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Analytical Method Development and Validation for the Quantitative Estimation of Dutasteride in Its Tablet Dosage Form by RP-HPLC Method

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ABSTRACT:

New Analytical reversed-phase high performance liquid chromatographic (RP- HPLC) method has been developed for the determination of Dutasteride (DTS) in pharmaceutical formulation and bulk drug The purpose behind this method development was to have a precise, reliable, robust analytical RP HPLC method for specific and accurate quantitative estimation of DTS from its bulk and single component formulation using BDS HYPERSIL C18 (4.6mmø×250mm) analytical column. The mobile phase composed of Acetonitrile: Water: Methanol 75:10:15 (V/V/V) and the flow rate was maintained at 0.7 ml/min at UV detection wavelength 274 nm. Dutasteride was well resolved and retained at 8.34minutes. Total run time was 10min and the temperature was maintained at 20°C. This newly developed analytical RP-HPLC method was validated as per the recommendations of ICH Revised Q2 (R1) guidelines of analytical method validation, in order to prove that the new analytical method, meets the reliability characteristics. The method characteristics showed the capacity of an analytical method to keep, all over the time, the basic standards for validation: selectivity, linearity, precision, accuracy and sensitivity. The calibration plot gave linear relationship over the concentration range 10 to 22µg/ml. The LOD and LOQ were 5.2724 µg/ml and 10.977 respectively. The repeatability testing for drug showed that the method is precise within the acceptable limit. %RSD of the results of robustness studies were found to be within acceptable limits. The validated method was successfully used for quantitative analysis of dutasteride in pharmaceutical formulations and bulk drugs.

Key words: RP-HPLC, Dutasteride (DTS), Benign prostatic hyperplasia, ICH Revised Q2 (R1) guidelines.

INTRODUCTION:

The development and validation of the analytical method is an important element in pharmaceutical discovery, development and production, with a view to ensuring the identity, purity, power, and performance of drug products. The steps involved in the development of the RP-HPLC method are the physicochemical properties of the drug, setting RP-HPLC conditions, sample preparation, method optimization, and method validation. Separation and quantification of the drug is the main goal in RP-HPLC method development.

RP-HPLC includes polar mobile phase and a non-polar stationary phase and. During the development process, the parameters vary depending on the column size, instrument specification, and wavelength at which asymmetric peaks and the mobile phase can be obtained.

A new, rapid, economical RP- HPLC method is used for the quantification of the marketed formulation.

Dutasteride (DTS) is a selective inhibitor of isoforms (type1 and type 2) of the enzyme 5-reductase, this enzyme converts testosterone to 5-dihydrotestosterone (DHT). DHT is an androgen which is mainly responsible for the prostate gland's initial growth and subsequent enlargement. Dutasteride prevents testosterone from being converted to DHT. (DTS) is commonly in the treatment of benign prostatic hyperplasia since it has less adverse effects than the other drugs available. It also lowers the chances of urinary retention.

The basic purpose behind proposed method development was to have a robust analytical RP-HPLC method for estimation of dutasteride from marketed formulation, so that it can be used effectively for its routine quality control analysis, stability assay and content uniformity assay.

MATERIALS AND METHODS:

Working standard of pharmaceutical grade Dutasteride was obtained as generous gift sample from local API manufacturing unit. Marketed formulation of tablets containing 0.5mg of DTS was procured from local pharmacy shop. Methanol and Acetonitrile purchased from S.D. Fine Chemicals, Mumbai, India.

DRUG PROFILE:

Dutasteride belongs to a class of drugs called 5α-reductase inhibitors, chemically known as Bis (trifluoromethyl) phenyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide. Dutasteride selectively inhibits steroid 5-reductase isoforms I and II, an intracellular enzyme that converts testosterone to 5-dihydrotestosterone (DHT). Dutasteride works by lowering the amount of DHT in the bloodstream. It is used to treat male scalp hair loss as well as hormone therapy in transgender women.

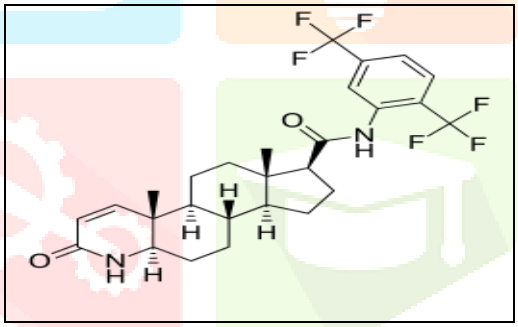

Figure1: Chemical structure of Dutasteride

Table No.1: PHYSICAL PROPERTIES OF DUTASTERIDE:

PROPERTIES	**DUTASTERIDE(DTS)**
Chemical name	Bis(trifluoromethyl)phenyl]-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide
Molecular formula	C27H30F6N2O2
Molecular weight	528.539 g·mol−1
Therapeutic category	5α reductase inhibitor
State	Whit crystalline powder
Melting point	242 to 250°C
Log P	5.09
Solubility	Practically insoluble in water, Soluble in ethanol(44mg/ml),methanol(64mg/ml), Polyethylene glycol 400(3mg/ml)

PKa	12.56(acidic) 2.7(basic)
bioavailability	60%
λ max	235
Half life	3-5 weeks

The objective of the proposed work was to develop reliable analytical method and validate the same as per the recommendations of ICH Revised Q2 (R1) guidelines of analytical validation. The purpose behind this method development was to have a robust analytical HPLC method for estimation of Dutasteride in single component formulation. It was planned to use the method for content uniformity and Quality control analysis marketed formulation of DTS.

Method:

Reversed-Phase High Performance Liquid Chromatographic (RP- HPLC) method.

Instrument Used:

The HPLC system; make: Shimadzu UFLC series employed with LAB SOLUTION software (Version 6.72 SP1).

EXPERIMENTAL STUDIES:

Analytical Method Development:

Preparation of standard stock and working solution for RP-HPLC method development studies:

10mg quantity of DTS weighed accurately and transferred into 10ml volumetric flask labeled as 'Standard/Stock solution A' and volumes was made up with methanol.

Working solution (Solution B) to be used for method development for estimation of DTS (Solution B: 100 µg/ml of a drug) was prepared from standard solution, 1 ml from stock solution were pipetted out and transfer to 10 ml capacity volumetric flask and volume was made up to mark with mobile phase. Solution B was diluted 20 times with mobile phase was used for sample injection during experimental trials of method development studies.

Selection of detection wavelength:

A UV spectrum of 10 ppm solution of DTS was generated scanning over the range of 200-800 nm using Double Beam Spectrophotometer (Shimadzu UV1801). Drug showing higher absorbance was analyzed selected as detection wavelength for estimation of DTS by UV detector in HPLC system (Shimadzu UFLC series).

Optimization of chromatographic conditions:

Many preliminary trials were conducted in order to select and optimize the stationary phase, mobile phase, flow rate, injection volume, and column temperature

Analytical Method Validation:

Performance characteristics of analytical HPLC method were statistically validated as per ICH revised Q2 (R1) guideline for analytical method validation.

Table No.2: Protocol of analytical method validation parameters with its method to be followed according to ICH revised Q2 (R1) guideline. (ICH Q2(R1))

Parameter	Purpose	Recommendation as per ICH revised Q2(R1) guideline	Acceptable criteria
Accuracy	Assay (Content/potency): Recovery studies	By adding known added quantities of analyte to the synthetic mixture of drug product components and the combined dosage form, accuracy was established across the specified range of analytical procedure. As per ICH, Accuracy should be assessed using a minimum of 9 determinations over a minimum of three concentration levels covering the specified range i.e. 3 concentration levels in triplicate. (e.g. 3 Concentration/3 replicates each). The method's accuracy is expressed as a percentage of the known added amount of analyte in the sample.	98-102 % Recovery of known added amount of analyte.

Precision	Assay(Content/potency): Repeatability and Reproducibility	**Repeatability**	**Intermediate Precision**	
		Repeatability was assessed by using minimum of 9 determination covering the specified range for the procedure (e.g. 3 concentration/3 replicates each)	The intermediate precision was established to investigate the effects of random events, such as days, on the analytical procedure's precision. Intraday and inter day precision studies were carried out by taking 9	RSD≤ 2%

			determinations of 3 concentration/3 replicates each, three times on the same day and three times on different days	

		Precision is reported as relative standard deviation (coefficient of variation) for each type of precision investigation.	
Specificity	Identification, Testing for impurities and Assay(Content/potency)	As per ICH revised Q2 (R1), Specificity should be carried out ensure identification tests, the determination impurity and assay.	No interference In analyte determination
Detection Limit and Quantification Limit.	Testing for impurities Sensitivity of analytical method: and assay Determination of minimum detectable and quantifiable concentration of analyte solution	The detection and quantification limits are determined by the response standard deviation and slope. **LOD**=3.3×σ/S **LOQ**=10×σ/S σ = Standard deviation of response estimated based on calibration curve. S = Slope of the calibration curve.	NA
Linearity	Testing for impurities and assay(content/potency):To check linear relationship of performed concentration.	As per ICH, for the establishment of linearity, a minimum of 5 concentrations are approved. Linearity is indicated by the correlation coefficient, y-intercept, and slope of the regression line, as well as a data plot.	$R^2 \geq 0.99$

Robustness	To establish reliability of analytical method	Robustness was assessed in order to demonstrate the dependability of an analytical method with respect to deliberate variations in method parameters. The following factors were investigated to determine the robustness of the analytical method: ➢ Influence of variations in pH of a mobile phase. ➢ Influence of variations in mobile phase composition ➢ Flow rate ➢ Detection wavelength ➢ Injection volume ➢ Change in column temperature.	Pooled RSD ≤3% in every change item

Lab studies for establishment of analytical method validation parameters:

Accuracy:

Accuracy experiments were performed as per method validation protocol (Table 2) by conducting recovery studies of known added amount of DTS over three concentration levels viz. 80%, 100% and 120%. Recovery studies were also performed using marketed formulations.

Precision:

Experimental determinations for establishment of repeatability and reproducibility of analytical method were carried out as per method validation protocol (Table 2).

Intra-day precision:

Replicate analysis was performed in triplicate at three different concentration levels low [LQC: 10ppm], mid [MQC: 16 ppm] and high [HQC:22 ppm] for DTS respectively within the same day at three different times (Session 1, 2, 3).

Specificity:

To determine specificity chromatograms were obtained for blank (mobile phase), DST, placebo, marketed formulations, in-house formulations. All chromatograms were analyzed and evaluated for any interference with analyte of interest.

Linearity and Range:

Experimental determinations were carried out on seven serial dilutions of working solution (Solution B) prepared using mobile phase as diluting solvent. As per method validation protocol (Table 2), linear relationship was checked by plotting average peak areas against sample concentrations. It was evaluated across the range of 10-22 ppm for DST.

Robustness:

To evaluate and check reliability of newly developed analytical RP-HPLC method deliberate changes were made in critical method parameters as listed in table 3.

Table No.3: Robustness studies- variations in method parameters and levels of variation.

Method Parameters and Variations	Levels of Variation	Actual values of method parameters after changes
Injection volume (50µl)	-30µl	20µl
	+50µl	100µl
Flow rate(0.7µl/ml)	-0.2 µl/ml	0.5µl/ml
	+0.3µl/ml	1.0µl/ml
Temperature(20°C)	+10°C	30°C
	+20°C	40°C
Wavelength(235nm)	-10nm	240nm
	+10nm	274nm
Mobile phase (75:10:15)	45:10:45	-
	30:10:60	-

Application of Newly Developed and Validated RP-HPLC Method for Routine Sample Analysis of Marketed Formulations:

Content uniformity assay:

10 tablets of drug were accurately weighed and powdered for assay of analyte and for estimation from marketed formulation. The weight of powder equivalent to label claim of DTS was transferred into individual 100 ml volumetric flask and dissolved completely in methanol with the aid of sonication for 10 mins. The solution was filtered through 0.45µm filter paper. Further dilutions were made up using mobile phase. Then these solutions were filtered through 0.45µ syringe filter and 50µL of this filtered solution were injected into HPLC column and corresponding chromatograms were recorded. The data was statistically processed for calculation of percent drug content of the stated amount.

RESULT AND DISCUSSION

Analytical Method Development:

Detection of wavelength:

UV absorbance spectra for 10 ppm solution of DTS was analyzed and 235 nm was selected as a detection wavelength for estimation of chromatographic determination of DTS.

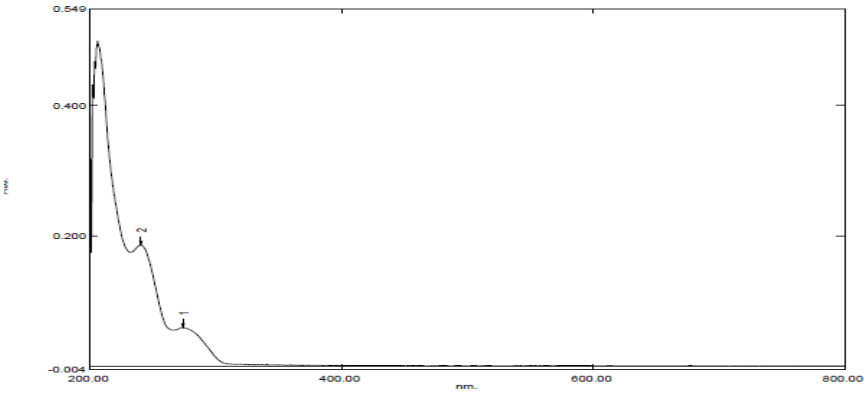

Figure 2: UV absorbance spectra

Optimization of chromatographic condition:

According to the referred scientific analytical literature it is found that the drug when estimated or analyzed by HPLC from single component formulation or bulk this drug get separated and retained on Octadecyl silane (ODS) C-18 HPLC columns. Thus, in order to get optimum resolution during simultaneous chromatographic estimation C18 column [BDS HYPERSIL C18 (4.6mmø×250mm) analytical column] was used. Experiments were designed and experimental trials were carried out for selection of mobile phase; some of these are tabulated in (**Table 3)**

TableNo.3: Experimental trials for selection of mobile phase.

Mobile phase components	Compositions
Methanol: Acetonitrile: Water	25:25:50 (V/V/V)
Methanol: Acetonitrile: Water	50:20:30 (V/V/V)
Methanol: Acetonitrile: Water(pH)	60:30:10 (V/V/V)
Methanol: Acetonitrile: Water(pH)	60:30:10 (V/V/V)
Acetonitrile: Water: Methanol	45:10:45 (V/V/V)
Acetonitrile: Water: Methanol	75:10:15 (V/V/V)

During experimental trials of RP-HPLC method development different flow rates varying from 0.5 to 1.5 ml/min as well as variable injection volumes in the range of 10 µl to 50 µl were tried. At the end of all experimental trials, on the basis of results and experimental observations with respect to response, resolution, peak sharpness, peak symmetry etc. chromatographic condition was finalized**. (Table 4)**

Table No.4: Optimized chromatographic condition.

Mobile Phase	Acetonitrile: Water: Methanol(75:10:15)
Stationary Phase	BDS HYPERSIL C18 (4.6mmø×250mm) analytical column
Flow rate	0.7ml/min
Detection wavelength	235nm
Injection volume	50µl

Chromatograms obtained using this optimized chromatographic condition showed that drug namely GLP was well resolved and retained at min respectively. Representative chromatogram of GLP is shown in figure 3.

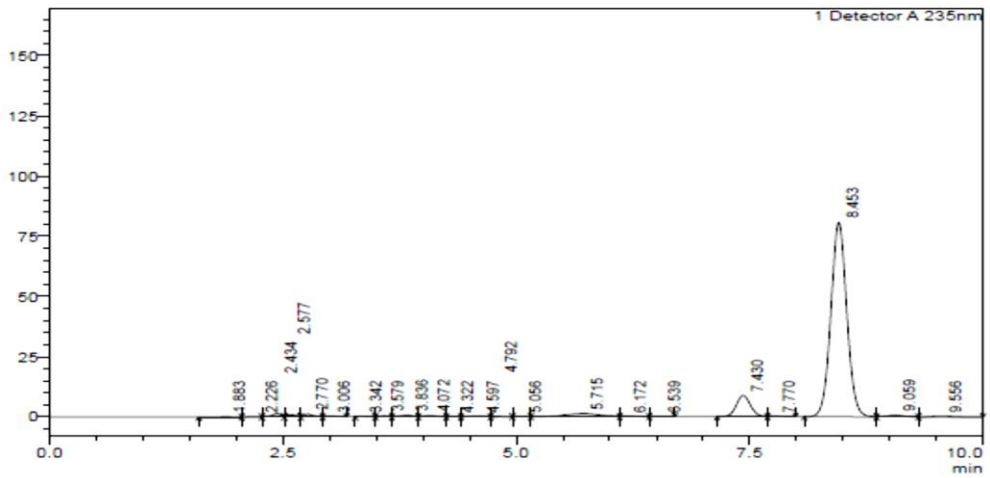

Figure 3: Representative chromatogram of DTS.

Analytical Method Validation:

Lab studies for establishment of analytical method validation parameters:

I. Accuracy:

Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. Experimental Recovery studies was also performed on tablets containing DTS. The marketed tablets of DTS were triturated and sample solution was prepared which yield a concentrations of 10 µg/ml DTS. To this solution known amount of DTS were added at three concentration levels viz. 80%, 100%, 120% and dilutions were carried out with mobile phase and injected for RP-HPLC analysis. Experimental observations and results are tabulated in table 5.

Table No. 5: Accuracy: Recovery studies on tablet formulation.

Observations						
Drug	**% Level**	**Concentration before spiking (µg/ml)**	**Total concentration after spiking (µg/ml)**	**Amount Recovered (µg/ml)**	**% Recovery**	**Inference**
DTS	80	2	2.8	2.6	96.1	Acceptable recovery hence accurate
	100	2	3	2.9	96.6	
	120	2	3.2	3.21	100	

II. Precision:

The results of intraday and inter-day precision studied are tabulated in table 6 and 7 respectively. Percent RSD values for both intraday and inter-day precision were found within acceptable limit.

Table No.6: Intra-day Precision Results

		DTS			
Levels		LQC	MQC	HQC	
Amount (µg/ml)		10	16	22	
Peak area	**1**	999774	1446172	2076099	Acceptable %RSD, hence precise
	2	997833	1447254	2078692	
	3	997268	1442981	2073650	
Average Peak Area		998291.7	1445469	2076147	
S.D.		1314.454	2221.551	2521.343	
%RSD		0.13167	0.153691	0.121443	

Table No.7: Inter day precision Results

		DTS			
Levels		**LQC**	**MQC**	**HQC**	
Amount		10	16	22	
Peak area	**1**	999851	1403827	2036077	
	2	996806	1416991	2045231	
	3	997833	1446172	2076099	Acceptable %RSD, hence precise
Average Peak Area		998163.3	1422330	2052469	
S.D.		1549.144	21671.49	20969.78	
%RSD		0.155199	1.523661	1.021686	

III. Specificity:

Separate chromatograms were obtained for blank (mobile phase), DTS, and Formulation overlaid chromatograms ensure the method is found to be selective and specific for analyte (DTS). Overlayed chromatogram of mobile phase, DTS, in-house formulation and placebo has shown in figure 4**.**

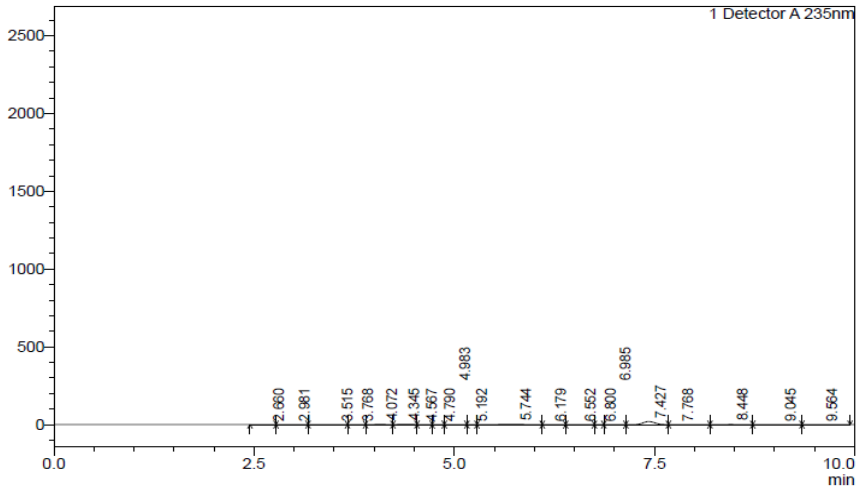

Figure 4: Chromatogram of Mobile Phase

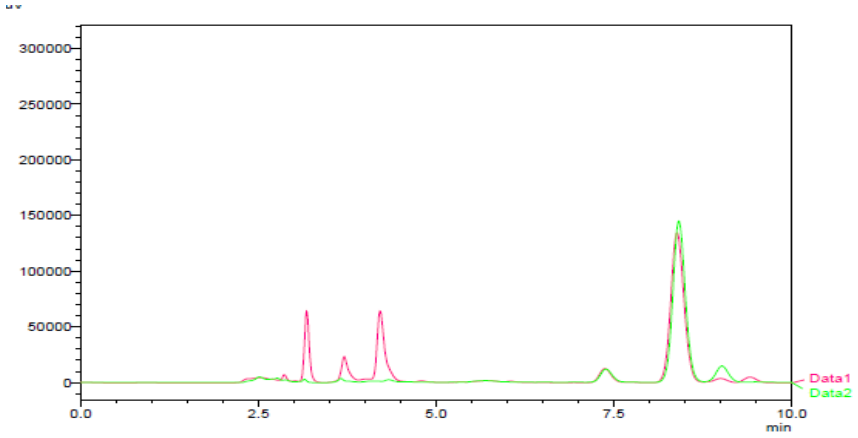

Figure 5: Overlay of Chromatogram of formulation and bulk drug

IV. Linearity:

Seven serial dilutions of DTS were prepared using a standard stock solution and dilutions were made with mobile phase. Replicate analysis was performed in triplicate and the average peak areas were plotted against concentrations to obtain the calibration curve. DTS were found linear across the range of 10-22 ppm. The linearity plot of DTS is given in figure 5. The values of correlation coefficient, y intercept and slope of regression line are shown in table 8.

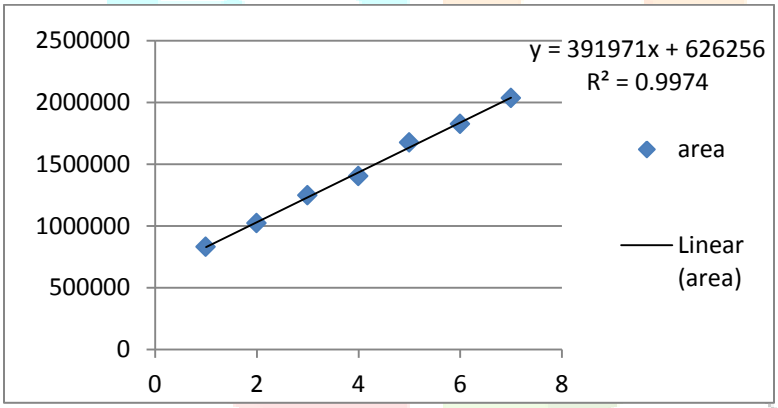

Figure 6: Linearity: Calibration plot for DTS

TableNo 8: Linear regression data of calibration plot.

Drug	**DTS**
Range	10 µg/ml-22 µg/ml
R^2	0.9974
Y Intercept	626256
Slope	201971

V. Limit of Detection and Limit of Quantification

Values for detection limit and quantification limit were determined based on the standard deviation of the response and the slop of regression line. The calculated values of limit of detection (LOD) and limit of quantitation (LOQ) for DTS is shown in table 9.

TableNo.9: Limit of Detection and Limit of Quantification

	DTS

LOD	5.2724
LOQ	10.977

VI. Robustness:

To determine robustness of analytical HPLC method changes observed in retention time and response were recorded. Method was found to be reliable and robust as method performance (retention time and response) is not much affected by deliberate variations column temperature, Injection volume and flow rate, and wavelength The results obtained are tabulated in table 10.

Table 10: Robustness: Effect on retention time and response by variation in flow rate and injection volume, and wavelength

Method Parameters and Variations	Level of Variation	Actual values of method parameters after changes	DTS	
			%RSD of recorded response (Area)	Retention time (Min)
Injection volume (50µl)	-30µl	20µl	0.01227	8.389
	+50µl	100µl	0.50045	8.392
Flow rate(0.7µl/ml)	-0.2 µl/ml	0.5µl/ml	0.19558	11.872
	+0.3µl/ml	1.0µl/ml	0.01265	5.913
Temperature(20°C) Wavelength(235nm)	+10°C	30°C	0.00152	8.438
	+20°C	40°C	0.01525	8.399
	-10nm	240nm	0.51021	8.467
Mobile phase (75:10:15)	+10nm	274nm	0.18523	8.496
	45:10:45	-	0.01231	8.356
	30:10:60	-	0.18562	8.469

Application:

Application of newly developed and validated RP-HPLC Method for routine laboratory analysis.

The method was conveniently adopted for content uniformity and Quality control analysis bulk drugs as well as marketed formulations of dutasteride.

Conclusion:

An analytical RP-HPLC method for quantitative estimation and content uniformity assay of dutasteride (DTS) from their bulk, single component formulations was successfully developed and statistically validated. The amount of dutasteride in the Tablet formulation was estimated.

Validation studies were performed as per the validation protocol developed following the recommendations of ICH revised Q2 (R1) guidelines in order to prove that the new analytical method, meets the reliability characteristics. Validation studies assured that newly developed RP-HPLC method is specific, accurate, precise and robust.

The newly developed and validated RP-HPLC method was successfully applied for quantitative estimation of DTS from marketed formulation (Tablets) and also in a routine laboratory analysis.

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