World Journal of Pharmaceutical research

Volume 3, Issue 1, 1010-1018. Research Article ISSN 2277 – 7105

A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSIN IN TABLET DOSAGE FORMS

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Article Received on
21 October2013
Revised on 23 November 2013,
Accepted on 26 December 2013

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ABSTRACT

An accurate, precise and reproducible high performance liquid chromatographic method was developed for quantitative estimation of dutasteride and tamsulosin simultaneously in tablet dosage forms. Separation of the drugs was achieved within 10 min on a Hypersil BDS C18, (250 x 4.6 mm, 5µ) column by gradient elution using mixtures of 0.02M phosphate buffer and acetonitrile (30:70v/v) as the mobile phase at a flow rate of 1.0 mL/min. The analytes in the eluate were monitored at 210 nm. Under the optimized conditions, the retention times obtained for dutasteride and tamsulosin were 3.142 min and 7.045 min respectively.The linearity of quantification was observed in the concentration range of 2.5-15 µg/mL for dutasteride and 2-12 µg/mL for tamsulosin. The performance of the method was validated according to ICH guidelines. The method was found to be suitable for accurate determination of these drugs in tablet dosage forms without any interference from normal excipients.

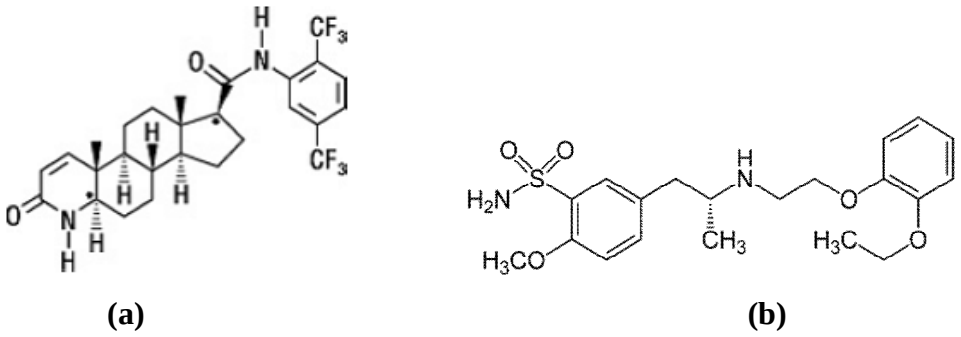
Key words: Dutasteride, Tamsulosin, Determination, HPLC, Gradient elution.

INTRODUCTION

Dutasteride, (5α, 17β) – N - {2, 5 bis (trifluoromethyl) phenyl}- 3 - oxo - 4 – azaandrost -1 – ene - 17- carboxamide, is a synthetic 4 - azasteroid compound and a 5-alpha-reductase inhibitor that inhibits the conversion of testosterone into dihydrotestosterone (DHT). It is used to treat benign prostatic hyperplasia (BPH). Dutasteride inhibits Type I and Type II isoforms of 5- alpha reductase, [1, 2]

Tamsulosin, 5- [(2R) – 2 - {[2- (2-Ethoxyphenoxy) ethyl] amino}propyl] -2- methoxy benzene sulfonamide , is an α_1-selective alpha blocker used in the symptomatic treatment of benign prostatic hyperplasia (BPH) [1-3]. Alpha blockers provide symptomatic relief within 48 hours, with maximum effect in 4–6 weeks, but do not reduce prostate size [4].

Dutasteride and tamsulosin combination is used to treat men with symptoms of an enlarged prostate gland, which is also known as benign prostatic hyperplasia (BPH). BPH is a progressive disease that affects nearly half of all men aged 50 years and older, and more than 90% of those older than 80 years. Common urinary symptoms are classified as irritative (frequency, urgency, and nocturia or obstructive (hesitancy, incomplete emptying, intermittency, and weak stream) [5].



(a) **(b)**

Figure 1.Chemical structures of Dutasteride (a) and Tamsulosin (b)

Detailed literature survey was carried out and revealed that few HPLC techniques [6-8] have been reported for the simultaneous determination of dutasteride and tamsulosin in pharmaceutical dosage forms. We have developed a new accurate and precise RP-HPLC method with short retention and run times for the determination of dutasteride and tamsulosin in tablet dosage forms. The proposed method was duly validated as per ICH guideline [9].

MATERIALS AND METHODS

Drugs, Chemicals and Solvents

The reference standard samples of dutasteride and tamsulosin were obtained from SL Drugs & Pharmaceuticals, Hyderabad, Andhra Pradesh. The commercial tablet formulation of dutasteride (0.5mg) and tamsulosin (0.4mg) 'Urimax D' manufactured by Cipla Limited, Mumbai was procured from the local market. Potassium dihydrogen orthophosphate, orthophosphoric acid, triethylamine, HPLC grade acetonitrile and methanol were purchased

from Rankem Fine Chemicals Ltd., Mumbai. HPLC grade water was prepared by using Millipore Milli-Q system.

Equipment and Chromatographic Conditions

A Waters Alliance liquid chromatograph (Model 2695) fitted with a diode array detector (Model 2996) and running on Empower2 data handling system was employed in the study. A Hypersil BDS, C_{18} column (250 x 4.6 mm, 5µ) was used for resolving the drug. All the chromatographic runs were carried out by using a mobile phase consisting of a mixture of phosphate buffer (0.02M) and acetonitrile (30:70 v/v) in isocratic mode at a flow rate of 1.0 mL/min. The injection volume of the samples was 20 µL. The detector wavelength was set at 210 nm for monitoring the drug. The chromatographic run time was set as 10.0 min.The chromatography was carried out at 30°C. Under these optimized conditions, the retention time obtained for dutasteride and tamsulosin were 3.142 min and 7.045 min respectively.

Preparation of the phosphate buffer

The phosphate buffer was prepared by dissolving 2.72 gm of potassiumdihyrogen ortho phosphate in a beaker containing 1000mL of water. 1ml of triethylamine was added and the contents sonicated . The pH of the solution was then adjusted to 2.8 with dilute orthophosphoric acid solution. It was then filtered through a 0.45µ membrane filter.

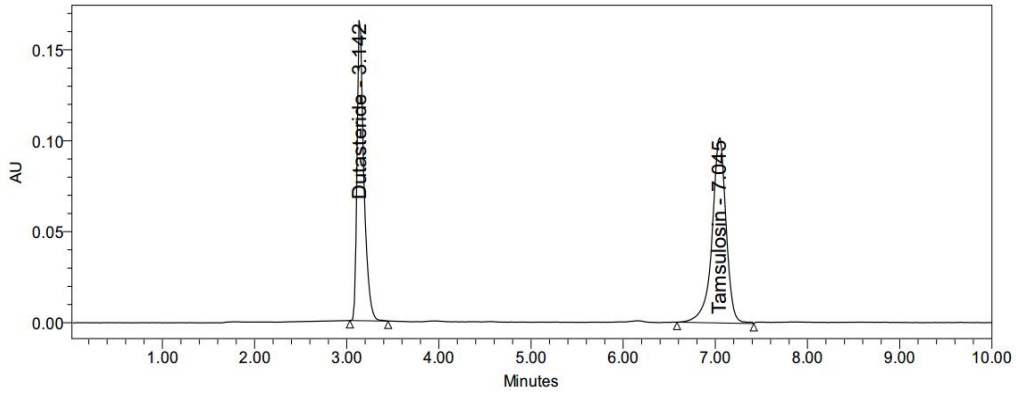


Figure 2.Representative chromatogram showing the separation of dutasteride and tamsulosin from the working standard solution.

Preparation of the mobile phase

The optimized mobile phase consisted of a mixture of the above-mentioned phosphate buffer (pH 2.8) and acetonitrile in the ratio of 30:70 v/v.

The diluent

A mixture of acetonitrile and water in the ratio of 90:10 v/v was used as the diluent for preparing the drug solutions.

Preparation of the mixed working standard solution of dutasteride and tamsulosin

4 mg of dutasteride and 5 mg of tamsulosin reference standards were separately weighed and transferred into a 100 mL volumetric flask. To this 70.0 mL of diluent was added, sonicated for 5 minutes and the volume was made up with a further quantity of diluent. This was used as the standard stock solution. The working standard solution was prepared by diluting 2.0 mL of the standard stock solution to 10 mL with diluent in a volumetric flask to get concentrations of 8 and 10 µg/mL of dutasteride and tamsulosin respectively. This was used as the mixed working standard solution.

Calibration Curve

Various dilutions of the mixed standard stock solution of dutasteride and tamsulosin were prepared at different concentration levels including the working concentration. Twenty microlitres of each concentration were injected into the HPLC system. The response was read at 210 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak area were constructed for the individual drugs.

Estimation of the drug from the tablet dosage forms

Ten tablets of 'Urimax D' [dutasteride (0.5mg) and tamsulosin (0.4mg)] were crushed and ground to a fine powder. A quantity of powder equivalent to 4 mg of dutasteride was transferred into a 100 mL volumetric flask. 80mL of the diluent was added and sonicated for 30 min. The volume was made up with the diluent and the contents were mixed well. This mixture was filtered through a 0.45µ membrane filter (discarding the first few mL of the filtrate). 2.0 mL of the filtrate was transferred into a 10 mL volumetric flask and made up to volume with the diluent. This solution was then chromatographed six times. From the chromatograms obtained, the average drug content in the formulation was calculated by using the regression equation method.

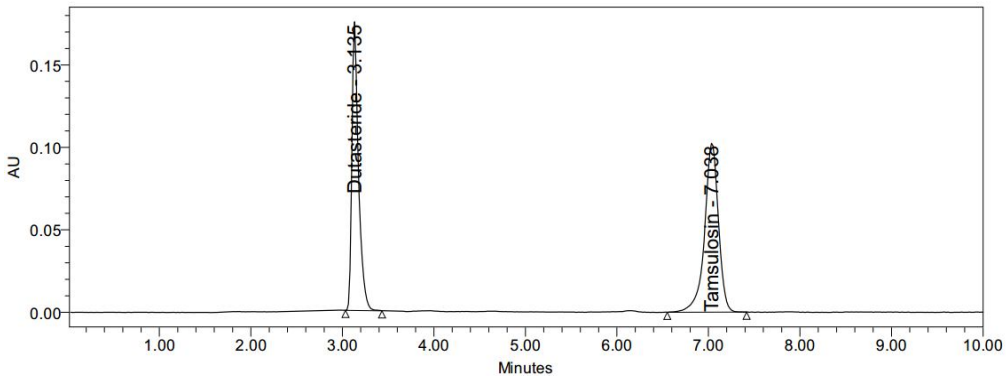


Figure 3. Representative chromatogram obtained from analysis of dutasteride and tamsulosin from formulation sample solution.

RESULTS AND DISCUSSION

During the method optimization studies, various combinations and proportions of the solvents and buffers were examined on a Hypersil BDS C_{18} (250 x 4.6 mm, 5µ) column for efficient separation of dutasteride and tamsulosin. Using a mobile phase consisting of a mixture of phosphate buffer (pH 2.8) and acetonitrile in the ratio of30:70 v/v, a good resolution and baseline separation of the drug peak was obtained. All the chromatographic conditions were optimized by evaluating the column efficiency parameters like theoretical plates and tailing factor (Table 1). Under these optimized conditions, the retention times obtained for dutasteride and tamsulosin were 3.142 min and 7.045 min respectively (Figure 2) in a run time of 10.0 min.The method was then validated as per the ICH guideline. The proposed method was also found to be applicable for the analysis of dutasteride and tamsulosin in their tablet formulations.

Table 1. Optimized chromatographic conditions

Stationary Phase	: Hypersil BDS C18 (250 x 4.6 mm, 5µ)
Mobile Phase	: Phosphate buffer : Acetonitrile =30:70 v/v
Flow Rate	: 1.0 mL/min
Column Temperature	: 30°C
Injection Volume	: 20 µL
Detection Wavelength	: 210 nm
Run time	: 10.0 min

Specificity

The chromatogram shown in Figure 2 reveals good baseline separation of dutasteride and tamsulosin from working standard solution. A good analytical method should be able to measure the drug accurately in the presence of probable interferences from the excipients of its formulation. Figure 3 thus demonstrates that no interfering peaks arising due to the excipients of its tablet were observed at the retention times of dutasteride and tamsulosin.

Linearity

The regressions of the plots were computed by least square regression method and were shown in the Figures 4 and 5. The calibration curves (n=3) constructed for each drug were linear over the concentration range of 2.5-15 µg/mL for dutasteride and 2-12µg/mL for tamsulosin. The correlation coefficients were greater than 0.999 and the %RSD for each concentration studied was less than 2%.

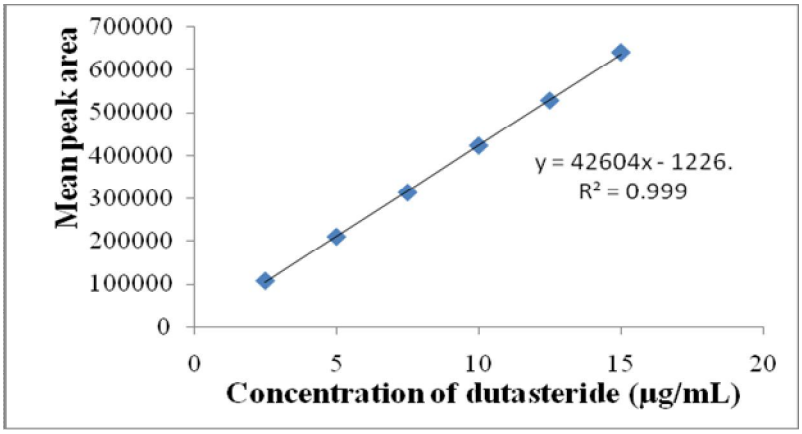


Figure 4. Linearity plot for dutasteride

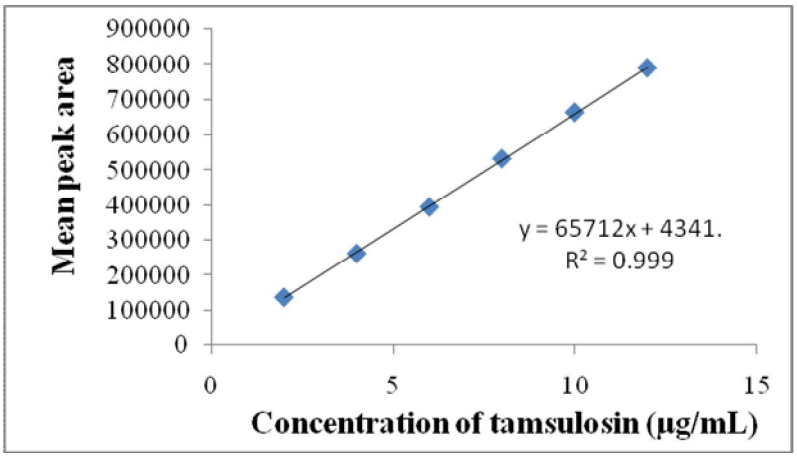


Figure 5. Linearity plot for tamsulosin

Accuracy and Precision

The accuracy of the method was determined by recovery experiments from standard addition method. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in Table 2. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and represented in Table 3 and Table 4. From the data obtained the developed RP-HPLC method was found to be precise.

Table 2. Accuracy data of the proposed method

Analyte	Amount of the analyteadded (µg/mL)	Amount of the standardadded (µg/mL)	Amount recovered (µg/mL)	Mean recovery (µg/mL)	% Mean recovery
Dutasteride	5	10	15.00	5.00	100.09
	10	10	20.03	10.03	100.32
	15	10	24.97	14.97	99.81
Tamsulosin	4	8	12.02	4.02	100.62
	8	8	16.08	8.08	101.04
	12	8	20.02	12.02	100.19

Table 3. Precision data for dutasteride

S.No.	Intra-day precision	Inter-day precision
1	398030	397954
2	393913	395154
3	397159	398077
4	397984	396452
5	392432	391456
6	391755	395461
Average	395212	395212.2
SD	2856.20	2855.96
%RSD	0.70	0.72

Table 4. Precision data for tamsulosin

S.No.	Intra-day precision	Inter-day precision
1	497116	501978
2	497396	502185
3	497387	499045
4	504980	502454
5	502331	502884

6	502152	502785
Average	500227	501888.5
SD	3361.00	1434.99
%RSD	0.70	0.29

System suitability parameters

System suitability parameters were studied with six replicate injections of the standard solution and the results are presented in Table 5.

Table 5. System suitability parameters of the proposed method

Parameter	Dutasteride	Tamsulosin
Retention time (min)	3.142	7.045
Tailing factor	1.1	1.12
Theoretical plates	7548	6594
HETP	0.0331	0.0379

Method suitability

The commercial tablet formulation, 'Urimax D' [dutasteride (0.5mg) and tamsulosin (0.4mg)] was analyzed by the proposed method. The recovery obtained (100.1%) by the proposed method was found to be in good agreement with the labeled amount of the drug, which confirms the suitability of the method for the analysis of dutasteride and tamsulosin in tablet dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of the College of Pharmaceutical Sciences, Andhra University, for providing laboratory facilities for carrying out this study.

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