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Advances in Bioresearch

ORIGINAL ARTICLE

A Comparative Study of RP-HPLC and UV-Visible Spectrophotometric Method and Validation for Dutasteride Estimation in API And Tablet Dosage Form

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ABSTRACT
The present research work illustrates method development and validation of UV spectrophotometric and HPLC for the estimation of dutasteride. A simple, specific, accurate, reproducible and economical UV-HPLC method was developed and validate for the determination of dutasteride in Bulk and tablet dosage form. The UV spectrum recorded between 200-400 nm and the wavelength 242nm was selected for the determination of dutasteride. UV-HPLC analysis was carried out using Luna – C-18 (250 mm x 4.6 mm, 20 µL) column and Shimadzu Pharmaspec-Model-1700 UV spectrophotometer and mobile phase composed of Acetonitrile: Water (80:20 v/v, p^H adjusted to 3.5 with orthophosphoric acid) at a flow rate of 1.0 mi/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 for HPLC and Y = 0.020X + 0.012 for UV. Accuracy values were (99.96%-100.70%) for UV Method and (99.93% to 100.63%) for HPLC Method. Intra-day precision from 0.246 to 1.725 and Inter-day precision from 0.124 to 0.291 for HPLC and Intra-day precision from 0.115 to 0.859 and Inter-day precision from 0.0396 to 0.686 for UV. For both method the LOD, LOQ and % Recovery was determined. The present method can be recommended for determination of dutasteride in routine control analysis of drug. The proposed UV-Visible spectrophotometric and HPLC method was validated according to ICH guidelines for linearity, precision, accuracy, specificity, LOD and LOQ.
Keywords: *HPLC, UV spectrophotometer, Dutasteride, LOD, LOQ, ICH guidelines.*

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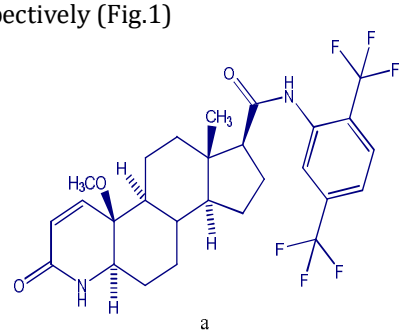
INTRODUCTION

High Performance Liquid Chromatograph was derived from the classical column chromatography and most important tools of analytical chemistry today. HPLC methods development and validation play important roles in new discovery, development, manufacture of pharmaceutical drugs. [1,2,3] HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability [4,5]. Ultraviolet spectroscopy is advanced analytical instrument in pharmaceutical industries and used since last 35 years. The method of analysis is based on measuring the absorption compounds in the near ultraviolet path of spectrum (200-400 nm). The principle of UV visible spectroscopy is based on absorption of ultraviolet light or visible light by a chemical compound, which give spectra [6,7]. There are many studies conducted for the determination of tablet dosage forms of dutasteride and also with the combination of other drugs by HPLC and UV spectrophotometry individually reported but to the best of our knowledge, no RP-HPLC and UV-Visible spectrophotometric method comparative study reported of dutasteride. There is urgent need to develop a simple, sensitive, accurate and precise UV-HPLC method of dutasteride in pure and pharmaceutical dosage form and compare both the method. The results of the analysis were validated by latest guidelines set by International Conference on Harmonization (ICH) [8,9].

Drug Profile

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 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 [10,11]. Chemical name and empirical formula of dutasteride are: (5α, 17 β)- N-{2,5 bis (trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide and $C_{27}H_{30}F_6N_2O_2$ respectively (Fig.1)



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(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

Fig1. Chemical structure of Dutasteride

Physicochemical Characterization of the Drug

Dutasteride is a white powder freely soluble in acetonitrile, ethanol, methanol, and insoluble in water. Melting point of the drugs was noted (242-250ºC). Physiochemical properties shown in Table 1.

Table 1. Physicochemical properties of used drug.

Drug Category	Anti-baldness Agents Ant hyperplasia Agents Enzyme Inhibitors
Appearance	White and pale-yellow powder
Solubility	Ethanol, Methanol Polyethylene glycol
Chemical Formula	$C_{27}H_{30}F_6N$
Molecular Weight	528.53 gm/mol
pka value	4.6
M.P.	242-250ºC

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. Instrumentation & Chromatographic Condition. (RP-HPLC & UV)

Instruments	Chromatographic Condition
HPLC System: Shimadzu (model-LC-20AT)	Flow Rate: 1ml/min.
Shimadzu UV spectrophotometer (Model-UV-1700)	UV range: 200 to 400 nm
Colum: Phenomenex Luna C18 column	Wavelength:242 nm
Pump: Shimadzu (Model- LC-20AT)	Injection Volume: 20µL
Detector: UV-Visible Spectrophotometer (SPD-20A)	Column Temperature: 25°C
PH Meter: Digital pH meter (Cyber Labs, USA)	Mobile Phase: Acetonitrile: Water

Preparation of Standard solution

Stock Solution of drug

For the creation of the calibration curve, Stock solution of dutasteride was prepared in mobile phase. 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 up to 100 ml with mobile phase. Different working solutions of dutasteride were prepared from the stock solution using appropriate dilutions.

Preparation of standard sample

10 mg of dutasteride was transferred to a 100 ml calibrated flask and dissolved in 75 ml of mobile phase. The content was shaken for 10 min. Final volume was made up to 100 ml with mobile phase. Different working solution of dutasteride was prepared from the sample solution using appropriate dilutions. The final solution has been sonicated and filtered through 0.45-µm Millipore filter.

Preparation of sample for different test

Aliquots of the sample solution were transferred to the 10ml volumetric flask containing 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 From the UV spectra the wavelengths 242, nm was selected for monitoring of the drugs.

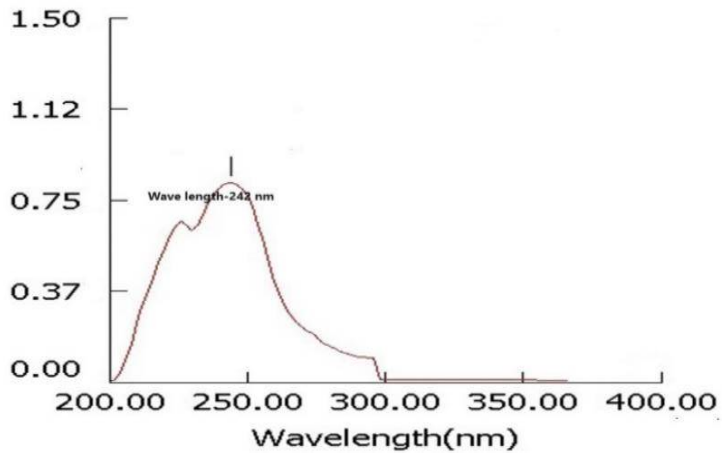

Fig 3. UV spectra of Dutasteride showing wavelength at 242 nm

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).

Table 3. Chromatographic parameters in different mobile phase compositions.

Parameter	(CH_3OH: H_2O)		(ACN: Water: THF)		
(Mobile Phase)	70:30	85:15	45:45:10	55:35:10	65:25:10
Retention Time	13.793	8.077	17.793	9.863	6.19
Tailing Factor	1.245	1.241	1.46	1.435	1.516
No. of theoretical Pt.	7948	7467	18956	16035	11940

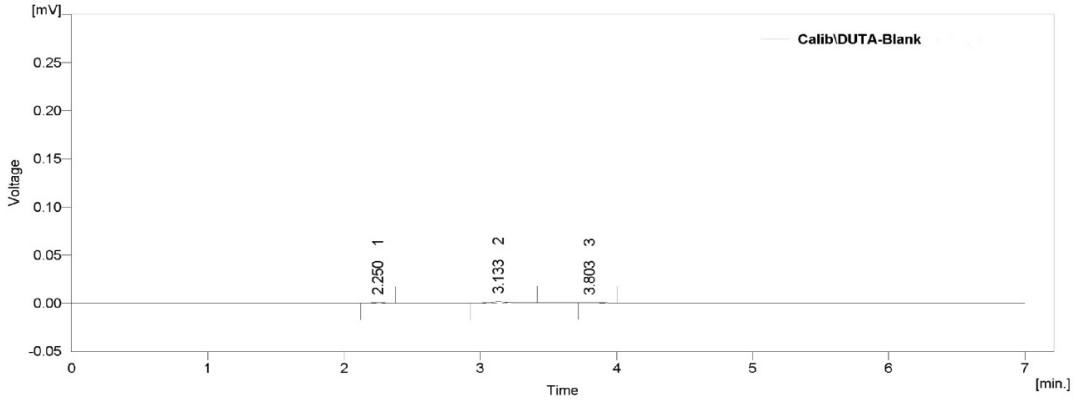

Fig 4. Chromatogram of mobile phase used for the preparation of sample

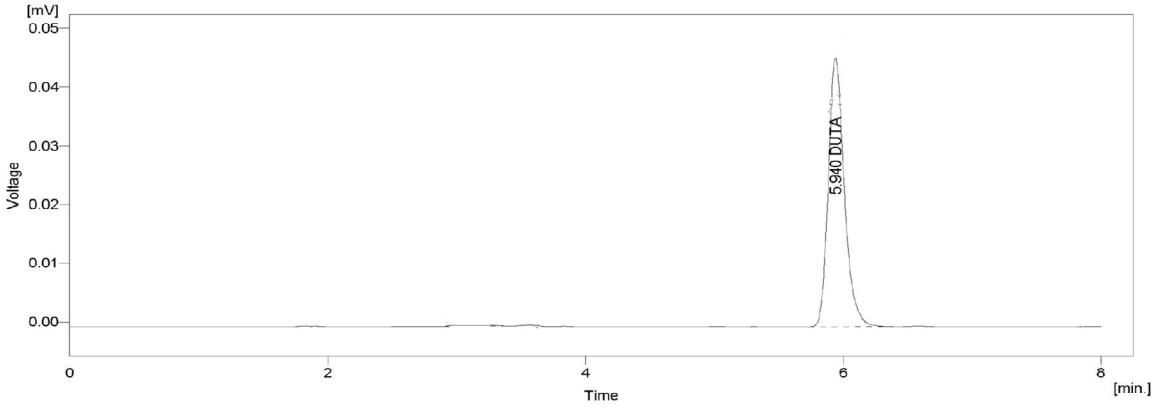

Fig 5. Chromatogram of Dutasteride (25µg/ml)

System suitability

System suitability was performed by injecting repetitive injection (n=6) of dutasteride (25 mcg/ml) 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. The data were represented in Table 4 and the chromatogram were represented in Fig. 6.

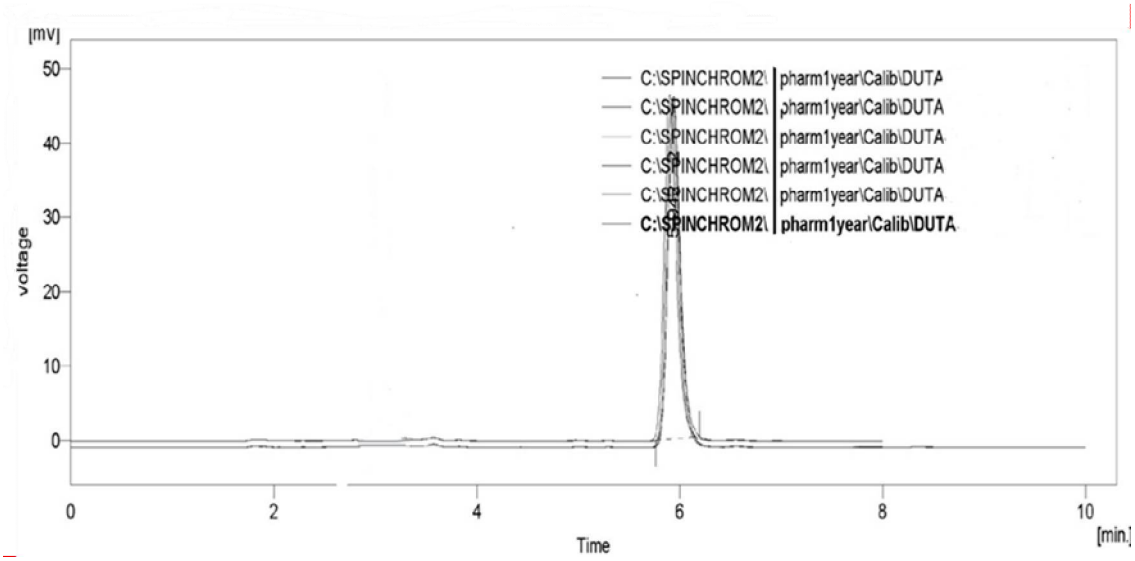

Fig 6. System Suitability Curve showing SST result

Table 4. SST Result for Component for Dutasteride

Chromatogram (Serial no.)	RT (min.)	Area (mV)	Height (mV)	Amount (µg/ml)	Tailing factor	Resolution
Duta-1	5.920	396.841	46.61	24.70	1.23	16.15
Duta-2	5.938	398.278	46.05	25.04	1.25	16.12
Duta-3	5.935	392.381	45.42	25.04	1.26	16.13
Duta-4	5.94	396.841	45.84	25.06	1.25	16.11
Duta-5	5.903	399.659	46.49	25.05	1.26	16.05
Duta-6	5.942	400.838	46.22	25.02	1.26	16.15
Mean	5.93	397.344	46.11	24.98	1.25	16.12
%RSD	0.25	0.75	0.95	0.57	0.79	0.24
Limit of % RSD	2.0 %	2.0 %	2.0 %	2.0 %	2.0 %	2.0 %
Result	Pass	Pass	Pass	Pass	Pass	Pass

Table 5. Validation parameters of method development.

Parameter	UV	HPLC
Wavelength	242	242
Linearity Range (µg/mL)	5-100	5-100
Standard Regression Equation	Y = 0.020X + 0.012	Y = 0.0618 X – 0.008
Regression coeifficient (r^2)	0.9998	0.9998
Accuracy (% Recovery ±SD)	99.96%-100.70%.	99.93%-100.63%.
Precision (Intra-day)	0.115 to 0.859	0.246 to 1.725
Precision (Inter-day)	0.0397 to 0.686	0.124 to 0.291
LOD µg/ml	0.242 µg/ml	4.0714 µg/ml
LOQ µg/mL	0.732 µg/ml	12.337 µg/ml

Linearity

The calibration curves were plotted over the concentration range of 5 to 100 µg/ml were prepared in triplicate. The regression equation for the calibration plot of HPLC and UV was Y = 0.0618X - 0.008 (regression coefficient (r^2) 0.9998) and Y = 0.020X + 0.012 (regression coefficient (r^2) 0.9998) respectively as shown in Table **6** and Figure **7**.

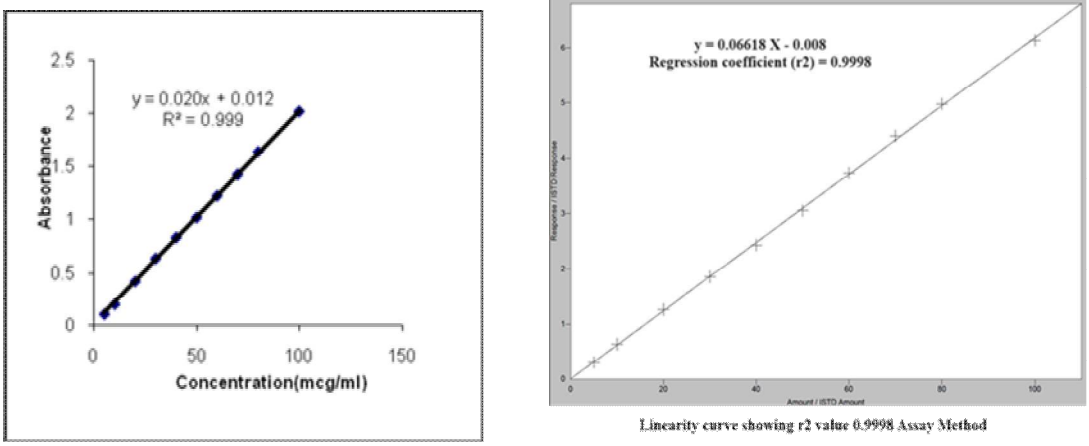

Fig 7. Linearity curve showing r2 value 0.999 for both UV and HPLC method

Table 6. Calibration curve data of UV and HPLC Method (Conc. Range: 5-100 µg/ml):

UV: value of parameters (r^2, slope & intercept)					
Conc.	**CC1**	**CC2**	**CC3**	**CC4**	**CC5**
r^2	0.9996	0.9995	0.9998	0.9998	0.9995
Slope	0.0208	0.0206	0.0201	0.0200	0.0209
Intercept	0.0229	0.0032	0.0129	0.0151	0.0141
HPLC: Value of parameters (r^2, slope & intercept)					
r^2	0.9997	0.9998	0.9997	0.9998	0.9998
Slope	0.0609	0.0620	0.0614	0.0629	0.0629
Intercept	0.0022	0.0225	0.0028	0.0001	0.0039
	1st Day		2nd Day		3rd Day

Accuracy

A known quantity of standard solution has been added to the sample solution previously analyzed at three different levels (25, 50, 75µg/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. The calculated recovery and percentage recovery was within 100±2.0% the acceptable range which indicated method was found to be accurate.

Table 7. The accuracy of the method development by the measurement of recovery.

UV: Accuracy data:					
Theoretical	Measured	Abs./Area	SD	% COV	% Accuracy
25*	24.991	0.5118	0.0008	0.1563	99.96
50*	50.353	1.0190	0.0007	0.0687	100.70
75*	74.978	1.5116	0.0005	0.033	99.97
HPLC: Accuracy data:					
25*	24.983	389.629	0.014	0.914	99.927
50*	50.120	806.001	1.449	1.449	100.205
75*	75.471	1157.647	0.492	0.492	100.629

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

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. . Intra- day and Inter-day precision for both the methods were within the acceptable range ± 2, indicative of good method precision.

Table 8. Intra-day precision for the determination of dutasteride:

UV Intra-day precision data:						
Conc.	00 Hrs.		3 Hrs.		6 Hrs.	
µg/mL)	SD	RSD	SD	RSD	SD	RSD
25*	0.0008	0.1563	0.0008	0.1560	0.0011	0.1151
50*	0.0007	0.0687	0.0011	0.108	0.0012	0.1178
75*	0.0005	0.033	0.0011	0.0727	0.0013	0.0859
HPLC Intra-day precision data:						
25*	4.451	1.134	2.977	0.754	6.795	1.725
50*	0.737	0.092	5.763	0.724	8.234	1.038
75*	0.999	0.599	6.051	0.515	2.902	0.246

*Every value is the mean of three analysis parameters

Table 9. Inter-day precision for the determination of dutasteride:

UV Inter-day precision data:						
Conc.	**DAY 1st**		**DAY 2nd**		**DAY 3rd**	
µg/mL)	SD	%RSD	SD	%RSD	SD	%RSD
25*	0.0008	0.1563	0.001	0.1955	0.0022	0.4283
50*	0.0007	0.0687	0.001	0.0981	0.0070	0.6858
75*	0.0005	0.033	0.001	0.0659	0.0006	0.0396
HPLC Inter-day precision data:						
25*	4.451	1.134	2.2858	0.584	0.565	0.145
50*	0.737	0.092	5.750	0.723	0.980	0.124
75*	0.999	0.599	5.023	0.429	3.443	0.291

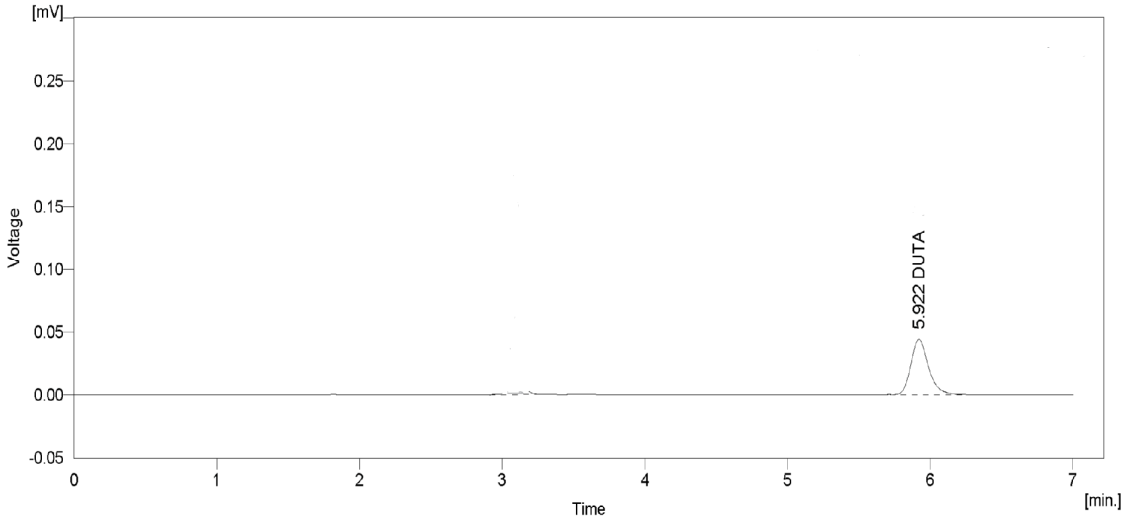

Fig 8. Chromatogram of Dutasteride (25µg/ml)

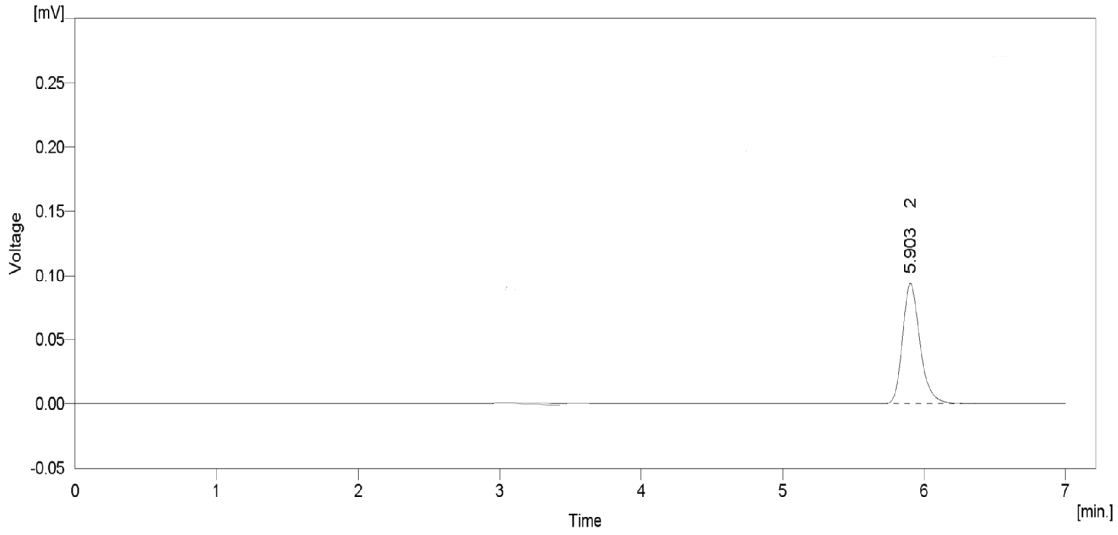

Fig 9. Chromatogram of Dutasteride (50µg/ml)

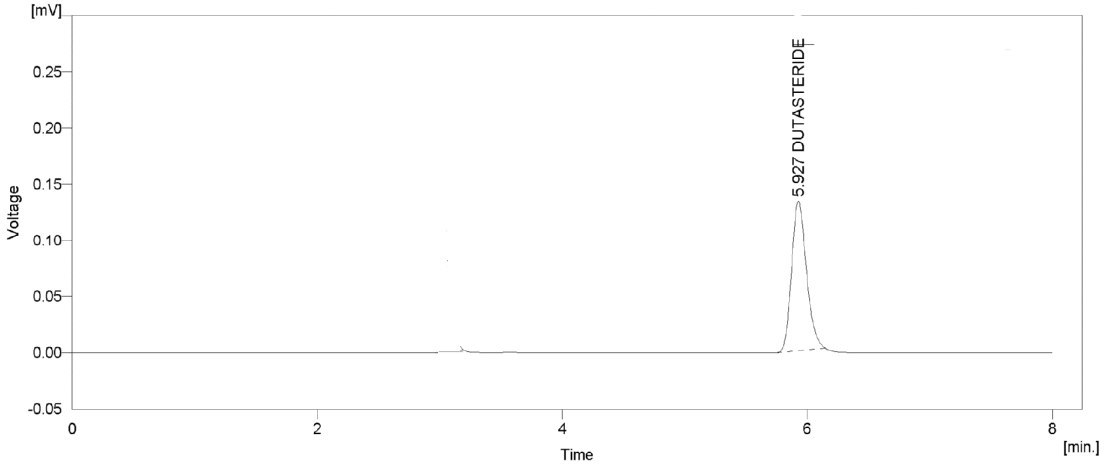

Fig 10. Chromatogram of Dutasteride (75µg/ml)

Assay of Tablet Formulation

Average weight of the 20 tablets was determined. These tablets were crushed to a fine powder. Powder equivalent to 10 mg was weighed and transferred to a 100 mL volumetric flask. It was dissolved in mobile

phase. Six replicates of the required dilution were prepared from tablet stock solution and sonicated for 10 min. These solutions (25 µg/mL) were analyzed and mean, standard deviation and relative standard deviation (RSD) were calculated for both UV and HPLC.

Table 10. Recovery for the assay of Method development of stock and dosage form.

		Theoretical (µg/mL)	Measured (µg/mL)	Area of drug	S.D.	% Recovery	%RSD
UV	Stock	25*	24.91	0.5118	0.008	99.96	0.1563
	Dosage	25*	24.80	0.5117	0.012	99.94	0.2345
HPLC	Stock	25*	24.92	398.760	1.502	99.68	0.377
	Dosage	25*	24.84	387.674	2.260	99.36	0.583

*Every value is the mean of three analyses parameters

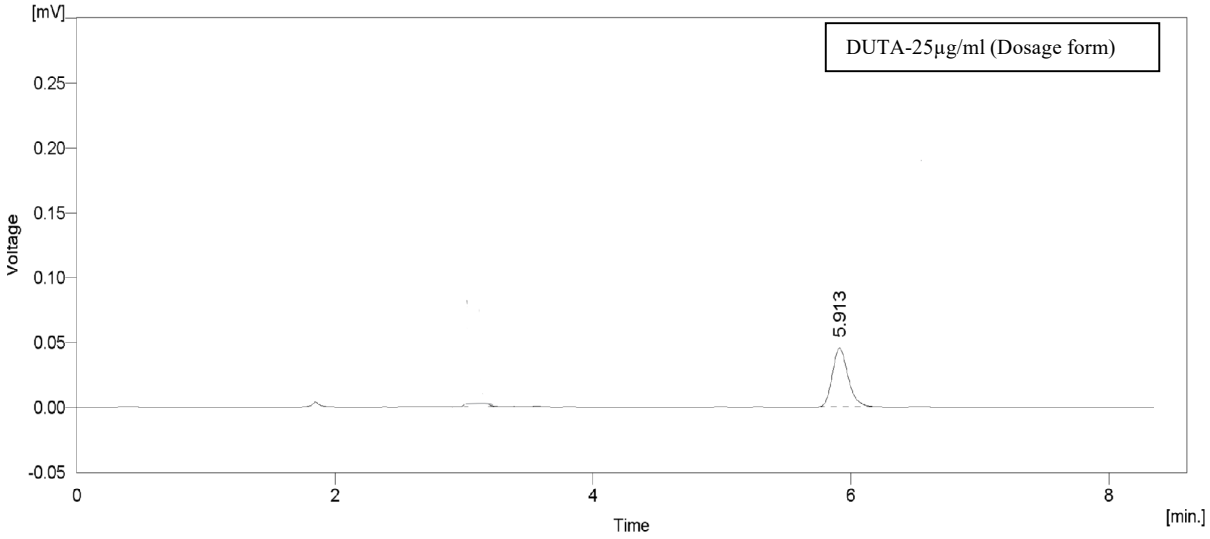

Fig.11 Chromatogram of Dutasteride (Dosage form, 25µg/ml)

Recovery Studies

Recovery study carried out for the drug was performed by spiking the standard drug in powder formulation. 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.11 are within ±2.0 of the true values in intra-day assay experiments for both UV and RP-HPLC method.

Recovery level1 (75% level): Accurately pipette and transfer the stock solution (3.0ml, 100 µg/ml), sample solution (4.0ml, 100 µg/ml) 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 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) to a 10ml volumetric flask and dilute with mobile phase to volume and mix.

Table 11. Recovery for the analysis of dutasteride in the Sterdu table

UV:	Conc. (µg/ml)					
Level	Taken*	Labeled*	Added*	Found*	Abs/Area	% Recovery
75%	70	40	30	69.78	1.409	99.69
100%	80	40	40	80.33	1.619	100.41
125%	90	40	50	89.98	1.812	99.98
HPLC:						
75%	70	40	30	69.37	1120.94	99.10
100%	80	40	40	79.09	1251.88	98.87
125%	90	40	50	89.17	1449.78	99.07

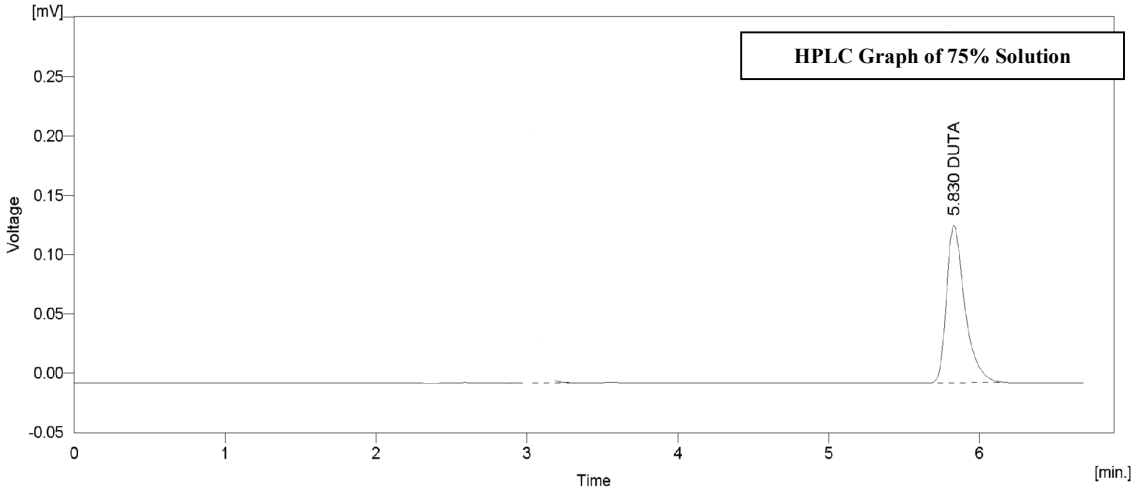

Fig 12. HPLC graph of 75% solution

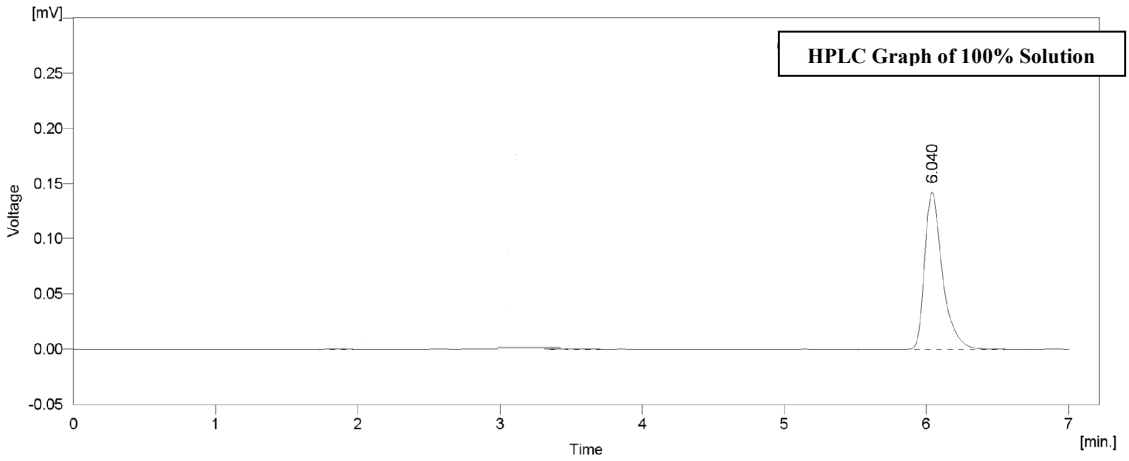

Fig 13. HPLC graph of 100% solution

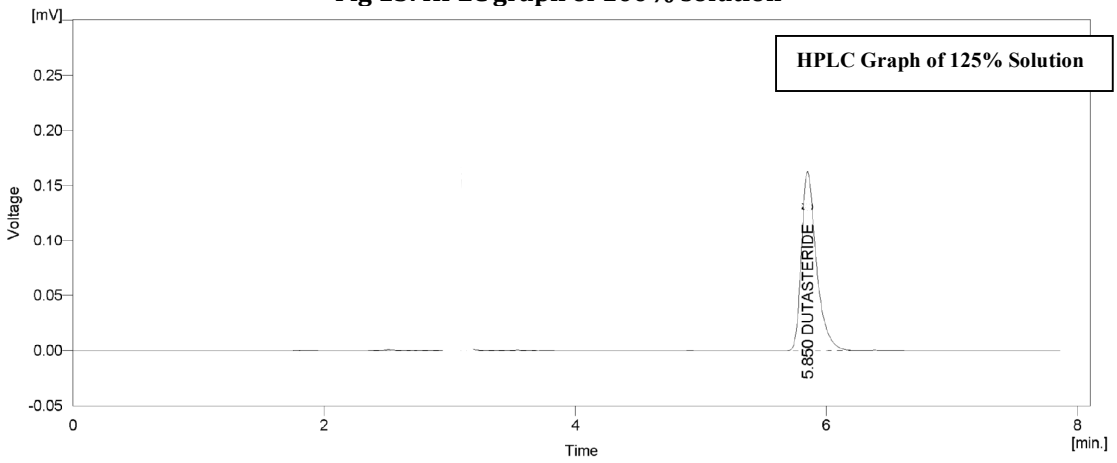

Fig 14. HPLC graph of 125% solution

Limit of Detection and Limit of Quantitation (Sensitivity)

For both the Method 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 (for HPLC) and same conc. was placed in the UV Spectrophotometer, the absorbances were measured for the drug solution (For UV), both the method was based on the SD of response and slope. The data were represented in Table.12

Table 12. LOD and LOQ determination of dutasteride.

	Concentration (µg/mL) Theoretical	Measured	Slope of Drug	Abs/Area	S.D.
UV	5*	4.800	0.0205	0.1080	0.0015
HPLC	5*	4.769	0.0616	79.964	0.0760
LOD =0.242 µg/mL and LOQ =0.732 µg/ml (UV) LOD =4.0714 µg/mL and LOQ =12.337 µg/ml (HPLC)					

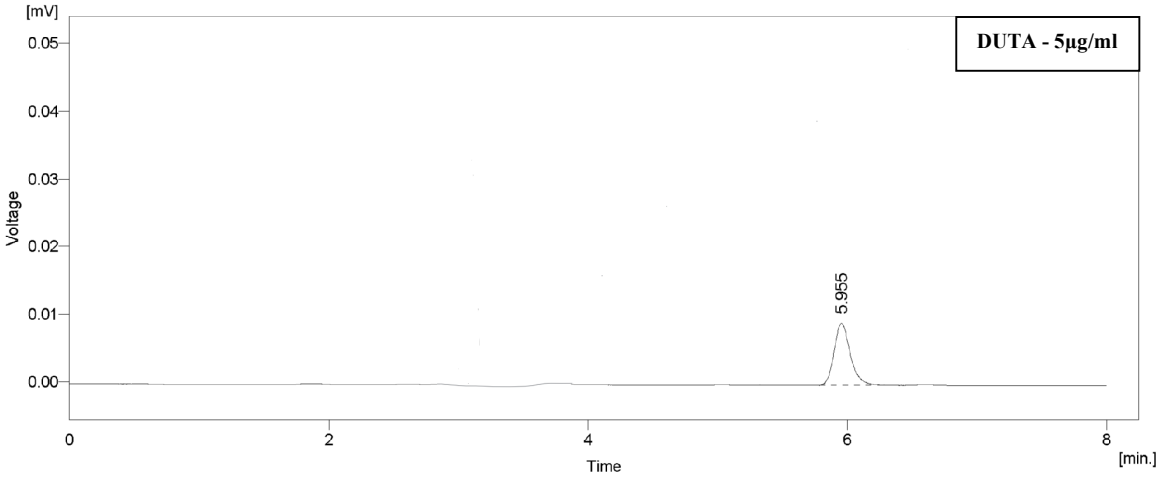

Fig.15Chromatogram of Dutasteride(5µg/ml)

Ruggedness and Robustness

The ruggedness of a method is its ability to remain unaffected by small, unintentional changes in experimental conditions. Robustness of the method shall be demonstrated changing the chromatographic parameters and system suitability parameters under each condition. The method was assessed by study of day-to-day 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 and ruggedness were 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.

Table 13. Data for Ruggedness and Robustness Test

	UV			HPLC		
	Recovery (%)	Wave Length	Absorbance	S. D	%RSD	Recovery (%)
Developer	99.92	242	1.0184	0.029	0.525	99.07
analyst #2	100.7	272	0.3138	0.007	0.128	98.40

Robustness (HPLC Method)

For the robustness of the analytical method at same Chromatographic Condition (Mobile Phase: ACN: Water: 80:20), changed the flow rate, p^H and wavelength. To study the effect of the flow rate, it was changed to 0.1 unit i.e. 0.9 and 1.1 ml/min. The change in wavelength 242 to 272 and pH (2.7 to 7.2). Data were shown in Table 14.

Table 14. Data for Robustness Test (HPLC)

Condition: Mobile Phase; ACN: Water (80:20), Flow Rate: I ml/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	5.922	1.00	1644.763
-	-	-	-	5.243	1.10	1644.763

RESULT AND DISCUSSION

System Suitability

The tailing factor for the peak due to dutasteride in stock standard solution not be more than 1.5. The system suitability of the method was checked by injecting six different preparations of same concentration of the dutasteride standard. The peak area and retention time for the drug were within 2% indicating the suitability of the system. The data were represented in Table 4 and the chromatogram were represented in Fig. 6.

Linearity

The Correlation Coefficient r^2 should not be less than 0.999. The correlation coefficient obtain was 0.999 which was in the acceptance limit. The linearity was established in the range of 5 to 100 µg/ml. The data were represented in Table 6 and the chromatogram were represented in Fig. 7.

Accuracy

The calculated recovery and percentage recovery values were (99.96%-100.70%) for UV Method and (99.93% to 100.63%) for HPLC Method. Percent recovery was within 100±2.0% the acceptable range which indicated method was found to be accurate. The calculated recovery and percentage recovery values listed in Table 7.

Precision

The result was found to be Intra-day precision from 0.115 to 0.859 and Inter-day precision from 0.0396 to 0.686 for UV-spectrophotometric method and 0.246 to 1.725 and 0.124 to 0.291 for HPLC Method respectively. Intra- day and Inter-day precision within the acceptable range ± 2, indicative of good method precision. The data were represented in Table 8, 9 and the chromatogram were represented in Fig.8, 9, 10.

Assay

In assay studies the % recovery of dutasteride from API and dosage form were 99.96% and 99.94% for UV spectrophotometric method and 99.68 % and 99.36%. for HPLC method. The data were represented in **Table.10** and the chromatogram were represented in Fig. 11.

Recovery Studies

In recovery studies the % recovery of dutasteride from API and dosage form were 99.69%, 100.41% and 99.98% for UV spectrophotometric method and 99.10%, 98.87% and 99.36% for HPLC method. The data were represented in Table.11 and the chromatogram were represented in Fig.12, 13, 14.

Limit of detection and Limit of quantitation

LOD and LOQ of described method were observed as 0.242 µg/ml and 0.732 µg/ml for UV spectrophotometric method and 4.071 µg/ml and 12.34 µg/ml for RP-HPLC method, based on the SD of response and slope. The data were represented in Table 12 and the chromatogram were represented in Fig.15.

Ruggedness

(For HPLC): The assay result with analyst #1 and Analyst #2 were % Assay = 98.40% (%RSD = 0.13%) and % Assay = 99.07% (%RSD = 0.53) respectively. %). (For UV) at wave length (272 & 242 nm) the absorbances of dutasteride were 0.314 and 1.018 respectively. The data were represented in Table 13.

Robustness

[for HPLC Method]: When the wave length and pH was adjusted to 273 and 7.2 the retention time of dutasteride were 6.193 min. and at 242 and 2.7 the retention time of dutasteride was 5.922 min. When the flow rate was changed ± 0.1 unit the retention time of dutasteride was 6.523 and 5.243 min respectively. The data were represented in Table 14.

CONCLUSION

A combined UV Spectrophotometric method and RP-HPLC method for dutasteride was developed and validated as per ICH guidelines for the determination of DSE in Bulk and dosage formulations. It was shown above that, the proposed method was linear, accurate, reproducible, repeatable, precise, selective, specific and cost effective proving the reliability of the method. In this study, the spectrophotometer instrument is simple and not of high cost, on the other hand, in terms of simplicity and expense, the proposed methods could be considered superior in comparison with the previously reported methods. The apparatus and reagents used are easily accessible even for the simple laboratories and the procedures do not involve any critical reaction. it is concluded that the proposed methods are simple, sensitive, reproducible, accurate and precise and can be recommended for routine and quality control analysis of dutasteride.

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