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RP-HPLC Technique for Dutasteride Quantitative Estimation in Tablet Dosage Form: Formulation and Validation of Analytical Technique

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Abstract: RP-HPLC is a novel analytical technique that can be used to detect dutasteride in pharmaceutical formulations and bulk pharmaceuticals. The aim of developing this technique was to provide an analytical RP HPLC method based on the Shiseido C18 analytical column that is accurate, dependable, and analytical for determining the concentration of dutasteride in both bulk and single component forms. The phase of mobile phase MeOH: ACN: H₂O ratio was 75:10:15 (V/V/V) at a UV detector wavelength of 274 nm, and the flow rate remained constant at 0.7 ml/min. At 8:34 min, the dutasteride case was concluded amicably. The run lasted 10 min, with the temperature remaining at 20°C. Tests were conducted to ensure that the recently developed analytical RP-HPLC technology satisfies the reliability requirements. In compliance with the ICH Revised Q2 (R1) guidelines for analytical technique validation, it was verified. The analytical technique's method features demonstrated its ability to maintain its sensitivity, accuracy, precision, selectivity, and consistency throughout time. A straight line relationship between the concentration range of 10 to 22 ppm was displayed in the calibration plot. 5.3010 µg/ml was the LOQ, while 10.999 µg/ml was the LOD. The drug's consistency test indicated that the procedure is most likely rather excellent. Reliability tests revealed that the %RSD was within allowable bounds. The authorised technique quantified dutasteride in pharmaceutical formulations and bulk drugs satisfactorily.

Keywords: Dutasteride, RP-HPLC, ICH Revised Q2, Validation, Development

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Introduction

To ensure that medicines are the appropriate ones and that they function properly, it is crucial to develop and test the analytical method during the discovery, formulation, and production of pharmaceuticals (Rajesh *et al.*, 2014). The development of the RP-HPLC technique involved examining the physical properties of the medication, establishing the RP-HPLC settings, preparing the sample, refining the procedure, and confirming its effectiveness (Paljashuva and Ramarao, 2019). The drug's separation and measurement are the primary objectives of creating an RP-HPLC technique (Aher *et al.*, 2023).

In RP-HPLC, there are two phases: a polar mobile phase and a non-polar stationary phase. The parameters vary during the development process according to the size of the column, the characteristics of the apparatus, and the wavelength at which the asymmetric peaks and mobile phase are present (Yadav *et al.*, 2017; Vyas *et al.*, 2022).

A novel low-cost, rapid, and simple RP-HPLC technology is utilised to determine the volume of sold goods. The medication dutasteride specifically inhibits the 5-reductase enzyme's type 1 and type 2 isoforms (Yeola *et al.*, 2023). This enzyme converts 5-dihydrotestosterone (DHT) from testosterone. DHT, an androgen, is the primary cause of the prostate gland's growth and subsequent enlargement (Kanthale *et al.*, 2020). Dutasteride prevents testosterone from converting to DHT. Due to its lower side effect profile compared to other medications, dutasteride is typically used to treat benign prostate hyperplasia. Additionally, it lessens the possibility that you may hold your pee. One kind of medication known as a 5 α -reductase inhibitor is

dutasteride (Bahiram *et al.*, 2023). The activity of steroid 5-reductase types I and II is specifically inhibited by dutasteride. This enzyme is responsible for converting testosterone in cells into 5-dihydrotestosterone (DHT). Dutasteride functions by reducing the blood's concentration of DHT. It is used to treat male pattern hair loss and hair loss in transgender women receiving hormone therapy (Bhavana *et al.*, 2019; Sonawane *et al.*, 2023a).

The main goal of the suggested approach creation was to create a reliable RP-HPLC method for measuring dutasteride (Fig. 1) from a market formulation. So, it could be used effectively for regular checks on quality, tests on stability, and tests on content uniformity (Sarkar *et al.*, 2006).

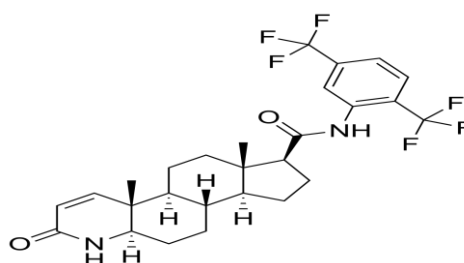


Fig. 1: Structure of Dutasteride.

Developing a reliable analytical method and testing it in accordance with the ICH Revised Q2 (R1) analytical validation standards was the aim of the suggested study (Maheshwari *et al.*, 2010). To determine the amount of dutasteride in a single-component mixture, a dependable analytical HPLC approach was the primary motivation behind the creation of this technique. The dutasteride that was sold in stores was to be tested using the method for content consistency and quality control analysis (Sonawane *et al.*, 2023b). Table 1 represents properties of dutasteride.

Table 1: Properties of Dutasteride

PROPERTIES	DUTASTERIDE(DTS)
Chemical name	Bis(trifluoromethyl)phenyl]-3-oxo-4-aza-5 α -androst-1-ene-17 β -carboxamide
Molecular formula	C ₂₇ H ₃₀ F ₆ N ₂ O ₂
Molecular weight	528.539 g·mol ⁻¹
Therapeutic category	5 α reductase inhibitor
State	Whit crystalline powder
Melting point	242 to 250°C
Log P	5.09
Solubility	Practically insoluble in water, Soluble in ethanol(44 mg/ml), methanol (64 mg/ml), Polyethylene glycol 400(3 mg/ml)

Materials and Methods

A local API production facility gave a working standard of pharmaceutical grade dutasteride as a present. A commercial kind of dutasteride tablets (0.5 mg) was purchased from a local pharmacy. Methanol and acetonitrile were purchased from Sigma Aldrich (India) (Veeraswami and Naveen, 2019).

Development of Analytical Methods:

Standard stock and working solution Preparation:

A precise 10 mg dose of dutasteride was measured out and put into a 10 ml volumetric flask that was marked as Standard/Stock solution A. Methanol was then used to fill in the gaps.

We made a working solution (Solution B) from the standard solution to figure out how much dutasteride was in it (Solution B: 100 μ g/ml of the drug). We pipetted 1 ml of the stock solution into a 10 ml volumetric flask. After that, we added enough mobile phase to completely fill the flask. During the method development studies, Solution B that had been diluted 20 times with mobile phase was used to inject samples (Basak, *et al.*, 2019; Muthyala and Naresh, 2019).

Selection of detection wavelength:

The Shimadzu UV1801 Double Beam Spectrophotometer was used to get a UV spectrum of a

dutasteride solution with 10 ppm. The spectrum covered wavelengths from 200 to 800 nm. The drug with the highest absorbance was looked at, and the Shimadzu UFLC series UV detector in the HPLC machine was used to find the right wavelength for measuring dutasteride (Bagal *et al.*, 2021).

Optimization of chromatographic conditions:

To choose and optimise the mobile phase, stationary phase, injection volume, column temperature, and flow rate, numerous preliminary trials were carried out (Ravisankar *et al.*, 2013).

Analytical Method Validation:

The performance parameters of the analytical HPLC technique were statistically validated by adhering to the ICH revised Q2 (R1) guideline for analytical method validation (Jadhav *et al.*, 2013).

Accuracy:

The accuracy experiments were conducted by doing recovery investigations of known added dutasteride amounts over three concentration levels, i.e. 80%, 100%, and 120%, in accordance with the technique validation approach (Table 1). Trials of recovery using commercial medications were also conducted (Peraman *et al.*, 2015).

Precision:

According to the process for method validation,

Table 2: Selection of Mobile Phase

Mobile phase components	Compositions
MeOH:ACN:H ₂ O	25:25:50(V/V/V)
MeOH:ACN:H ₂ O	50:20:30(V/V/V)
MeOH:ACN:H ₂ O (pH)	60:30:10(V/V/V)
MeOH:ACN:H ₂ O (pH)	60:30:10(V/V/V)
MeOH:ACN:H ₂ O	45:10:45(V/V/V)
MeOH:ACN:H ₂ O	75:10:15(V/V/V)

experimental determinations were made to establish the reproducibility and repeatability of the analytical method (Table 2) (Nyola and Jeyabalan, 2012).

Intra-day precision:

Three different concentration levels of dutasteride low (10 ppm), mid (16 ppm), and high (22 ppm) were analysed in triplicate during the course of the same day at three distinct times (Kesharwani *et al.*, 2020).

Specificity:

Chromatograms were obtained for the following: placebo, DST, commercial formulations, in-house formulations, and blank (mobile phase) in order to assess specificity. Every chromatogram was examined to look for any interference with the target analyte (Oza *et al.*, 2012).

Linearity and Range:

Seven consecutive dilutions of the working solution (Solution B), which was made utilising mobile phase as a diluting solvent, were used for the experimental determinations. Plotting average peak areas versus sample concentrations allowed for the linear connection to be verified in accordance with the technique validation process (Table 1). For DST, it was assessed over a range of 10–22 ppm (Ahire *et al.*, 2022, 2023).

RP-HPLC Technique for Regular Sample Examination of Sold Formulations:

Content uniformity assay:

10 tablets were precisely weighed, then powdered for use in both estimation from the marketed

formulation and analyte testing. One 100 ml volumetric flask was filled with methanol and sonicated for 10 min to completely dissolve the powder, which was the weight of powder comparable to the dutasteride label claim. Using 0.45 µm filter paper, the solution was filtered. Mobile phase was used to make up additional dilutions. 50 µl of the filtered solution was injected into the HPLC column after these solutions had been filtered using a 0.45 µ syringe filter. The corresponding chromatograms were then recorded. The percentage of drug content in the specified amount was calculated by statistically processing the data (Jarouliya *et al.*, 2015; Jain *et al.*, 2023; Jaiswal *et al.*, 2023).

Results and Discussion

Development of Analytical Methods:

Wavelength Detection:

After studying the UV absorbance spectra of a dutasteride solution at a concentration of 10 ppm. The detection wavelength for the chromatographic quantification of dutasteride was selected at 235 nm. Figure 2 shows UV absorbance spectrum of dutasteride.

Optimization of chromatographic condition:

The scientific analytical literature that has been mentioned states that when measured or analysed by HPLC from bulk or single component formulation, the drug separates and stays on Octadecyl silane (ODS) C-18 HPLC columns. Therefore, a C18 column was chosen to get the best resolution possible during simultaneous chromatographic estimation. To choose the mobile

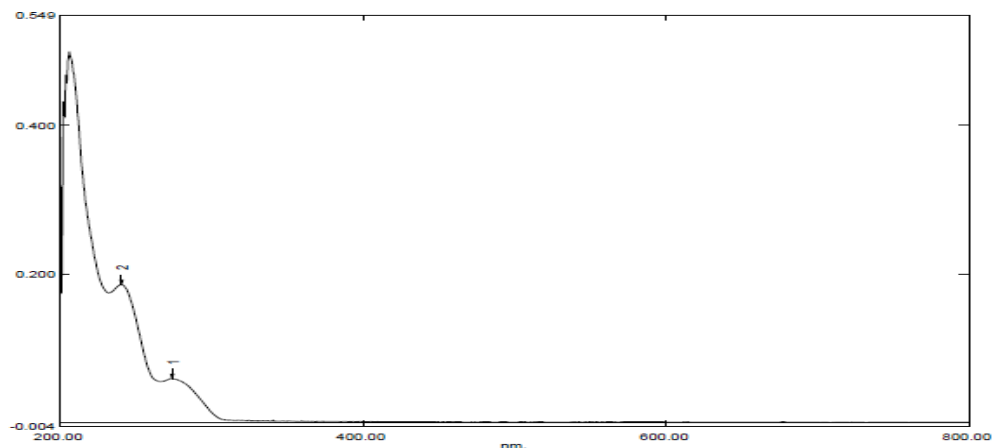


Fig. 2: UV absorbance spectrum of dutasteride.

Table 3: Optimized Chromatographic Condition

Mobile Phase	MeOH:ACN:H ₂ O (75:10:15)
Stationary Phase	Shiseido C18analytical column
Flow rate	0.7 ml/min
Wavelength detection	235 nm
Injection volume	50 µl

phase, experiments were planned and trials conducted; a few of these are listed in Table 2.

Throughout the RP-HPLC technique development's experimental trials, several injection volumes and flow rates between 0.5 and 1.5 ml/min were investigated. The injection volumes ranged from 10 µl to 50 µl. The chromatographic condition was determined at the conclusion of each experimental trial based on the findings and experimental observations regarding responsiveness, resolution, peak sharpness, peak symmetry, etc. (Table 3).

Chromatograms produced under these chromatographic conditions of choice demonstrated that the medication, GLP, was retained at min and well resolved at min.

Validation of Analytical Methods:

Analytical method validation parameters:

Accuracy:

The method's accuracy is expressed as the percentage of the sample's known additional

analyte recovery. Dutasteride tablets were also the subject of experimental recovery research. The commercially available dutasteride pills were triturated, and a sample solution containing 10 µg/ml of dutasteride was prepared. This solution was supplemented with dutasteride at known concentrations of 80%, 100%, and 120%. Mobile phase was used to dilute the mixture before it was injected for RP-HPLC analysis. Table 4 is a tabulation of the experimental findings and outcomes. Table 4 is a tabulation of the experimental findings and outcomes.

Precision:

Tables 5 and 6 illustrates the findings of the study on intraday and interday precision, respectively. The percentage RSD readings were found to be within an acceptable range for both intraday and interday precision.

Specificity:

Dutasteride, blank (mobile phase), and formulation superimposed chromatograms were

Table 4: Recovery Studies on Tablet Formulation Based on Accuracy

Observations						
Drug	% Level	Concentration before spiking ($\mu\text{g/ml}$)	Total concentration after spiking ($\mu\text{g/ml}$)	Amount Recovered ($\mu\text{g/ml}$)	% Recovery	Inference
Dutasteride	80	2	2.9	2.5	95.90	Acceptable recovery
	100	2	3.0	2.8	96.80	Acceptable recovery
	120	2	3.1	3.20	100	Hence accurate

Table 5: Intra-day Precision Results

		Dutasteride			Acceptable %RSD, hence precise
Levels		10 ppm	16 ppm	22 ppm	
Peak area	1	999774	1446172	2076099	
	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 6: Inter-day Precision Results

		Dutasteride			Acceptable % RSD, hence precise
Levels		10 ppm	16 ppm	22 ppm	
Peak area	1	999849	1403830	2036069	
	2	996810	1416993	2045229	
	3	997836	1446169	2076098	
Average Peak Area		998162.9	1422329	2052471	
S.D.		1550.100	21670.50	20970.11	
% RSD		0.155200	1.523669	1.021652	

obtained separately. This ensures that the approach is determined to be specific and selective for Dutasteride. Figure 3 displays an overlapping chromatogram of the mobile phase, dutasteride, proprietary formulation, and placebo. Figure 4 shows Overlay of Chromatogram of formulation and bulk drug.

Linearity:

A standard stock solution was used to prepare seven serial dilutions of dutasteride, and mobile

phase was used to make the dilutions. Plotting the average peak areas against concentrations during a triplicate duplicate investigation resulted in the calibration curve. Dutasteride levels were found to be linear between 10 and 22 ppm. The dutasteride linearity plot, y intercept, correlation coefficient, and slope of the regression line data are displayed in Figure 5.

Limit of Quantification and Limit of Detection:

The values for the detection and quantification

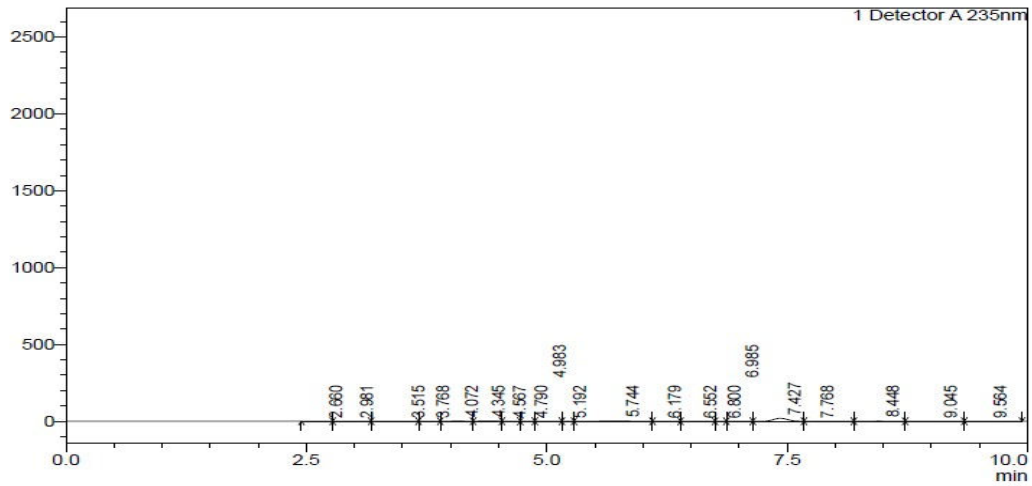


Fig. 3: Chromatogram of Mobile Phase.

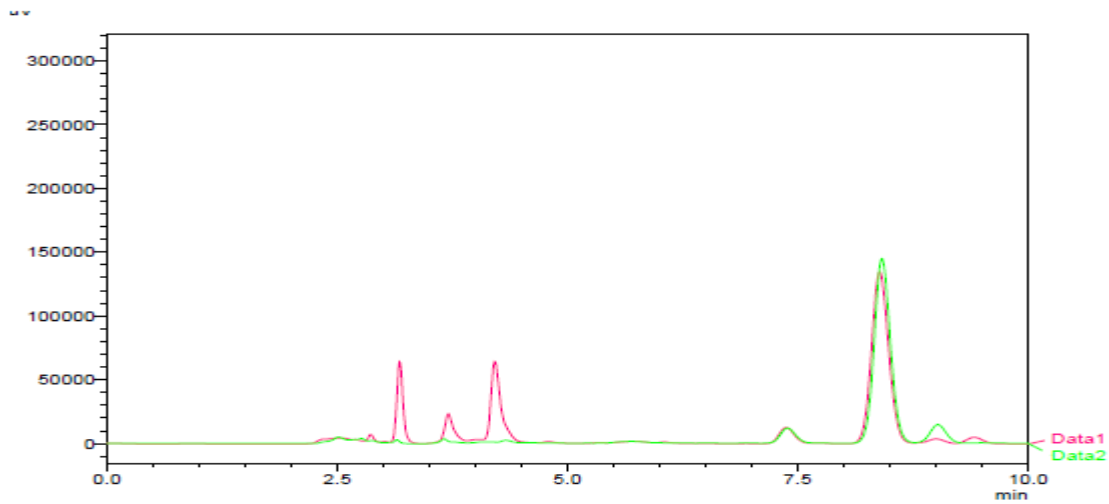
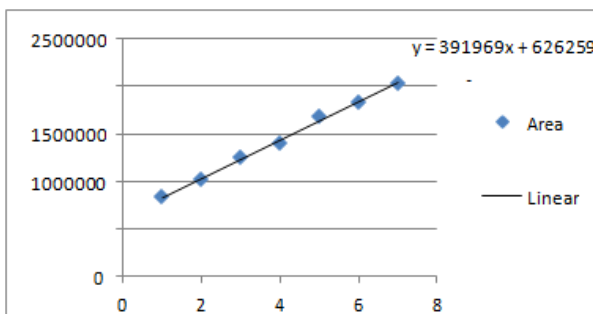


Fig. 4: Overlay of Chromatogram of formulation and bulk drug.



Drug	Dutasteride
Range	10 PPM to 22 PPM
R ²	0.9990
Y Intercept	626259
Slope	201970

Fig. 5: Linearity: Calibration plot for dutasteride.

Table 7: Limit of Detection (LOD) and Limit of Quantification (LOQ)

Drug	Dutasteride
LOD	5.3010
LOQ	10.999

limits were computed using the slope of the regression line and the response standard deviation. The established limits of quantitation (LOQ) and detection (LOD) for dutasteride are shown in Table 7.

Conclusion

The method was easily applied for checking the quality and consistency of bulk medicines as well as dutasteride preparations that were sold in stores. An analytical RP-HPLC method was used to successfully find out the uniformity of dutasteride's content and get a quantitative estimate from bulk single component formulas. These results were then statistically confirmed. A calculation was made to find out how much dutasteride was in the tablet. Validation tests were conducted utilising the modified ICH Q2 (R1) standards validation methodology to demonstrate that the new analytical method satisfies the dependability requirements. Validation studies have shown that the new RP-HPLC technology is strong, precise, exact, and specific. This study employed the recently established and approved RP-HPLC technology to accurately anticipate its potential applications in routine laboratory research. There was no trouble using the method to check the quality and uniformity of bulk medicines, dutasteride formulations from marketed formulations (Tablets), and in a normal lab study.

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