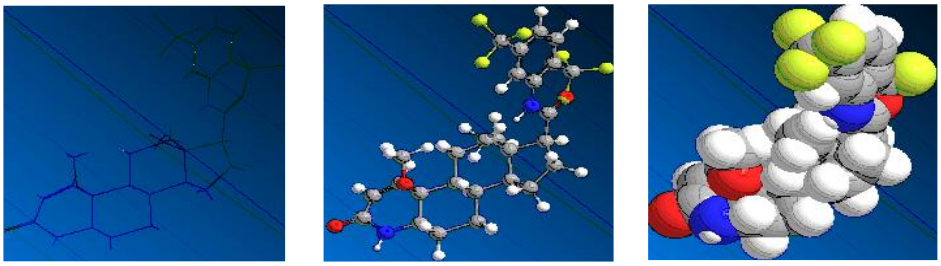
1. Introduction

Dutasteride capsules have been approved by the FDA for the treatment of symptomatic benign prostatic hyperplasia (BPH) in men with an enlarged prostate gland. It is a synthetic 4-azasteroid compound having antiandrogenic activity [1-5]. It belongs to a class of drugs called 5-alpha-reductase inhibitors, which blocking both type 1 and type 2,5-alpha-reductase isoenzymes block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone [6, 7]. There are many studies conducted for the determination of tablet dosage forms of dutasteride and also with the combination of other drugs. But internal standard method of dutasteride is quite uncommon, in this method Dexamethasone use as internal standard for minimising the systematic errors during method validation. There is urgent need to develop a simple, cost effective, rugged, accurate and precise RP-HPLC method using Shimadzu (Model- LC-20AT)for the estimation of dutasteride inpure and Pharmaceutical dosage form.The results of the analysis were validated by latest guidelines set by International Conference on Harmonization (ICH) [8, 12]. The chemical structure, Chem. 3D Preview by Wire Frame, Ball & Stick Model and Space Filling Model of Dutasteride was illustrated in Figure 1.



a

(4a*S*,4b*S*,6a*S*,7*S*,9a*S*,11a*R*)-*N*-(2,5-bis(trifluoromethyl)phenyl)-4a-methoxy-6a-methyl-2-oxo-2,4a,4b,5,6,6a,7,8,9,9a,9b,10,11,11a-tetradecahydro-1*H*-indeno[5,4-*f*]quinoline-7-carboxamide



b c d

Figure 1. (a) Chemical Structure of Dutasteride, Chem 3D Preview, (b) Wire Frame Model (c) Ball & Stick Model (d) Space Filling Model.

Methods Used For Physicochemical Characterization of the Drug:

Melting Point Determination: Melting point of the drugs was determined by taking a small amount of drug in a capillary tube closed at one end and was placed in theil's melting point apparatus and the temperature at which the drug melts was noted. Average of triplicate readings was noted (242-250ºC).

Solubility Studies: The solubility of dutasteride was determined in distilled water, Ethanol, Methanol, Polyethylene glycol viz., pH 3.0, pH 6.0 and pH 8.0. Triplicate readings were taken and average was calculated and others physiochemical properties shown in Table 1.

Table 1: Physicochemical properties of used drug

Drug Category	Anti-baldness Agents, Antihyperplasia Agents, Enzyme Inhibitors
Appearance	White to pale yellow powder
Solubility	Water Insoluble, Ethanol, Methanol, Polyethylene glycol
Chemical Formula	$C_{27}H_{30}F_6N$
Molecular Weight	528.53 gm/mol
Pka value	4.6
Melting point	242-250ºC

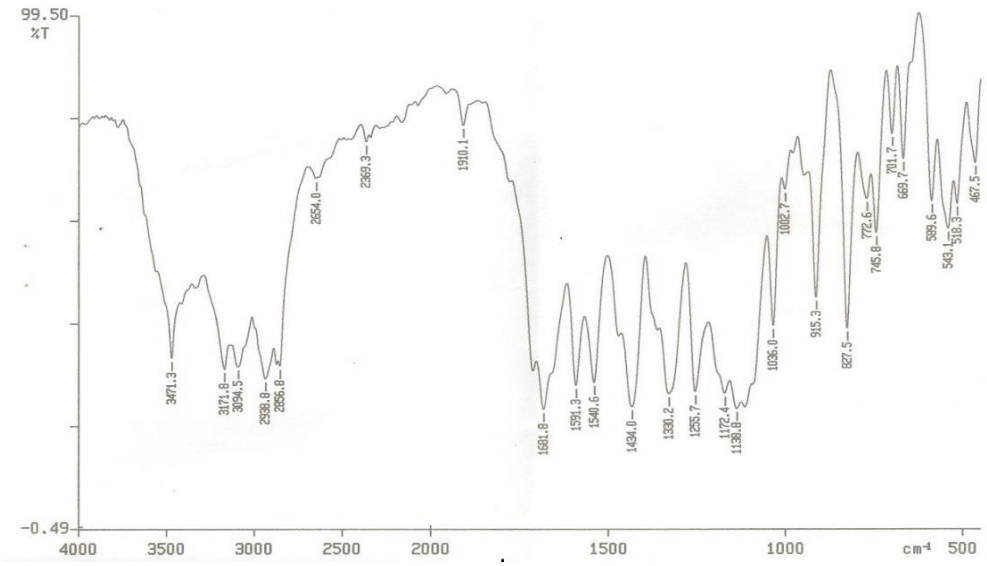


Fig 2: IR Spectra of Dutasteride

2. Materials And Methods

Dutasteride substance was obtained as gift sample from Local API manufacturing unit. Tablet dosage forms of DSE such as Veltride (0.5 mg/tablet, Intas Pharmaceutical Ltd), Dutas Capsule (0.5 mg/tablet, Dr. Reddy's Lab. Ltd., Hyderabad, India) and Sterdu (0.5 mg/tablet, Alkem Lab. Ltd., Mumbai, India) were purchased from local pharmacy market. The mobile was freshly prepared and filtered through a 0.45µm Millipore filter made of polyamide and degassed in an ultrasonic bath. All the chemicals used for mobile phase were of HPLC grade.

Table 2: RP-HPLC Instrumentation &Chromatographic Condition

Instruments	Chromatographic Condition
Hplc System: Shimadzu (Model- LC-20AT)	Flow Rate: 1 ml/minute
Colum: Phenomenex Luna C18 column	Detection wavelength: 242 nm
Pump: Shimadzu (Model- LC-20AT)	Injection Volume: 20 µL
Detector: U.V.Visible Spectrophotometer (SPD-20A)	Column Temperature: 25°C
U.V. Spectrophotometer: Shimadzu Pharmaspec (Model- U.V.-1700)	Run Time: 8 minutes
pH Meter: Digital pH meter (Cyber Labs, USA)	Mobile Phase: Acetonitrile :Water (80:20)

Preparation of Standard solution

Stock Solution of drug: Dutasteride (100mg) was weighed accurately and transferred to the 100 ml volumetric flask quantitatively. It was dissolved in 75 ml of mobile phase with the aid of sonication. The final volume was made upto 100 ml with mobile phase. Different working solutions of dutasteride were prepared from the stock solution using appropriate dilutions.

Stock solution of Dexamethsone (Internal Standard) Solution:Dexamethsone (100mg) was weighed accurately and transferred to the volumetric flask quantitatively. It was dissolved in 75 ml of mobile phase with the aid of sonication. The volume was made upto 100 ml with mobile phase. The concentration of the solution was 1000 µg/ml. This solution was used as internal standard solution. The final concentration of dutasteride samples were 5 µg/ml, 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50 µg/ml, 60µg/ml, 70 µg/ml, 80µg/ml, and 100µg/ml of Dutasteride. Each sample solution was containing IS

Preparation of standard sample: Powder equivalent to 10 mg of dutasteride was transferred to a 100 ml volumetric flask quantitatively. It was dissolved in 75 ml of methanol with the aid of sonication. Final volume was made upto 100 ml with methanol. Different working solution of dutasteride was prepared from the sample solution using appropriate dilutions.

Preparation of sample for different test: Aliquots of the sample solution were transferred to the 10ml volumetric flask containing (1 ml of 100 µg/ml of dexamethasone as IS) 25 µg/ml, 50µg/ml, 75µg/ml, of Dutasteride.

Method Development and Validation of Dutasteride in Bulk and Dosage form

By U.V Scan: showing wavelength maxima at 242nm and 272nm and From the UV spectra the wavelengths 242, nm was selected for monitoring of the drugs.

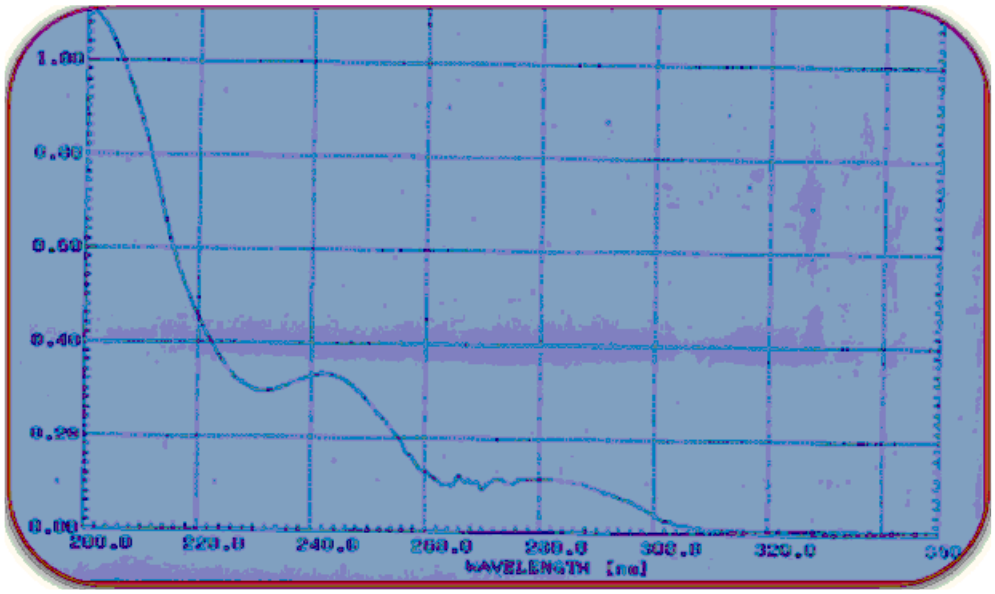


Figure 2. UV spectra of Dutasteride showing wavelength maxima at 242 nm and 272 nm

After trying different mobile phase, the final choice of the mobile please giving satisfactory resolution and run time was Acetonitrile and water in composition with (80:20).

Table3: Chromatographic parameters in different mobile phase compositions

Parameter	Mobile Phase (ACN : Water)			Mobile Phase (ACN : Water: THF)		
	60:40	**70:30**	**80:20**	**45:45:10**	**55:35:10**	**65:25:10**
Retention Time	14.88	9.637	5.983	17.793	9.863	6.19
Tailing Factor	1.696	1.818	1.412	1.46	1.435	1.516
No. of theoretical Plates.	12496	13899	11195	18956	16035	11940

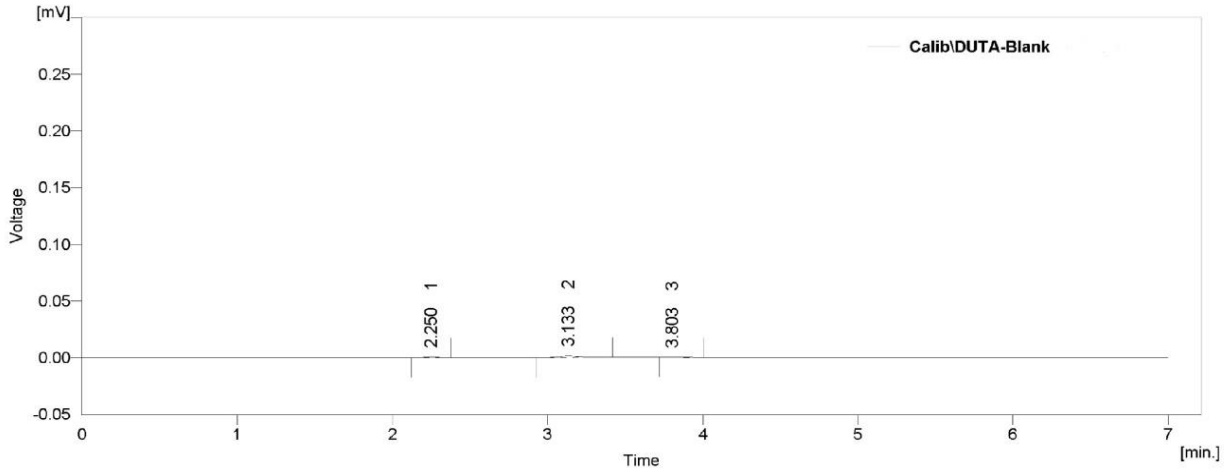


Fig 4: Chromatogram of mobile phase used for the preparation of sample

System suitability: System suitability was performed by injecting repetitive injection (n=6) of dutasteride (25 mcg/ml) and dexamethasone (10µg/ml IS) to the chromatograph, acceptance criteria: - % CV should be less than 2%. and the parameters were reported. 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 respectively. The % RSD of the peak area is not more than 1 as shown in Table 4. The Fig 4. And 5 represented the chromatogram blankandstandard of respectively.

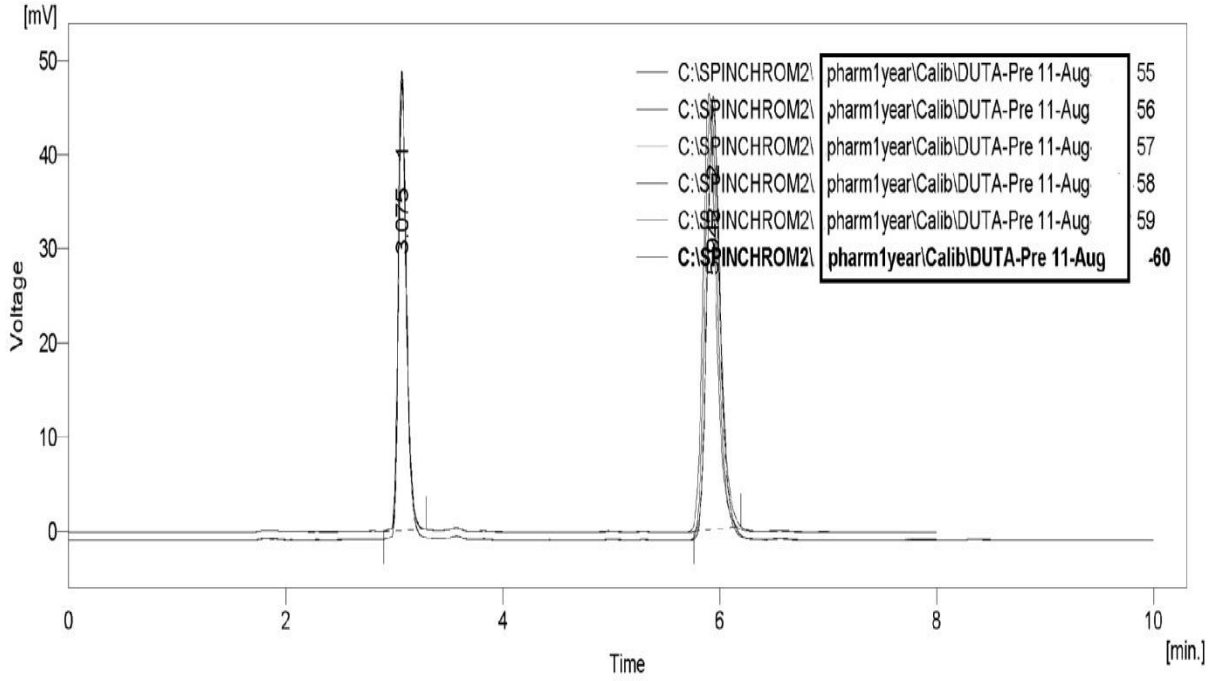


Fig 5: System SuitabilityCurve showing SST result

Table 4: SST Result of Dutasteride calculated by USP

✓ - SST Result for Component "Dutasteride", Calculate By : "USP"

X	Chromatogram	Retention Time [min.]	Area [mV.s]	Height [mV]	Amount	Width 05 [min.]	Asymmetry [-]	Symmetry /Tailing [-]	Efficiency [th. pl.]	Efficiency /Length	HETP [mm]	Resolution [-]
	Lower Limit											
	Upper Limit											
	%RSD Limit	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Mean	5.930	397.344	46.105	24.984	0.131	1.379	1.251	11566.006	231320.120	0.004	16.119
	RSD [%]	0.25	0.75	0.95	0.57	0.70	1.22	0.79	1.03	1.03	1.03	0.24
	Parameter Result	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Calib\DUTA-Pre 11-Aug ____-58	5.940	396.841	45.840	25.058	0.132	1.385	1.250	11664.000	233280.000	0.004	16.150
	Calib\DUTA-Pre 11-Aug ____-55	5.920	396.067	46.610	24.696	0.130	1.354	1.233	11763.141	235262.822	0.004	16.123
	Calib\DUTA-Pre 11-Aug ____-56	5.938	398.278	46.049	25.035	0.130	1.364	1.250	11482.814	229656.288	0.004	16.131
	Calib\DUTA-Pre 11-Aug ____-57	5.935	392.381	45.423	25.044	0.132	1.385	1.257	11469.927	229398.537	0.004	16.113
	Calib\DUTA-Pre 11-Aug-____-59	5.903	399.659	46.492	25.052	0.130	1.385	1.260	11520.444	230408.889	0.004	16.047
	Calib\DUTA-Pre 11-Aug ____-60	5.942	400.838	46.215	25.019	0.132	1.400	1.257	11495.709	229914.184	0.004	16.150

Table 5: Validation parameters of method development

Absorption Maxima(nm)	242
Linearity Range (µg/mL)	5-100
Standard Regression Equation	Y = 0.0618 X – 0.008
System Suitability	11566 ± 119.695
Accuracy (% Recovery ±SD)	99.93%-100.63%.
LOD µg/ml	0.242µg/ml
LOQ µg/mL	0.732µg/ml

Linearity:The calibration curves were plotted over the concentration range of 5 to 100 µg/ml with containing (0.1 ml of 1000 µg/ml of dexamethasone as IS). accurately measured standard solutions (5,10,20,30,40,50,60,70,80,90,100µg/ml) The concentration of IS in prepared solution was 10µg/ml. The regression equation for the calibration plot was Y = 0.0618X - 0.008 (regression coefficient (r^2) 0.9998) shown in Table **7 & 8** and Figure **6**. The average area ratio and height ratio of dutasteride at each concentration level was identified and the linearity graph was calculated by the area count method and height method.

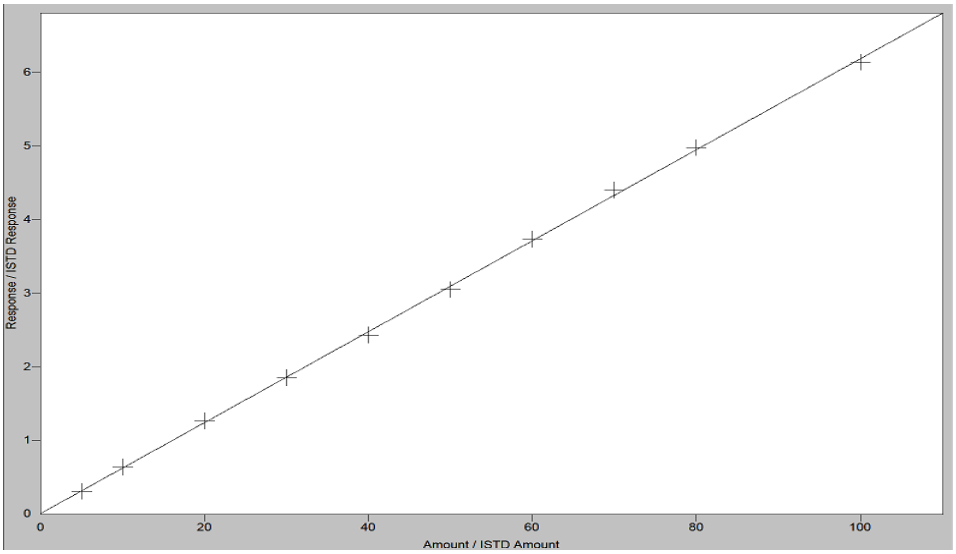


Fig 6: Lineariyt curve showing r^2 value 0.9998 assay method

Table 6: Weighting factors

Weighting factor	r2	Slope	Intercept	Subst. Equation
x	0.9998	0.0618	-0.0083	Y = 0.0618 – 0.0083
1/x	0.9998	0.0618	0.0069	Y = 0.0618 + 0.0069
1/x2	0.9998	0.062	0.0025	Y = 0.0618 + 0.0025

Table 7: Value of parameters (r^2, slope & intercept) using area count method

Conc. (5-100 µg/mL)	CC1	CC2	CC3	CC4	CC5	CC6
r^2	0.9997	0.9998	0.9997	0.9998	0.9998	0.9997
Slope	0.0609	0.062	0.0614	0.0608	0.0629	0.0611
Intercept	0.0022	0.0025	0.0028	0.0001	0.0039	0.0038
	1st Day		2nd Day		3rd Day	

Table 8: Value of parameters (r^2, slope & intercept) using Height method

Conc. (5-100 µg/mL)	CC1	CC2	CC3	CC4	CC5	CC6
r^2	0.9997	0.9998	0.9998	0.9998	0.9997	0.9997
Slope	0.0357	0.0369	0.3561	0.0370	0.0362	0.0368
Intercept	0.0084	0.0017	0.0101	0.0057	0.0060	0.0023
	1st Day		2nd Day		3rd Day	

r^2, slope, intercept were calculated using the height ratio method

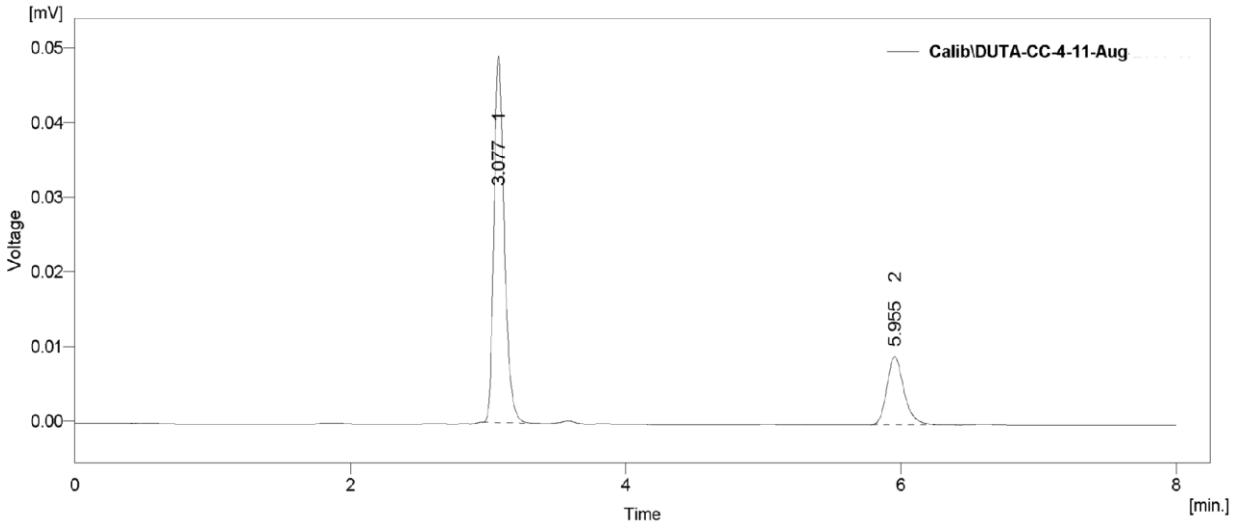


Fig7: Chromatogram of Dexamethasone (IS, 10µg/ml) and Dutasteride(5µg/ml)

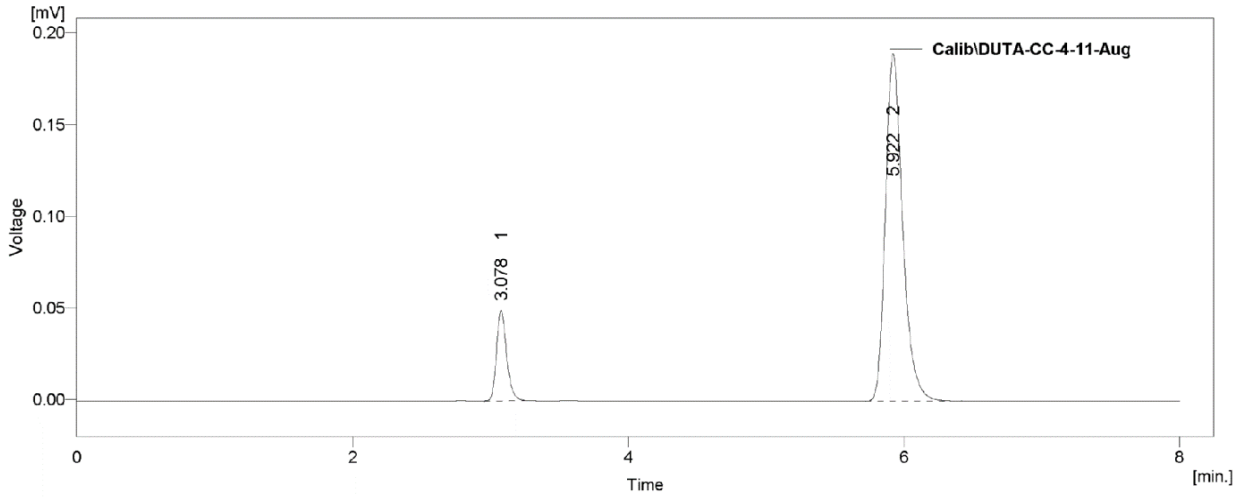


Fig 8: Chromatogram of Dexamethasone (IS, 10µg/ml) and Dutasteride(100µg/ml)

Accuracy: The accuracy of the method was determined by measurement of recovery. Recovery was calculated by use of the regression equation and a regression line graph was drawn using the amount added on the x-axis and the amount found on the y-axis. The calculated recovery and percentage recovery values listed in Table 9are within ±2.0 of the true values in intra-day assay experiments.The accuracy of the method for assay of Dutasteride was demonstrated at three concent rations 25, 50, and 75 µg /ml. Percentage recovery was calculated for the intra-day assay experiments. Standard addition and recovery experiments were also conducted to determine the accuracy of the method.

Table 9: The accuracy of the method development by the measurement of recovery

Theoretical (Conc.)	Measured (Conc.)	Area of drug	Area of I.S	Area Ratio (Drug/I.S)	SD	% COV	% Accuracy
25*	24.983	389.629	250.734	1.553	0.014	0.914	99.927
50*	50.120	806.001	259.552	3.106	0.045	1.449	100.205
75*	75.471	1157.647	247.665	4.674	0.023	0.492	100.629

*every value is the mean of three analyses parameters. All concentration measured in µg/ml

The calculated recovery and percentage recovery values were (99.93%-100.63%). Percent recovery was within 100±2.0% the acceptable range which indicated method was found to be accurate.

Precision:The precision of the method was assessed by study of repeatability and intermediate precision. Repeatability (intra-day variation) of the assay measured for different concentrations (25, 50, and 75 µg/ml) was expressed as RSD calculated from results from analysis on each of three days. Intermediate precision (inter-day variation) at the same concentrations was determined on successive days. For study of intra-day precision the concentration of both drugs calculated three times on the same day at interval of 3 hrs. In the inter-day study the drug concentration was calculated on three different days.

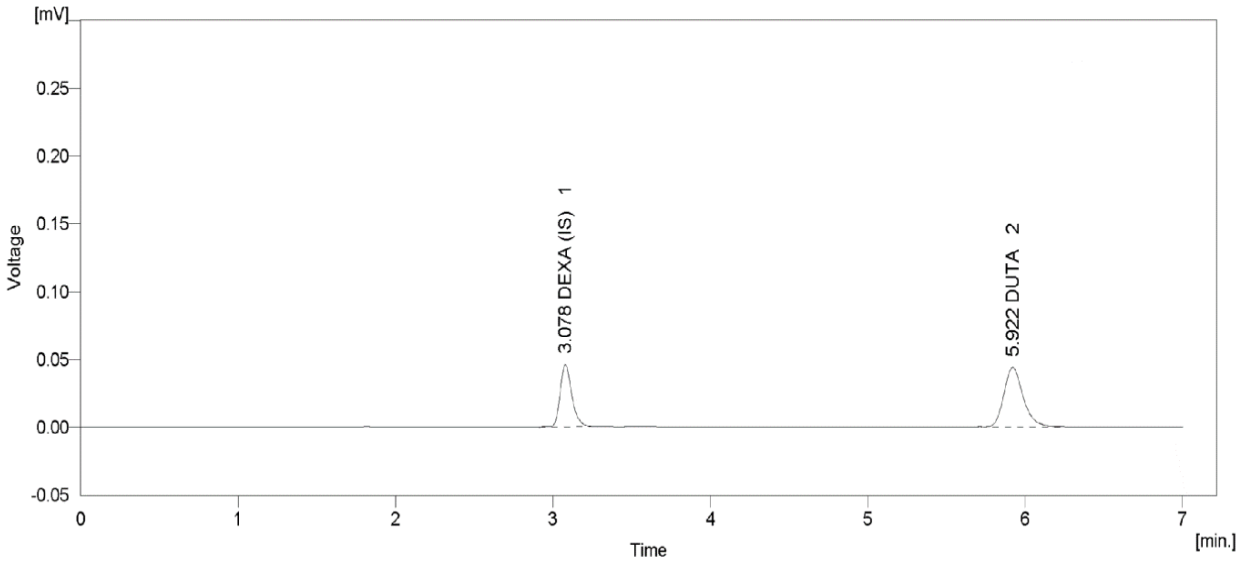


Fig 9: Chromatogram of Dexamethasone (IS,10µg/ml) and Dutasteride(25µg/ml)

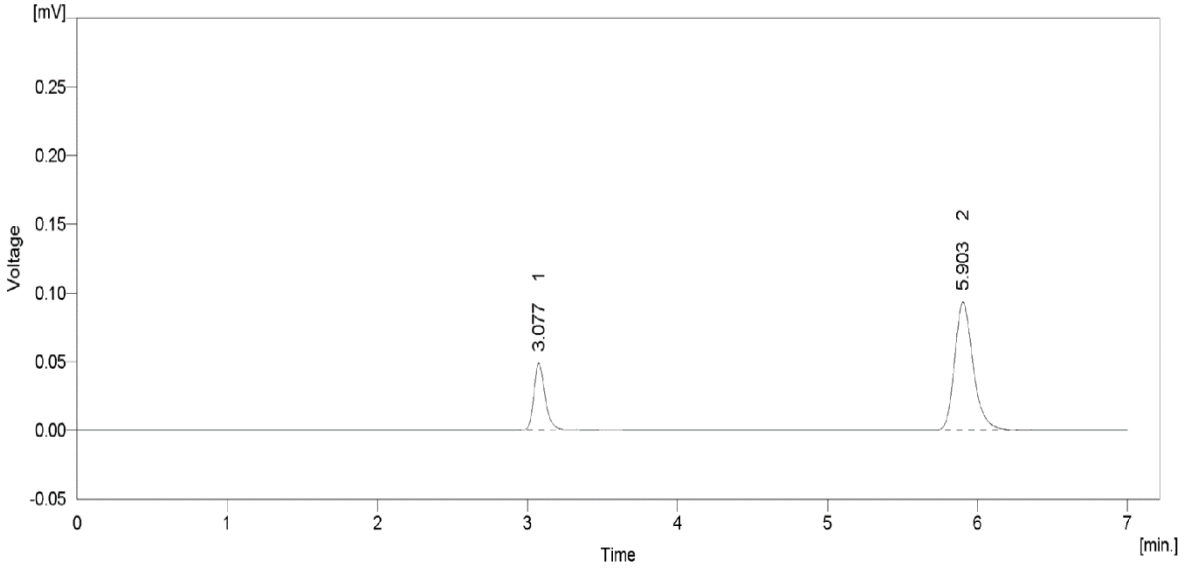


Fig 10: Chromatogram of Dexamethasone (IS,10µg/ml) and Dutasteride(50µg/ml)

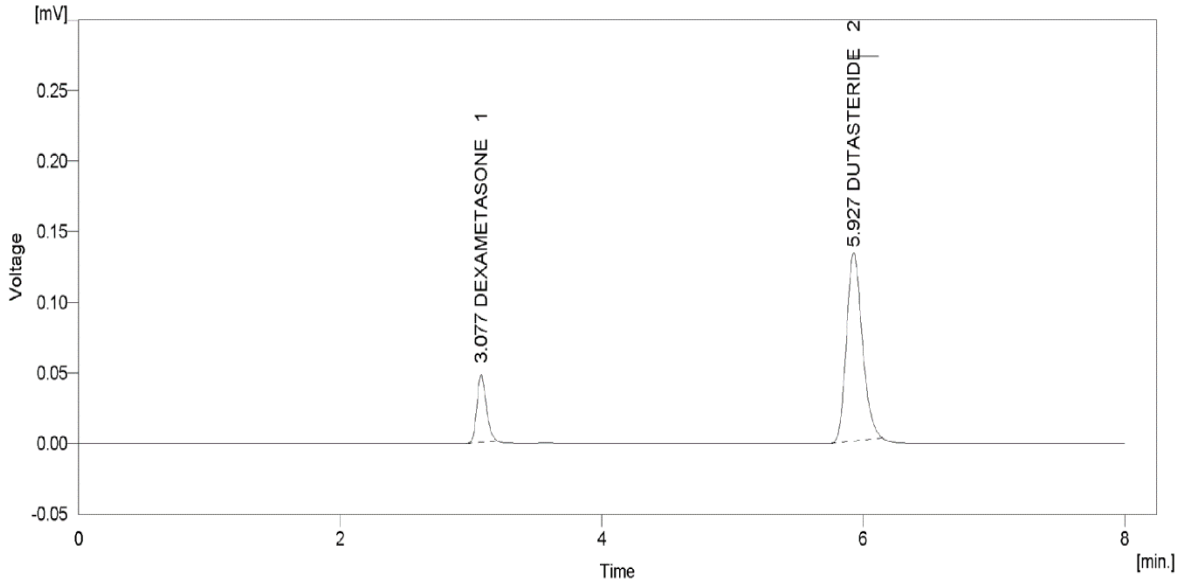


Fig 11: Chromatogram of Dexamethasone (IS,10µg/ml) and Dutasteride(75µg/ml)

Table 10: Intra-day precision for the determination of dutasteride

Conc. (µg/mL)	00 Hrs			3 Hrs			6 Hrs		
	Area (Drug/I.S).	SD	*RSD %*	Area (Drug/I.S).	SD	*RSD %*	Area (Drug/I.S).	SD	*RSD %*
25*	1.553	0.0142	0.914	1.547	0.0199	1.286	1.5560	0.0114	0.7330
50*	3.106	0.045	1.449	3.107	0.0326	1.049	3.1320	0.0240	0.7660
75*	4.674	0.023	0.492	4.691	0.0078	0.166	1.5126	0.0430	0.9290

*every value is the mean of three analyses parameters.

Table 11: Intra-day precision for the determination of dutasteride

Conc. (µg/mL)	1st Day			2nd Day			3rd Day		
	Area (Drug/I.S).	SD	***RSD %***	Area (Drug/I.S).	SD	***RSD %***	Area (Drug/I.S).	SD	***RSD %***
25*	1.553	0.0142	0.914	1.559	0.022	1.411	1.544	0.0130	0.842
50*	3.106	0.045	1.449	3.132	0.0070	0.208	3.108	0.0001	0.186
75*	4.674	0.023	0.492	4.660	0.0162	0.347	4.673	0.023	0.492

*every value is the mean of three analyses parameters

The result was found to be Intra-day precision from 0.166 to 1.449 and Inter-day precision from 0.186 to 1.449. Intra- day and Inter-day precision within the acceptable range ± 2, indicative of good method precision.

*Assay of Tablet Formulation***:** Average weight of the 20 tablets was determined. These tablets were crushed to a fine powder. Powder equivalent to 10 mgwas weighed and transferred to a 100 mlvolumetric flask. It was dissolved in mobile phase. Six replicate of the required dilution were prepared from tablet stock solution and sonicated for 10 min. These solution (25µg/ml) were analyzed, mean, standard deviation and relative standard deviation (RSD) were calculated dilution were prepared from tablet stock solution and sonicated for 10 min. These solution (25 µg/mL) were analyzed and mean, standard deviation and relative standard deviation (RSD) were calculated (Table 12).

Table 12: Recovery for the assay of Method development of stock and dosage form solution

	Theoretical (µg/mL)	**Measured (µg/mL)**	**Area of Drug/I.S**	**S.D.**	**% Recovery**	**%RSD**
Stock solution	25*	24.86	1.5647	0.0086	100.70	0.55
Dosage form solution	25*	24.74	1.547	0.0140	99.66	0.897

*every value is the mean of three analyses parameters

In assay Studies the Percent recovery of dutasteride stock solution and dosage form solution was found to be 100.70% and 99.66%

Recovery Studies:Recovery was calculated by use of the regression equation and a regression line graph was drawn using the amount added on the x-axis and the amount found on the y-axis. The

calculated recovery and percentage recovery values listed in Table No.12are within ±2.0 of the true values in intra-day assay experiments.

Recovery level1 (75% level): Accurately pipette and transfer the stock solution (3.0ml, 100 µg/ml) , sample solution (4.0ml, 100 µg/ml) and (0.1 ml of 1000 µg/ml of dexamethasone as IS) to a 10ml volumetric flask and dilute with mobile phase to volume and mix. Recovery level 2 (100% level): Accurately pipette and transfer the stock solution (4.0ml, 100 µg/ml) , sample solution (4.0ml, 100 µg/ml) and (0.1 ml of 1000 µg/ml of dexamethasone as IS) to a 10ml volumetric flask and dilute with mobile phase to volume and mix. Recovery **level 3 (125% level):** Accurately pipette and transfer the stock solution (5.0ml, 100 µg/ml) , sample solution (4.0ml, 100 µg/ml) and (0.1 ml of 1000 µg/ml of dexamethasone as IS) to a 10ml volumetric flask and dilute with mobile phase to volume and mix. (Table 13).

Table 13: Recovery for the analysis of dutasteride in the Veltride (0.5mg) tablet

Recovery	**Concentration (µg/mL)**				**Area of**	**%**
Level	Taken*	Labeled*	Added*	Found*	**Drug/I.S**	**Recovery**
75%	70	40	30	69.37	4.30	99.10
100%	80	40	40	79.09	4.89	99.86
125%	90	40	50	89.17	5.53	99.07

*Each value is the mean of three analyses.

In Recovery Studies the Percent recovery of dutasteride stock solution and dosage form solution was found to be 99.07% and 98.86%. The % CV was less than 2%.

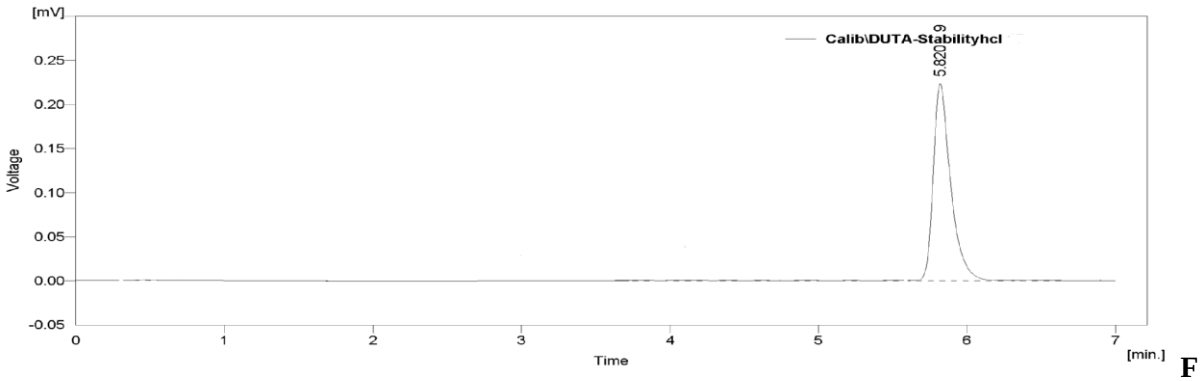
Stability: The stability of standard solution was demonstrated by injecting the standard solution at regular time intervals of 3 hours till at least for 12 hours at room temperature. The area count of dutasteride signal in standard and sample chromatogram at different time intervals was monitored to conclude the stability of standard solution. The mean, SD and %RSD are reported in table 14.

Table 14: Stability of Standard Solution at Room Temperature

Average Area* (n=3)	**SD**	**%RSD**	**Day**
1548.376	1.701	0.110	1
1533.455	1.063	0.070	2
1554.678	0.572	0.037	3

Specificity (Degradation Studies): ThisStudy was performed to demonstrate to non-interference from degradation products that are formed during acid stress, base stress, oxidative stress, thermal stress and light stress on the test sample. The method will be stability indicating if the degradation products do not interfere with the dutasteride peak.

Degradation in Acidic solution:The dutasteride (10 mg) was dissolved in 0.1N HCl (100 ml) and kept at room temperature. Various samples were collected and analyzed to check the stability of drug in acidic medium. In a separate experiment the solution were heated upto 100°C and then the samples were analyzed to check the effect of acid and temperatue on degradation of dutasteride. At room temperature no degradation was observed but the drug gradually underwent degradation with time on heating at 100°C. The degradation product can be observed at RRTs of 2.123, 2.580 min.



F

Fig 12: Chromatogram obtained before degradation with 0.1 N HC

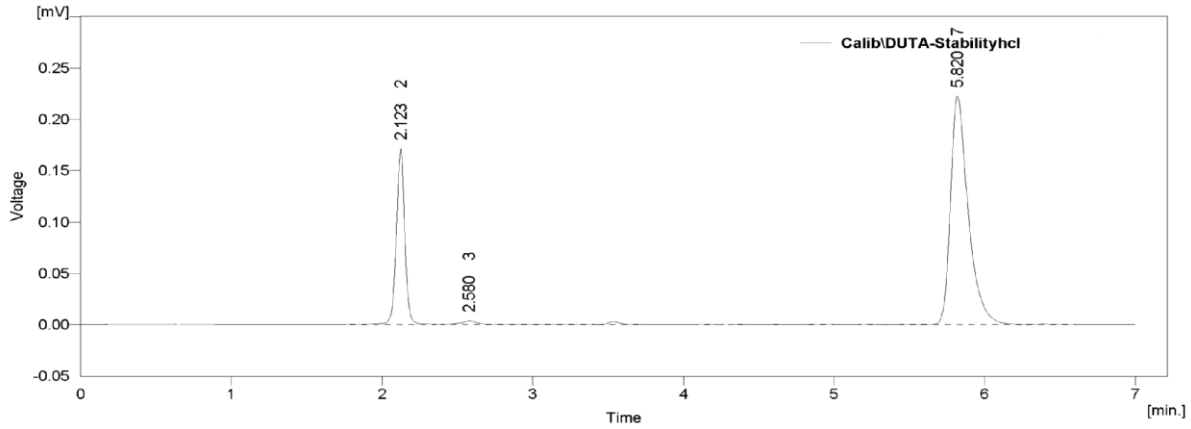


Fig 13: Chromatogram obtained after degradation with 0.1 N HCl

Degradation in Basic solution**:**The dutasteride (10 mg) was dissolved in 0.1N NaOH (100 ml) and kept at room temperature. Various samples were collected and analyzed to check the stability of drug in acidic medium. In a separate experiment the solution were heated upto 100°C and then the samples were analyzed to check the effect of base and temperatue on degradation of dutasteride. At room temperature no degradation was observed but the drug gradually underwent degradation with time on heating at 100°C. The degradation product can be observed at RRTs of 2.287, 2.550, 4.803, 5.143 min

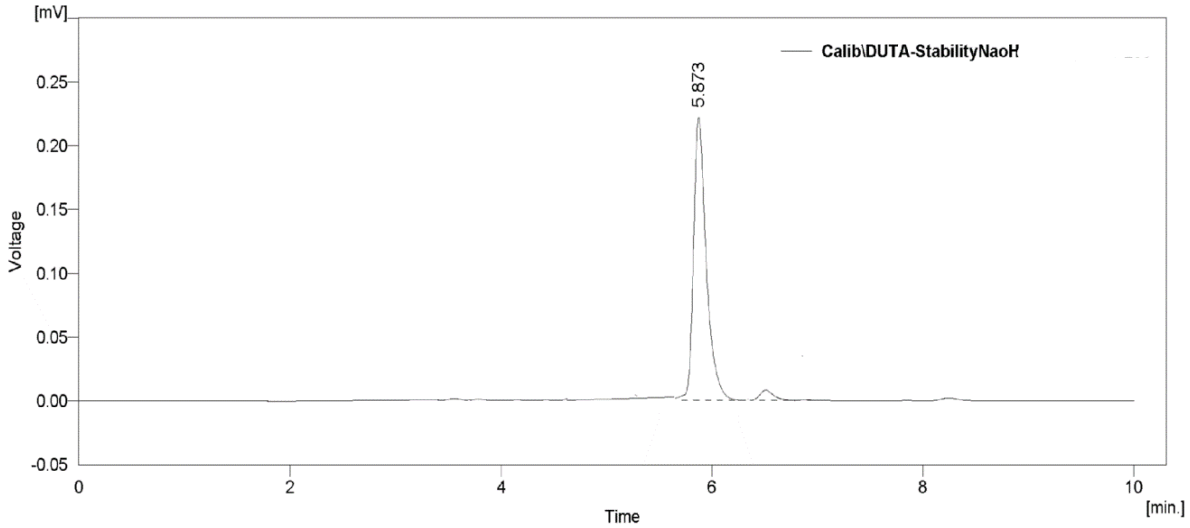


Fig 14: Chromatogram obtained before degradation with 0.1 N NaOH

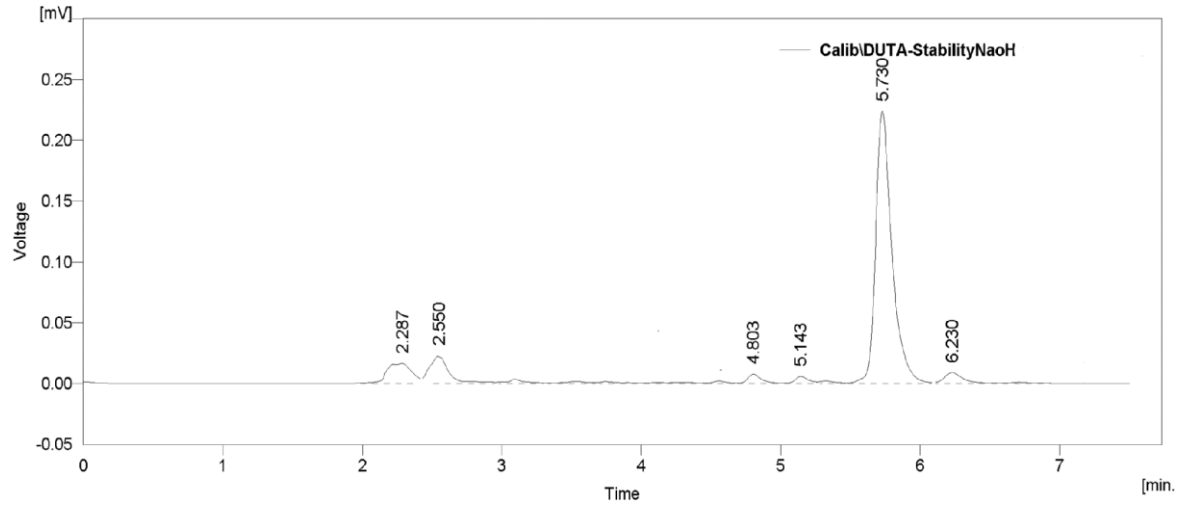


Fig 15: Chromatogram obtained after degradation with 0.1 N NaOH

Oxidative Condition: The drug was exposed to 10% H_2O_2 at room temperature mild degradation was observed but the drug gradually underwent degradation with time on heating at 100°C forming degradation product at RRTs 2.557.t room temperature no degradation was observed but the drug gradually underwent degradation with time on heating at 100°C. The degradation product can be observed at RRTs of 2.287, 2.550, 4.803, 5.143 min

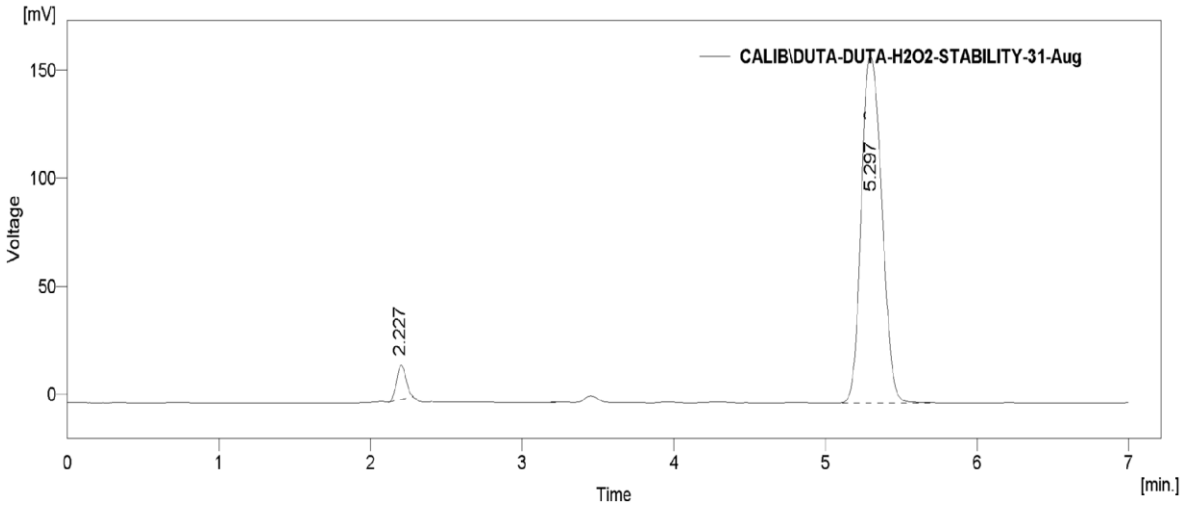


Fig 16: Chromatogram obtained before degradation with 0.1 N H_2O_2

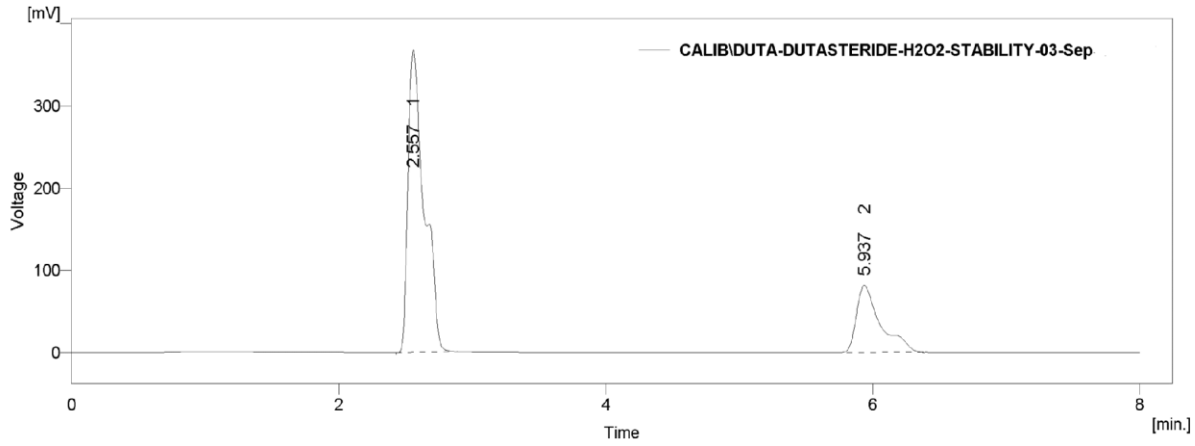


Fig17: Chromatogram obtained after degradation with 0.1 N H_2O_2

Photodegradation of dutasteride

The dry drug (10 mg) was subjected to UV irradiation for three Hrs. the drug was than dissolved in methanol (10 ml). to give 1000 µg/ml. This solution was filtered through a 0.45-µm syringe filter and analysed by HPLC. Sample did not produce any other signal than dutasteride indicating that the drug was stable under UV irradiation. The methanolic solution of the drugs was also stable under stressed conditions (UV light and heat))

Table 15: Stability of Standard Solution after UV Radiation and Reflux. At 50ºC;

Condition	Duration	Average Area(n=3)
Initial	0 Hrs	
Initial	0 Hrs	182.161
UV Radiation	3 Hrs	
UV Radiation	3 Hrs	180.214
Reflux.	3 Hrs	
Reflux.	3 Hrs	164.017

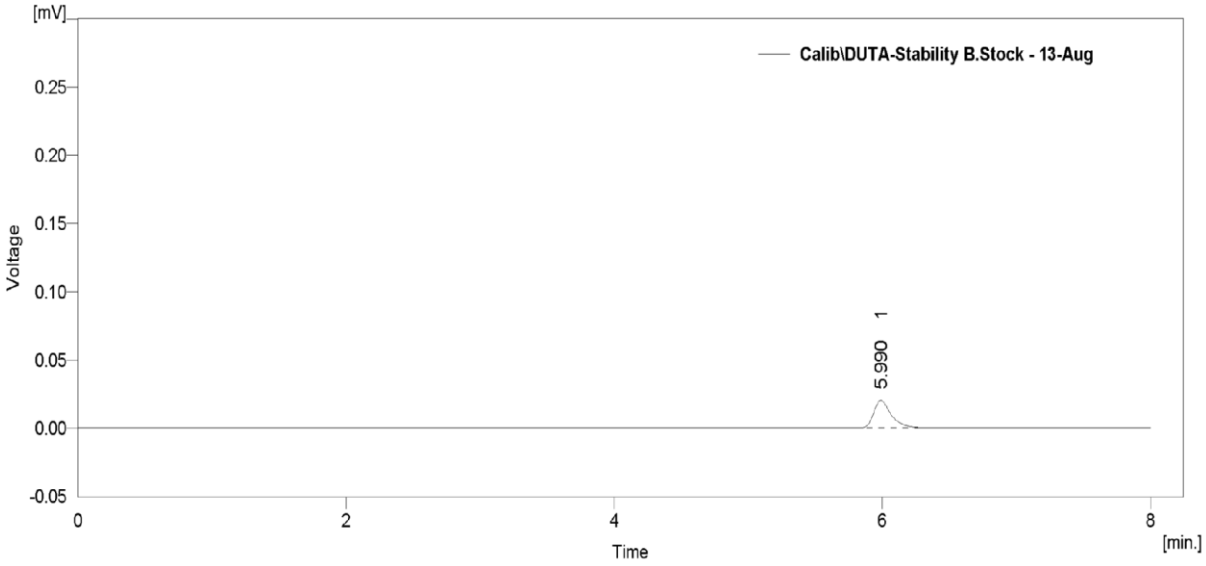


Fig 18: Chromatogram obtained before Reflux

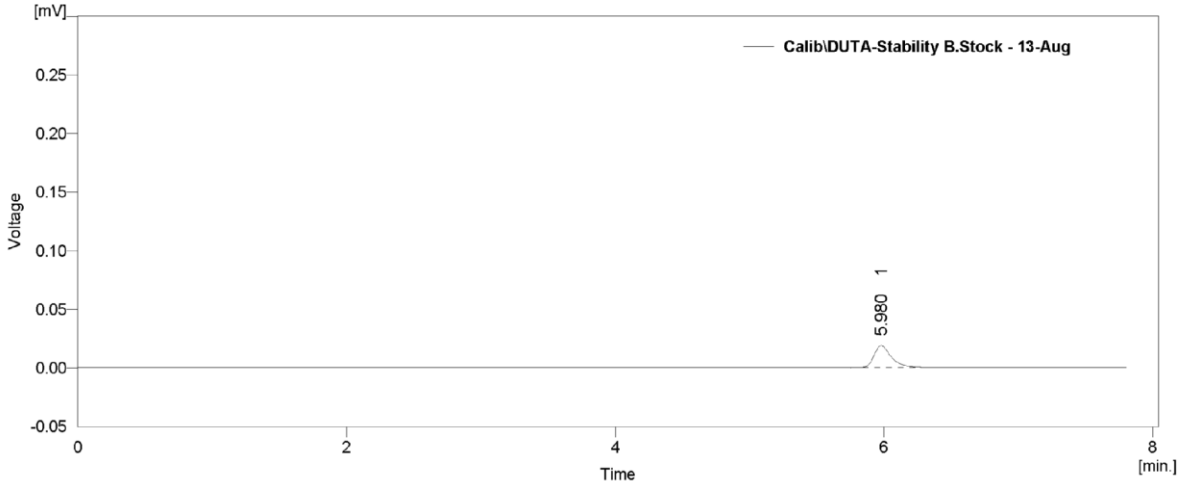


Fig 19: Chromatogram obtained after Reflux.(50ºC)

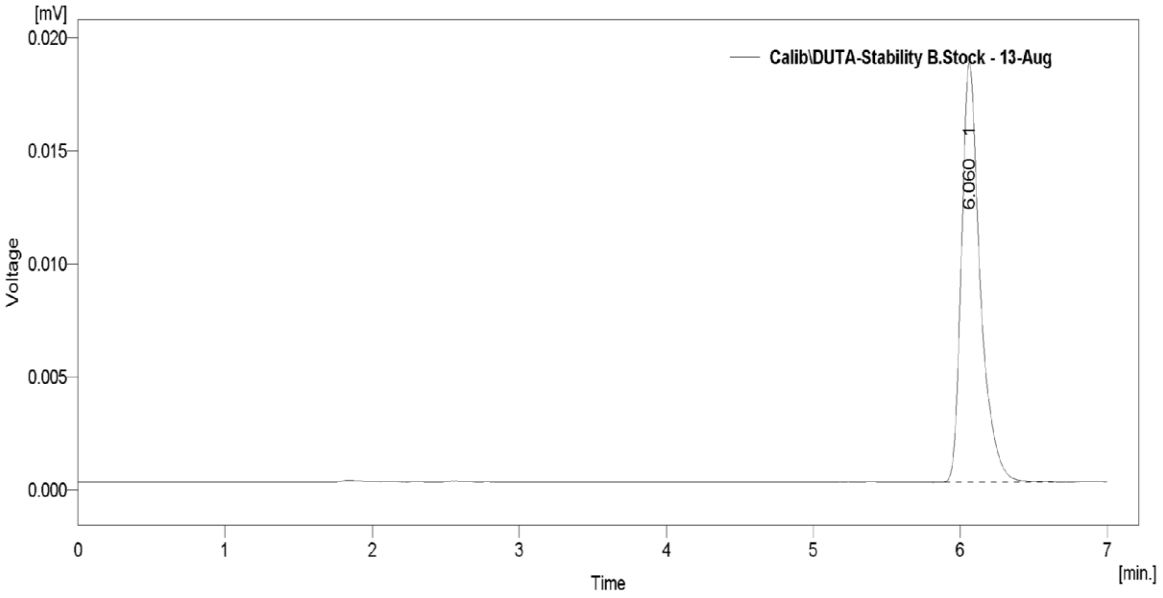


Fig 20: Chromatogram obtained before UV irradiation

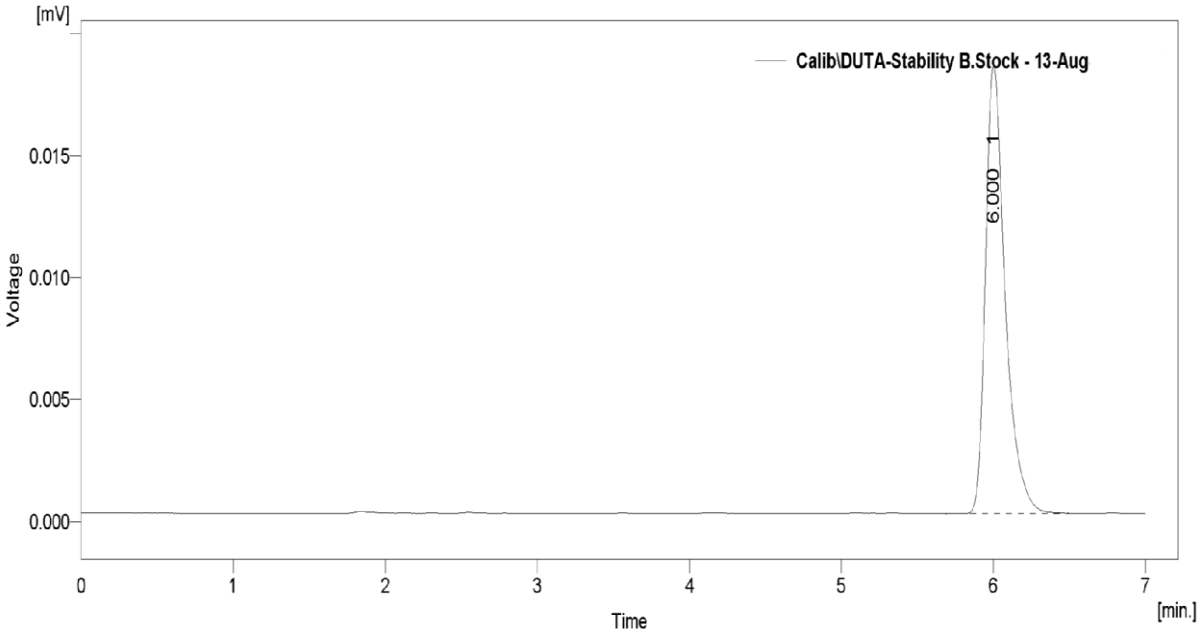


Fig 21: Chromatogram obtained after UV irradiation

*Limit of Detection and Limit ofQuantitation (Sensitivity):*The LOD and LOQ of the method were determined by injecting standard solution of progressively decreasing concentration under the chromatographic condition. A series of solutions in the range 0.2-1.0% of the assay concentration (10 µg /mL) were prepared by dilution of the standard solutions. Each solution (5 µg/mL) (n=5) injected five times, the mean areas, standard deviation and %RSD were calculated. The LOD and LOQ were calculated. (Table 16).

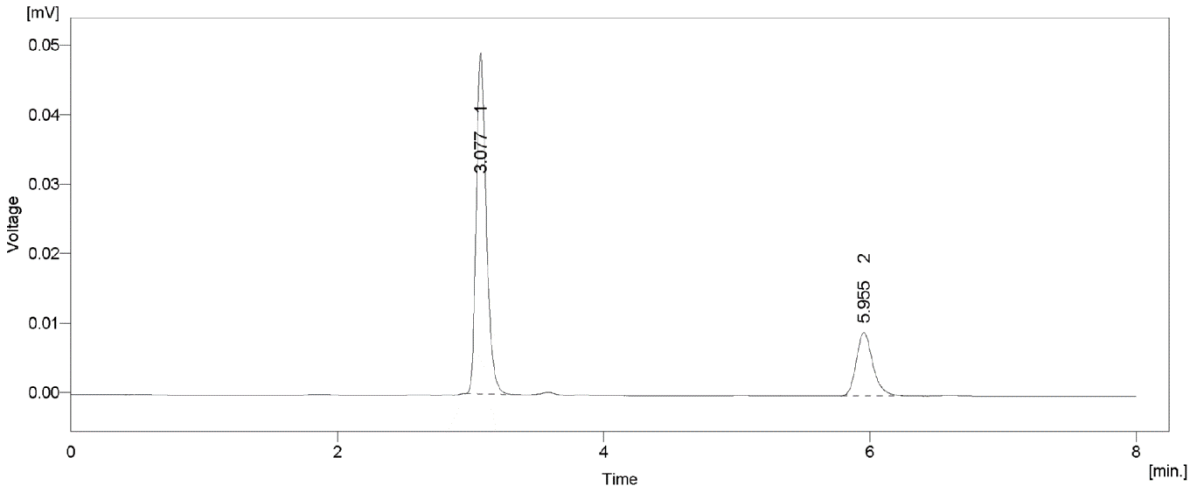


Fig 22: Chromatogram of Dexamethasone (IS,10µg/ml) and Dutasteride(5µg/ml)

Table 16.:LOD and LOQ determination of dutasteride.

Concentration (µg/ml)				
Theoretical	Measured	**Slope ofDrug**	**Area (drug/I.S)**	**S.D.**
5*	4.769	0.0616	0.303	0.0048
		LOD =0.257 µg/mL		
		LOQ =0.779 µg/mL		

limit of detection was 0.257µg and limit of Quantitation was 0.779 µg respectively.

*Ruggedness and Robustness:*The robustness and ruggedness of the method were assessed by study of daytoday variation, and analyst to analyst variation by use of a matrix design involving the estimation on two different days using two different analysts on two different days, with a total of four analyses. Under each of the conditions, samples were analyzed including a duplicate injection for each estimate.

Robustness: Robustness of the method was demonstrated by injecting the system suitability solution by deliberately changing the chromatographic parameters and monitoring the retention time and system suitability parameters under each condition. Data are presented in table 17

Table 17: Data forRobustnessTest

Chromatographic Condition : Mobile Phase : ACN : Water: (80:20)						
Flow Rate: Iml/min				pH : 2.7		
Retention Time(min.)	Wave Length	pH	Area of drug	Retention Time(min.)	Flow Rate (ml/min.)	Area of drug
6.193	273	7.2	1529.383	6.523	0.90	1636.809
5.922	242	2.7	1644.760	6.230	0.95	1606.773
6.076	273	2.7	1621.345	5.922	1.00	1644.763
6.012	242	7.2	1591.321	5.657	1.05	1621.070
-	-	-	-	5.243	1.10	1622.981

When the wave length was adjusted to (273 & 242) the retention time of dutasteride were 6.193 & 5.922 min. At pH-2.7 and pH-7.2 the retention time of dutasteride were 5.922 and 6.193.

When the flow rate was reduced to 0.95 and 0.90 ml/min. the retention time of dutasteride was increased to 6.230 min (5.2%) and 6.523 min (10.14%) respectively. On the other hand when flow rate was increased to 1.05 and 1.10 ml/min. the retention time of dutasteride was reduced to 5.657 (4.5%) and 5.243 min (11.5%).

Ruggedness: The ruggedness of a method was its ability to remain unaffected by small, unintentional changes in experimental conditions such as temperature, mobile phase composition and pH, When any method developed by the different analysts. Some variation is possible in method development.Data are presented in table 18.

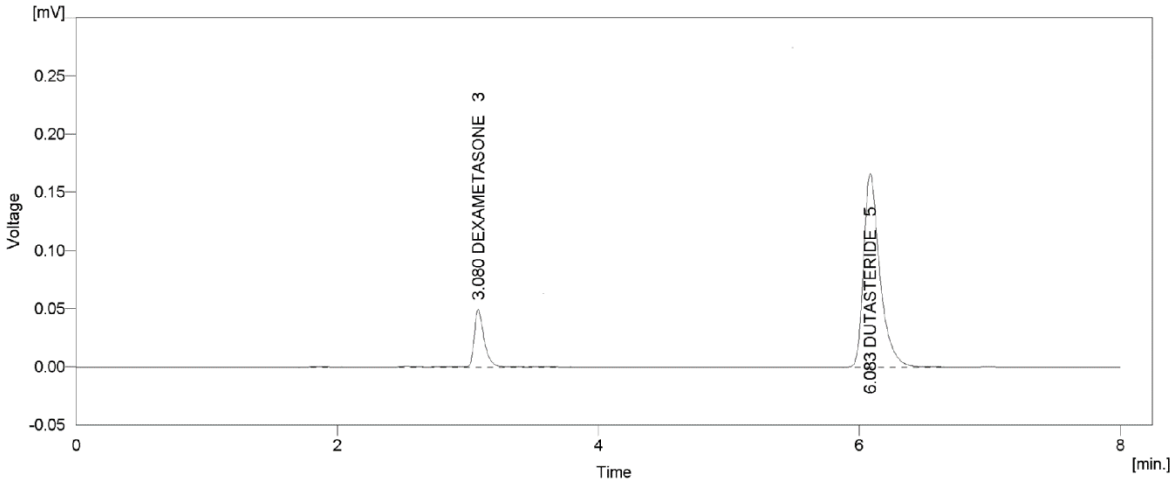


Fig 23: Chromatogram of Dexamethasone (IS,10µg/ml) and Dutasteride(90µg/ml) (Samples analysed by the developer)

Table 18: Data for Ruggedness Test

Ruggedness		**Robustness**	
	S.D	%RSD	Recovery (%)
Developer	0.029	0.525	99.07
analyst #2	0.007	0.128	98.40

3. Results and Discussion

*Linearity:*The regression equation for the calibration plot was Y = 0.020X + 0.012 and Regression Coefficient (R^2) 0.9998. These results showed there was a good linear relationship between absorbance and the amount of analyte in the range studied.

*Accuracy:*The calculated recovery and percentage recovery values were (99.96%-100.7%). Percent Recovery was within 100±2.0% range, indicates that the method is accurate.

*Precision:*Intra-day precision ranged from 0.033 to 0.1563 and inter-day precision ranged from 0.033 to0.6858. Intra-day and Inter-day precision were within the acceptable range (%CV ± 2%) indicative of good method precision.

*Assay:*In assay studies the percent recoveries of dutasteride from stock and dosage form solution were 99.96% and 99.94 %.

*Recovery Studies:*In recovery studies the percent recovery of dutasteride stock solution and dosage form solution was found to be 99.79% and 100.00%.

*Limit of detection and Limit of quantitation:*The limit of detection was 0.242µg and limit of quantitation 0.732 µg.

*Ruggedness and Robustness:*The present method is rugged and robust as the results (of analysis done different person) were within the acceptable range (%CV less than 2%, % accuracy 100±2%). At wave length (272 & 242nm) the absorbances of dutasteride were 0.314 and 1.018 respectively.

Robustness and Ruggedness was observed that results were well within acceptance limits of 98-102%, with RSD ±2.0%, indicating the method is rugged and provides consistent and reliable results which are not affected by small changes in experimental conditions

4. Conclusion

Fromthe experimental study we conclude that, this is a novel, simple, selective, precise, accurate, reproducible and suitable method for the analysis of dutasteride in the formulation and bulk drug. The

proposed methods can be successfully applied for dutasteride assay in tablet dosage forms without any interference in quality control.The proposed methods are used for the routine analysis of the drugs in the quality control. In view of these facts, the found method could be a subject of further investigations for developing a selective and accurate method for quality control.

Acknowledgements

The authors are thankful to Dr. D.K.Sharma (Director) *School of Pharmacy& Research Centre, Sanskriti University, 28 KM stone Chhata Mathura, UttarPradesh-281001*for providing necessary facility to carry out the Analytical study.

References:

1. Dipti B. Patel, N.J. Patel, S.K. Patel, A.M. Prajapati, S.A. Patel, RP-HPLC method for estimation of dutasteride in tablet dosage form, Indian Journal of Pharmaceutical Science, 2010, 72, 113-116.
2. Dipti B. Patel, N.J. Patel, S.K. Patel, A.M. Prajapati, S.A. Patel, Simultaneous analysis of tamsulosin hydrochloride and dutasteride in pharmaceutical dosage form, Acta Chromatographia, 2010, 22;419-431.
3. Subramanian P., S. Rajinikanth, S. Poonguzhali. Development and validation of a New RP-HPLC method for the estimation of dutasteride in bulk and pharmaceutical formulations.ISSN 2231-3354, JAPC, Volume 6 (12): 047-055. Dec.2016
4. V.C Amrutha., K. Shantha Kumari, S. Sushma, K. Prakash. development and validation of RP-HPLC method for simultaneous estimation of Alfuzosin hydrochloride and dutasteride in bulk and pharmaceutical dosage form. Inventi Rapid: Pharm Analysis and Quality Assurance, 2012; 4: 1-4.
5. N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, M. Koteshwara., M. Santosh, Tandem mass spectrophotometry assay of dutasteride in human plasma Journal Chromatography B, 2004, 809, 117-124.
6. S.Sudhir, B.Vishal, T. Vinayak., Determination of Dutasteride by LC: Validation and Application of the Method. Chromatographia Volume 67, Numbers 11-12 -June, 2008.
7. S. Budavari, editor. The Merck Index.13th ed. Whitehouse station, NJ Merck and Co, Inc; 2001:3504.
8. International Conference on Harmonization, Q1A (R2), Stability testing of new drug substances and products, Geneva, 2003.
9. M. Kazusaki, S. Ueda, N.Takeuchi., Review- Validation of analytical procedures by high-performance liquid chromatography for pharmaceutical analysis. Chromatography, 2012; 33:65-73
10. Chandrasekha K., A. Manikandan, novel rp-hplc method development and validation of tamsulosin and dutasteride in tablets by ratio's method ,j.chem, issn: 0974-1496,volume 14, june-2021
11. Varshini C., Shantha Kumari K, Sushma S, Prakash K. development and validation of RHPLC method for simultaneous estimation of Alfuzosin hydrochloride and dutasteride in bulk and pharmaceutical dosage form.Pharm Analysis and Quality Assurance, 2012; 4: 1-4.
12. International Conference on Harmonization of Technical Requirements for Human Use. Validation of analytical procedures: Text and Methodology, Q2 (R1), 2005.
13. Mohammed Ishaq B, Vanitha Prakash K, Krishna Mohan G. Simultaneous determination of dutasteride and tamsulosin in pharmaceutical dosage forms by RP-HPLC. Der Pharma Chemica, 2014,6:103-109.
14. Priyadarshani SB, Chetan SC, Preeti G, Chatrapal S, Ravindra K, Mahendra SR, Sujata SB. Analytical method development and validation of dutasteride with tamsulosin in pharmaceutical capsule dosage form by RP-HPLC method. International Journal of Pharmacy, 2015; 5: 493-499.
15. Saimalakondaiah D, Ranjeet Kumar V, Rama Mohan Reddy T, Ajitha, A, Uma Maheshwara Rao V. International Journal of Pharma Research &Review, 2014; 3:46-57.
16. Vishnu P, Choudhariand Anna PN. Stability indicating TLC method for the determination of dutasteride in pharmaceutical dosage form. Chromatographia, 2009; 70: 309-313.
17. B.M.Ishaq, K.Krishna, G.Mohan. Simultaneous determination of dutasteride and tamsulosin in pharmaceutical Vanitha Prakash dosage forms by RP-HPLC. Der Pharma Chemica, 2014,6:103-109. Kar, Minerva Press, 2001, 369-394.
18. G. Chatwal, S. Anand, Spectroscopy (atomic and molecular), Bombay, HimalayaPublishing House, 1996.
19. Validation of analytical methods:Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland, 1996.
20. D.V.S. Rao, P. Radhakrishnanand, Chromatographia, 2008, 67, 841-845.
21. M.M. Kamila, N. Mondal, L.K. Ghosh, IJPRIF, 2010, 3, 0974-4304.
22. V.V. Pande, Journal of Pharmacy Research, 2009, 3, 15-20.
23. S.S. Kamat, V.B. Choudhari, V.T. Vele, Chromatographia, 2008, 67, 11-12.
24. S.K. Sudhir, B.C. Vishal, Asian Journal of Chemistry, 2008, 20, 5514-5518.
25. A.K. Sangita, K.S. Amlan, apan Kumar Pal. Chromatographia, 2008, 67, 11-12.
26. S.S. Deshmukh, S.S. Dhaneshwar, Der Pharmacia Lettre, 2010, 2, 342-349.
27. B. Uslu, Electroanalysis, 2001, 14, 866-870.
28. www.scholarsresearchlibrary.com.