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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, rapid, accurate and precise method was developed and validated for the simultaneous estimation of Dutasteride and Tamsulosin hydrochloride in pharmaceutical dosage form. The method was based on RP-HPLC. Chromatographic separation was performed on Xterra-symmetry C_{18} (150mm x 4.6mm, 5µm particle size) column using a mobile phase consisting of a mixture of Phosphate buffer (pH 2.5 with dilute orthophosphoric acid): Acetonitrile (20:80%v/v) in an isocratic mode. The following system conditions were maintained throughout development and validation i.e., flow rate 0.8mL/min, column was maintained at room temperature and the detected by a UV-wave length at 274nm. The Dutasteride and Tamsulosin hydrochloride were well resolved on the stationary phase and the retention times were 2.0 and 5.0minutes respectively. The method was validated; Dutasteride and Tamsulosin hydrochloride both were shown to be linear over a range of 25-75µg/mL. The limit of quantification was 18.1µg/mL for Dutasteride and 18.7µg/mL for Tamsulosin hydrochloride and the limit of detection was 5.9µg/mL for Dutasteride and 6.2µg/mL for Tamsulosin hydrochloride. The Precision, Accuracy, Specificity, Ruggedness and Robustness were determined to validate the method.

Keywords: Dutasteride, Tamsulosin hydrochloride, RP-HPLC, Method development, Validation and C_{18} Column.

INTRODUCTION

Dutasteride (DU) (Fig. 1A) is chemically designated as (5α, 17β)-*N*-{2,5-bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide. It is a dual 5-α reductase inhibitor that inhibits conversion of testosterone to dihydrotestosterone (DHT). It is used for benign prostatic hyperplasia; it increases the risk of erectile dysfunction and decreased sexual desire. Tamsulosin hydrochloride (TA) (Fig. 1B) is chemically designated as (-)-(R)-5-[2-[[2-(o-Ethoxyphenoxy) ethyl]amino]propyl]-2-methoxybenzenesulfonamide, monohydrochloride[1-6]. It is α_{1a}-selective alpha blocker used in the symptomatic treatment of benign prostatic hyperplasia (BPH). It is used in the treatment of difficult urination, a common symptom of enlarged prostate.

According to the literature survey[7-15] it was found that few analytical methods on UV Spectrophotometer, HPLC, HPTLC, LC-MS/MS and Chiral separations were reported for the estimation of Dutasteride and Tamsulosin hydrochloride individually and in combination with other drugs. Hence there is need to develop and validate an analytical method to estimate drugs. The objective of the proposed method is to develop simple and accurate method for the simultaneous estimation of Dutasteride and Tamsulosin hydrochloride in pharmaceutical dosage form by RP-HPLC.

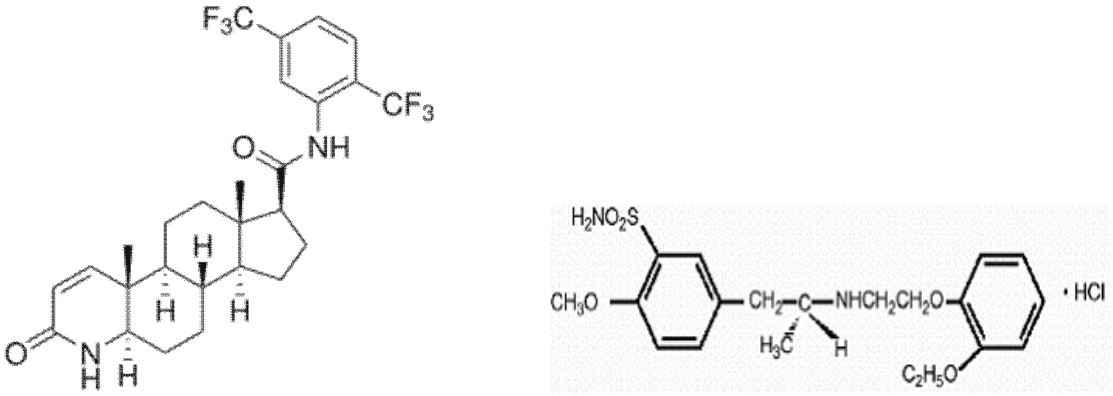

Figure 1: Chemical structures of Dutasteride (A) and Tamsulosin hydrochloride (B)

MATERIAL AND METHODS

Reagents and Chemicals: Dutasteride and Tamsulosin hydrochloride working standards were procured from Pharmatrain, Hyderabad. Combination tablets of Tamsulosin hydrochloride 400µg and Dutasteride 500µg (DUODART®) were purchased from local pharmacies. Purified water was obtained from Millipore system. Acetonitrile (HPLC grade) was obtained from E-Merck. All other chemicals used in the analysis were AR grade.

Instrumental and analytical conditions: The HPLC analysis was performed using a Waters 2998 model equipped with an autosampler, UV-Vis detector and running on empower software. Column used was Xterra-symmetry C_{18} (150mm × 4.6mm, 5µm particle size). UV detection was performed at 274nm. The injection volume of sample was 20µL. An isocratic mobile phase containing Acetonitrile and 0.05M potassium dihydrogen orthophosphate buffer (80:20%v/v), at the pH 2.5 with O-phosphoric acid was carried out with the flow rate of 0.8mL/min. Column was maintained at room temperature.

EXPERIMENTAL

Mobile Phase Preparation

Mixture of Phosphate buffer, pH 2.5 and Acetonitrile in the ratio of 20:80(%v/v) respectively was used.

Preparation of Standard Solution

Accurately weighed 50mg of Dutasteride working standard and 50mg of Tamsulosin hydrochloride working standard was transferred into two separate 100mL volumetric flasks. About 60mL of methanol was added and sonicated to dissolve. The volumes was made up with methanol and mixed. 1.5mL of these solutions were diluted to 10mL with mobile phase and mixed.

Preparation of Sample Solution

20 tablets were weighed accurately and the average weight was calculated.

Tablets were crushed to fine powder and equivalent to 50mg of Dutasteride sample was weighed and transferred into 100mL volumetric flask. 60mL of methanol was added and sonicated for 30min with intermediate shaking. Volume was made up with methanol. The above solution was centrifuged for 10min at 8000rpm. 1.5mL of this solution was diluted to 10mL with mobile phase and mixed.

METHOD DEVELOPMENT

An Xterra-symmetry C_{18} (150mm × 4.6mm, 5µm particle size) as a stationary phase with a mobile phase of Phosphate buffer, pH 2.5 and Acetonitrile (20:80) at a flow rate 0.8mL/min and a detection wavelength of 274nm afforded the best separation of drugs. The standard solution and sample solution prepared as above were injected into the 20µL loop and the chromatograms were recorded as shown in the “Fig. 2” and “Fig. 3” respectively. The

retention times of drugs, Dutasteride and Tamsulosin hydrochloride were found to be 2.0mins and 5.0mins respectively. The amount of drugs present in sample was calculated.

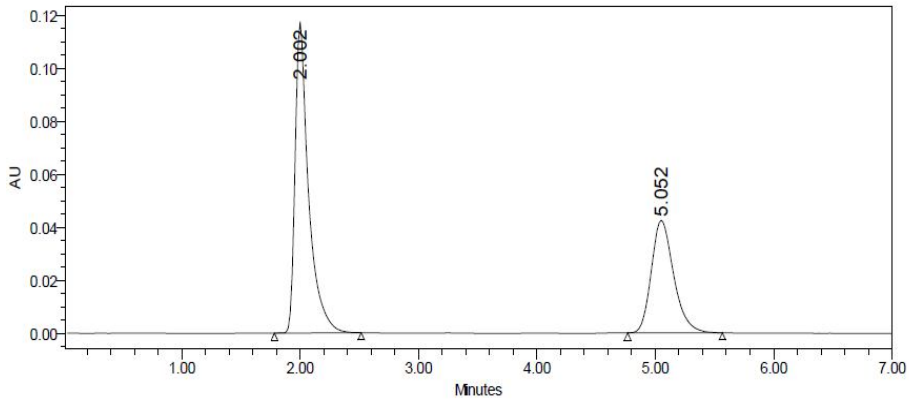

Figure 2: Standard Chromatogram

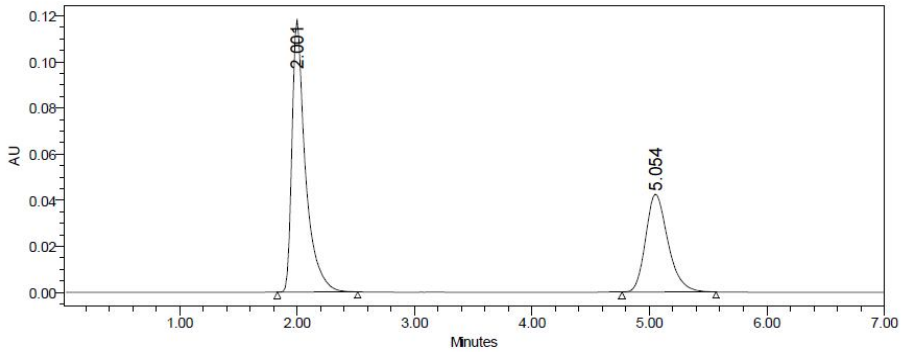

Figure 3: Sample Chromatogram

METHOD VALIDATION

The proposed method has been developed and validated for the determination of Dutasteride and Tamsulosin hydrochloride in pharmaceutical dosage forms. According to International Conference on Harmonization (ICH)[16,17] guidelines, validation of the method was carried out by using accuracy, linearity, suitability, range, LOD, LOQ, precision, ruggedness and robustness.

System suitability

A standard solution was prepared by using Dutasteride and Tamsulosin hydrochloride working standards as per test method and was injected 5 times into the HPLC system. The system suitability parameters were evaluated from the USP tailing and USP plate count values obtained from standard chromatograms as shown in Table 1.

Specificity

Specificity was evaluated by injecting standard solution and placebo solution individually

and it was observed that there was no interference from placebo.

Linearity of test solution

A series of solutions are prepared from standard stock solution at concentration levels from 25-125µg/ml for Dutasteride and Tamsulosin hydrochloride.

Accuracy

Drug assay was performed in triplicate as per test method with equivalent amount of drugs into each volumetric flask for each spike level to get the concentration of drugs equivalent to 50%, 100% and 150% of the labeled amount as the test method.

Precision

Repeatability: Repeatability of method was evaluated by calculating the %RSD of peak areas of six replicate injections for the standard concentration (100%) of drugs.

Ruggudness: The ruggedness was also evaluated by analyzing six samples of drugs by two analysts in the same laboratory using different HPLC systems.

Robustness

Standard solution was prepared as per the test method and analyzed using varied mobile phase composition (±5% of actual organic phase composition) and flow rate (±0.1ml/min of actual flow rate). System suitability parameters were evaluated.

Limit of Detection and Limit of Quantitation

The parameters LOD and LOQ were determined on the basis of standard deviation and slope of the regression equation.

RESULTS AND DISCUSSION

Selection of detection wavelength

From the overlain UV spectra, suitable wavelength considered for monitoring the drugs was 274nm as shown in “Fig. 4”.

A RP-HPLC method was developed by using an Xterra-symmetry C_{18} (150mm × 4.6mm, 5µm particle size) as a stationary phase with a mobile phase of Phosphate buffer, pH 2.5 and Acetonitrile (20:80) at a flow rate 0.8mL/min and a detection wavelength of 274nm afforded the best separation of drugs. The injection volume is 20µL and retention time for Dutasteride is 2.0min and for Tamsulosin hydrochloride was 5.0mins. The method was validated

according to ICH guidelines for various parameters like accuracy, precision, linearity, specificity, ruggedness, robustness, LOD and LOQ. Linearity was obtained in the concentration range of 25-125µg/mL for Dutasteride and Tamsulosin hydrochloride with correlation coefficient (r) of 0.999. The %recovery was found to be 99.91% for Dutasteride and 99.99% for Tamsulosin hydrochloride. The %RSD was found to be 0.42 for Dutasteride and 0.45 for Tamsulosin hydrochloride.

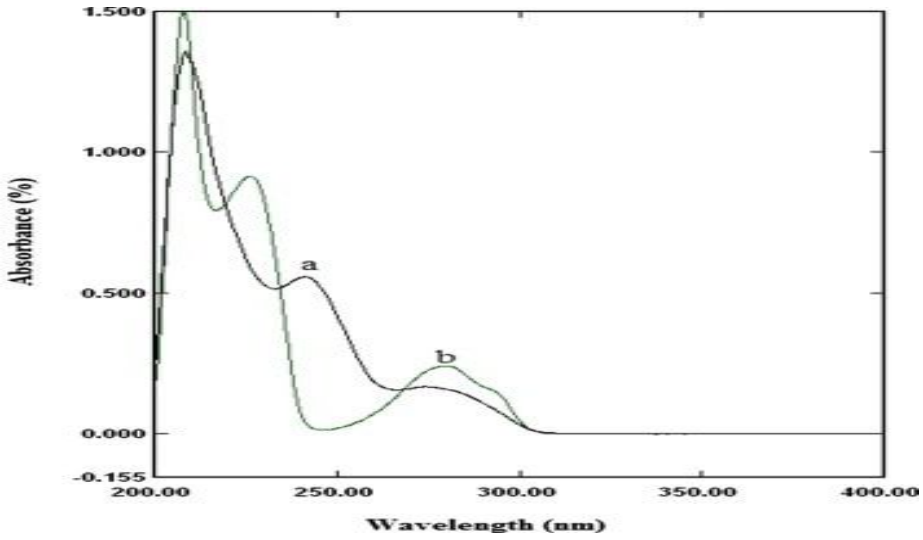

Figure: 4 Overlain spectra of (a) Dutasteride and (b) Tamsulosin hydrochloride

Table: 1 Data for Optimize Chromatographic condition

Name	Retention time (Min)	Area (µV*sec)	USP Plate Count	USP Tailing	Resolution
Dutasteride	2.006	921892	2712	1.8	11.0
Tamsulosin hydrochloride	5.060	551768	3428	1.3	

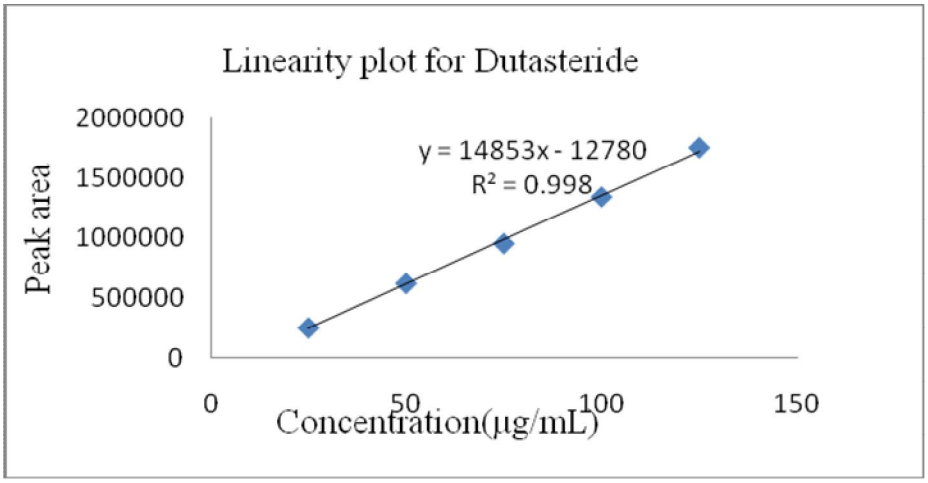

Figure: 6A Linearity plot of Dutasteride

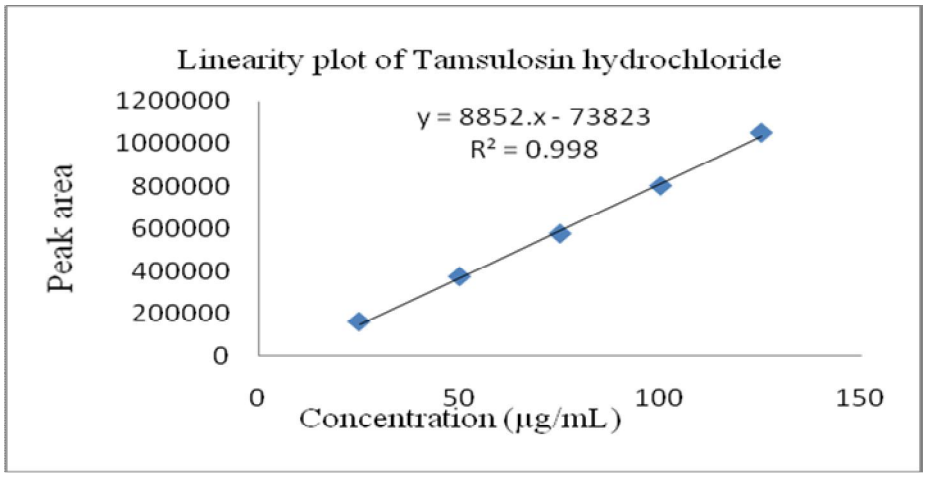

Figure: 6B Linearity plot of Tamsulosin hydrochloride

Table: 2 Result of different parameters

Parameters		Dutasteride	Tamsulosin hydrochloride
Specificity		Specific	Specific
Linearity	Regression equation, y=mx+c	y = 14853x – 127803	y = 8852.3x - 73823
	Correlation coefficient (r)	0.9992	0.9992
Accuracy (recovery) n=3	Level I (50%)	99.61%	100.08%
	Level II (100%)	99.97%	100.13%
	Level III (150%)	100.17%	99.76%
Precision (%RSD) n=6		0.42	0.45
Ruggedness (%RSD) n=6		0.46	0.31
Robustness (%RSD)	Variation in flow rate (±0.1)	< 2	< 2
	Variation in organic composition in mobile phase (±5%)	< 2	< 2
Limit of Detection (LOD)		5.9µg/mL	6.2µg/mL
Limit of Quantitation (LOQ)		18.1µg/mL	18.7µg/mL
System Suitability	USP Plate Count	2712	3428
	USP Tailing	1.8	1.3

CONCLUSION

A simple, precise, accurate, rapid, economical analytical method for simultaneous estimation of Dutasteride and Tamsulosin hydrochloride has been developed by using RP-HPLC method. The developed method was validated as per ICH guidelines. The proposed method shows good agreement with all validation parameters. The optimized method is precise, accurate, specific, rugged, and robust and a linear relation is observed between the concentration and the result. The developed method can be used for the analysis of routine quality control sample.

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