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Simultaneous determination of dutasteride and tamsulosin in pharmaceutical dosage forms by RP-HPLC

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ABSTRACT

A simple, rapid, precise and accurate isocratic reversed-phase HPLC method was developed and validated for the simultaneous determination of Dutasteride (DTA) and Tamsulosin (TMS) in commercial tablets. The method has shown adequate separation for DTA and TMS from their associated excipients of the tablets. Separation was achieved on a Waters C-18 Column with 250mm × 4.6 mm and 5 µm Particle size, using a mobile phase consisting of Acetonitrile (ACN): Water (90:10, v/v) at a flow rate of 1 ml/min and UV detection at 292 nm. The linearity of the proposed method was investigated in the range of 50-150 µg/ml (r = 0.9993) for DTA and 40-120 µg/ml (r = 0.9997) for TMS. The limits of detection were 0.17 ng/ml and 0.21 ng/ml for DTA and TMS, respectively. The limits of quantitation were 0.52 ng/ml and 0.65 ng/ml for DTA and TMS, respectively. This procedure was found to be convenient and reproducible for analysis of these drugs in tablet dosage forms.

Key words: Dutasteride, Tamsulosin, Acetonitrile, reversed-phase HPLC.

INTRODUCTION

Benign prostatic hyperplasia is the most prevalent urological disorder in men and is caused due to the enlargement of the prostate gland. [1] Tamsulosin hydrochloride, -(R) - 5-[2-[[2-(O-ethoxyphenoxy) ethyl] amino] propyl]- 2-methoxy benzene sulfonamide (Figure 1) hydrochloride, is a structurally new type of highly selective a1-adrenoceptor antagonist, having a molecular weight of 408.51. The drug has been used clinically for urinary obstructed patients with BPH. The α1-adrenoceptor antagonist activity of tamsulosin hydrochloride has been found to be more potent than other drugs such as prozosin. [2, 3]

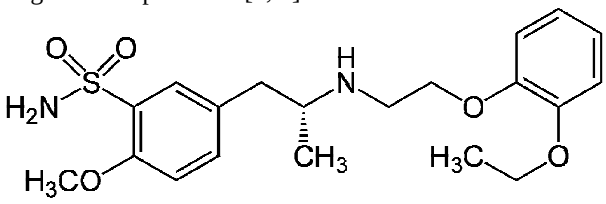

Figure 1: Chemical structure of Tamsulosin

Dutasteride is a synthetic 4-azasteroid compound (Figure 2) that is a selective inhibitor of both type 1 and type 2 isoforms of steroid 5α-reductase, an intracellular enzyme that converts testosterone to 5a-dihydrotestosterone (DHT). Dutasteride is chemically designated as (5a, 17b)-N-{2,5 bis (trifluoromethyl) phenyl}-3- oxo-4-azaandrost-l-ene-17-carboxamide. The empirical formula of dutasteride is $C_{27}H_{30}F_6N_2O_2$, representing a molecular weight of 528.5. Dutasteride inhibits the conversion of testosterone to DHT. DHT is the androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland. [4, 5, 6].

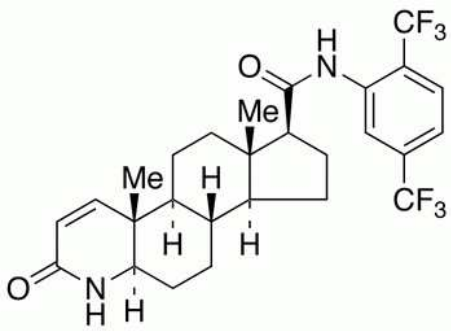

Figure 2: Chemical structure of Dutasteride

Detailed literature survey was carried out and revealed that very few analytical methods have been reported for the estimation of DTA and TMS individually and in combination with other drugs like UV [7,8,9], HPTLC [10, 11], LC–MS/ MS [12, 13] and chiral separations [14]. However the revere phase High performance liquid chromatographic method was not reported for the estimation of these drugs in combined dosage form. The objective of the present study was to develop a simple, precise, accurate and rapid Rp-HPLC method for the estimation of DTA and TMS in pharmaceutical dosage form. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines (15, 16).

MATERIALS AND METHODS

Chromatographic conditions:

The HPLC system consisted of a LC Waters (Waters, Milford, MA, USA) using a Water's C_{18} 250x4.6 mm, 5µ column, a quaternary gradient system (600 Controller), in line degasser (Waters, model AF). The system was equipped with a photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). The mobile phase was pumped at a flow rate of 1.0 mL min^{-1}. The detection wavelength for analytes was 292 nm respectively.

Chemicals and reagents:

Dutasteride and Tamsulosin were supplied by Dr. Reddy's laboratories Ltd., Hyderabad, Andhra Pradesh, India with 100% purity. Tablets (Brand: VELTAM – PLUS, Make: Intas Pharmaceuticals, Dehradhun, India) for analysis were purchased from local pharmacy. HPLC grade Methanol was procured from Merck, Mumbai. Water used in this study was prepared by Millipore milli Q (Bedford, USA) water purification system. An isocratic mobile phase consisting of a mixture of ACN: water in a ratio of 90:10 v/v was used throughout the analysis. The mobile phase was filtered through a 0.45 µm Millipore filter and degassed in an ultrasonic bath. Fresh mobile phase was prepared daily. The flow rate of the mobile phase was 1.0 mL/min. Detector signal was monitored at a wavelength of 292 nm. The column temperature was kept ambient and injection volume was 20 µL.

Solution preparation

Standard solution:

DTA standard stock solution was prepared by transferring 50.0 mg of DTS working standard into a 50 mL volumetric flask. A small portion of mobile phase was added and sonicated for 5 mins. The solution was diluted to the mark with mobile phase. 1ml of this solution was transferred to 10 ml volumetric flask and diluted upto the mark with the mobile phase to get a final concentration of 100 µg/ml.

Portion of 50.0 mg of TMS working standard was transferred into 50 mL volumetric flask and dissolved by adding mobile phase. 1ml of this solution was further diluted to 10ml with the mobile phase to get a final concentration of 80 µg/ml.

Sample solution
Sample solution was prepared by transferring 1149.50 mg of powdered tablet mass in a 10mL volumetric flask. 5 ml mobile phase was added to dissolve the tablet powder completely by using ultrasonicator for 20 min. The volume was diluted to the mark with mobile phase and mixed thoroughly. This solution was filtered through 0.45 µm membrane filter and 20 µL of this solution was injected into HPLC. The standard and sample solutions were found to be stable for at least 24 h.

Method Validation:
Specificity
The specificity defined as the ability of method to measure the analyte accurately and specifically in the presence of components present in the sample matrix, was determined by analysis of chromatograms of drug-free and drug-added placebo formulation.

Linearity
Five-point standard curves for both compounds were constructed by drawing peak area versus analyte concentration using 50-150 µg/ml (for DTA) and 40-120 µg/ml (for TMS) processed separately and run in duplicate daily on the 3 consecutive days. The concentration ranges were selected based on optimized drug concentration levels. Calibration curves were generated using weighted linear regression analysis with a weighting factor of 1/*x* over the respective standard concentration range.

Assay Accuracy and precision:
The accuracy of an analytical method is defined as the similarity of the results obtained by the analytical method to the true value and the precision as the degree of that similarity [17]. Accuracy of the method was performed by recovery study of formulations of three concentrations (one near to the limits of quantitation) on a single assay day to determine intra-day precision and accuracy. In addition, analyses of six samples of three concentrations on 3 consecutive days were used to determine inter-day precision and accuracy. Recovery studies of DTA and TMS were performed using the method of standard addition for measuring accuracy of method.

The assessment of assay precision was carried out using the data from the recovery study. The method of analysis of variance (ANOVA) was used for estimating the total variability, and between and within day variability of the analytical method.

Limits of detection (LOD) and quantitation (LOQ)
The detection limit and quantification limit for each analyte were determined based on signal-to-noise concept, as the lowest concentrations at which signal-to-noise ratio is between 3 or 2:1 and 10:1, respectively, with defined precision and accuracy under the given experimental conditions.

Robustness:
The robustness of the method describes the effect of the minor changes in the analytical parameters, such as pH value, eluent composition, temperature, flow rate, etc. on the separation.

Robustness testing was performed in the temperature range from 25 °C to 45 °C and altered flow rate of 0.8 ml/min to 1.2 ml/min.

Statistical analysis
Data collected in this study were analyzed using JMP statistical software package by one-way analysis of variance (ANOVA). Univariate linear regression analysis using least square method was applied to test the fitted model. Correlation coefficient was calculated and the results of the statistical analysis were considered significant if their corresponding p-values were less than 0.05.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

In order to achieve simultaneous elution of the two components, different chromatographic conditions were attempted. Stationary phases like C8, C18 and cyano were tested. DTA eluted in all the stationary phases, while TMS was retained with C8 and cyano columns using different mobile phase compositions of water and acetonitrile (65:35, 70:30, 85:15, 90:10 and 95:5 (v/v)). Both the components were eluted with C-18 column. To avoid merging of DTA peak (*RT* = 2.8 min) with TMS peak (*RT*= 3.8 min) and to reduce runtime, the mobile phase composition was selected as water: acetonitrile, in the ratio of 90:10 (v/v). To reduce the analysis time the gradient system was also employed but the peak area reproducibility for both the analytes were found to be very poor.

Under these optimized conditions, the analyte peaks were well resolved and free from tailing. The tailing factors were <1.5 for both the peaks. The elution order was DTA (*RT* = 2.8 min) and TMS peak (*RT*= 3.8 min), at a flow rate of 1.0 mL/min. The column temperature was maintained ambient. The chromatogram was recorded at 292 nm as the overlaid PDA spectrum of DTA and TMS showed maximum response at this wavelength. A chromatogram of tablet extract was recorded and shown in Figure 3.

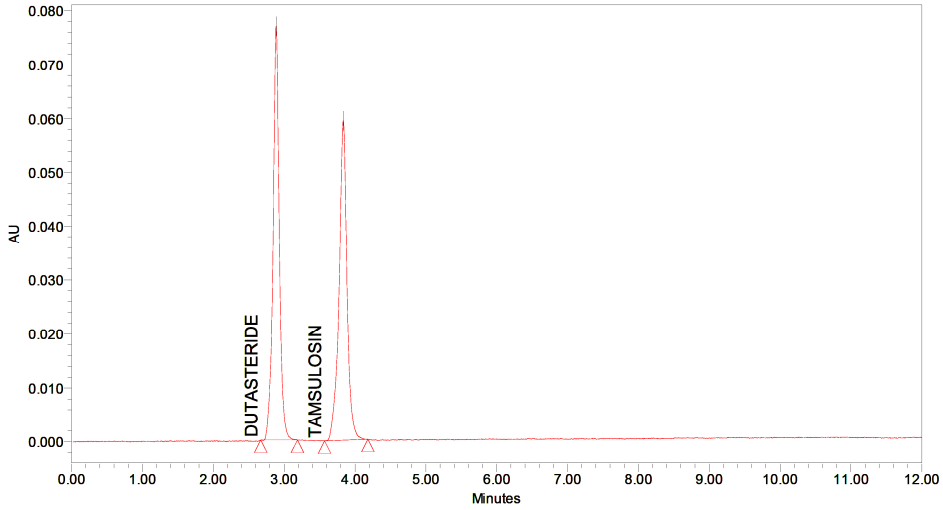

Figure 3: Chromatogram of tablet extract, showing well separated peaks of DTA (at RT 2.8) and TMS (at RT 3.8)

Method validation:

The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, LOD, LOQ and robustness. System suitability was established by injecting standard solution and results were shown in Table 1.

Table 1: System suitability parameters

S. No	Component (*n* = 6)	Peak Area	Peak symmetry	USP Tailing	Theoretical plates	USP resolution
1	Dutasteride	476123	1.55	1.00	5765	--
2	Tamsulosin	463685	1.68	0.90	6524	10

Specificity

The chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from the tablet excipients was found (figure 3). Peak purity was verified by confirming homogeneous spectral data for DTA and TMS.

Linearity

DTA and TMS showed linearity in the range of 50-150 µg/mL and 40-120 µg/mL, respectively. Linear regression equations and correlation coefficient (R2) are: y = 4775.7x - 1661.2, R^2 = 0.9993 for DTA and y = 5782.1x + 4579, R^2 = 0.9997 for TMS. The linearity curves were shown in figure 4 and 5.

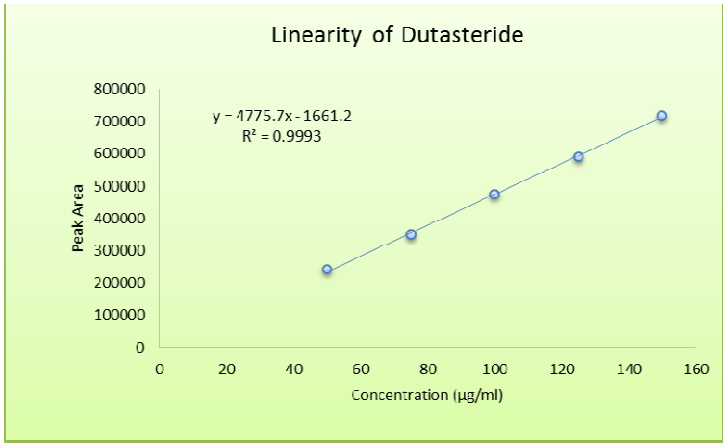

Figure 4: Linearity curve of Dutasteride

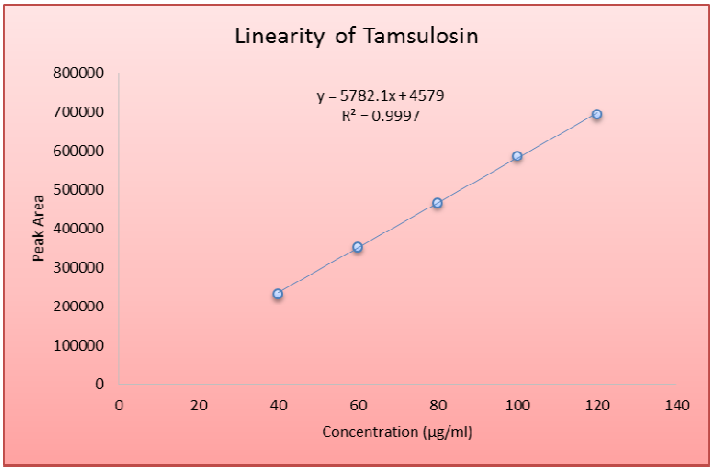

Figure 5: Linearity curve of Tamsulosin

Accuracy

The accuracy was expressed as the percentage of analytes recovered by the assay method. It was confirmed from results that the method is highly accurate (Table 2).

Table 2: Accuracy data (analyte recovery)

Analyte	Theoretical (% of target level)	Amount added (mg)	Amount recovered (mg)	Recovery (%)	Mean % Recovery
Dutasteride	50	50	50.62	101	100
	100	100	98.98	99	
	150	150	149.88	100	
Tamsulosin	50	40	39.80	99	100
	100	80	79.46	99	
	150	120	119.90	100	

Assay Precision:
The relative standard deviations (RSD) were 0.25% for DTA and 0.38% for TMS, which are well within the acceptable limit of 2.0%. The RSD's. for intermediate precision were found to be 0.53% for DTA and 0.64% for TMS. The results of Assay precision was shown in table 3.

Table 3: Assay Precision data

Sample No.	Dutasteride	% Assay - 1	Tamsulosin	% Assay - 2
1	473568	99.46	464546	100
2	476399	100.06	463691	100
3	475942	99.96	462730	100
4	475522	99.87	459573	99
5	474612	99.68	462335	100
6	473597	99.47	461228	99
Average Assay		**100**	**Average Assay**	100
STD		**0.25**	**STD**	0.38
% RSD		**0.25**	**% RSD**	0.38

Robustness
In all deliberately varied conditions, the RSD of peak areas of DTA and TMS were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be <1.5.

CONCLUSION

Proposed HPLC method is specific, accurate and precise for the simultaneous determination of DTA and TMS (Modified release type) from pharmaceutical dosage form. The described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs in combination.

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