Research Article

Development and validation of a Reversed Phase HPLC method for simultaneous determination of Tamsulosin and Dutasteride in tablet dosage form

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Abstract

Objective: A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Tamsulosin and Dutasteride in tablets. **Materials and methods**: Chromosil C_{18} column (250 mm x 4.6 mm, 5µ) mixture of Methanol, Acetonitrile and 2% O-phosphoric acid in the ratio of 60:20:20 v/v/v as a mobile phase; at a flow rate of 1.0 mL/min. UV detection was performed at 234 nm. **Results:** The retention times were 2.19 and 5.19 min for Tamsulosin and Dutasteride respectively. Calibration plots were linear (r^2=0.999) over the concentration range of 16-96 µg/mL for Tamsulosin and 20-120 µg/mL for Dutasteride. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. **Conclusion:** The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Tamsulosin and Dutasteride in bulk and tablet dosage form.

Keywords: Tamsulosin, Dutasteride, RP-HPLC, tablets

Introduction

Tamsulosin is a selective α_1 receptor antagonist that has preferential selectivity for the α_{1A} receptor in the prostate versus the α_{1B} receptor in the blood vessels. When alpha 1 receptors in the bladder neck and the prostate are blocked, this causes a relaxation in smooth muscle and therefore less resistance to urinary flow. Due to this the pain associated with BPH can be reduced (Figure 1). Chemically it is (*R*)-5-(2-{[2-(2-ethoxyphenoxy) ethyl] amino} propyl)-2 methoxybenzene-1-sulfonamide. Dutasteride belongs to a class of drugs called 5-alpha-reductase inhibitors, which block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone (DHT). Dutasteride inhibits both isoforms of 5-alpha reductase, type I and type II. Chemically it is (5α, 17β)-*N*-{2, 5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide (Figure 2) (Pande et al., 2009).

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Literature survey reveals that few spectrophotometric methods and chromatographic methods were reported for the estimation of Dutasteride & Tamsulosin HCl in single & combination with other drugs (Choudhari et al., 2012; Patel et al., 2011; Sujana et al., 2012). Therefore an attempt has been made to develop and validate simple, sensitive, precise & accurate RP-HPLC method for simultaneous estimation of Dutasteride & Tamsulosin HCl in combined tablet dosage form.

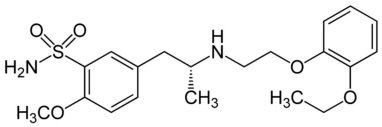

Figure 1. Molecular structure of Tamsulosin

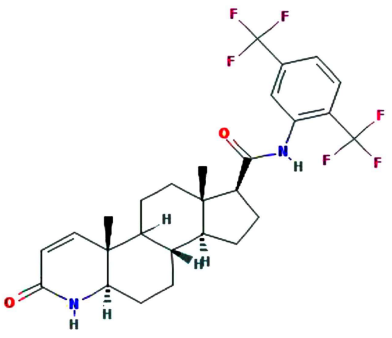

Figure 2. Molecular structure of Dutasteride

Materials and methods

Chemicals and reagents

Pharmaceutical grade of Tamsulosin and Dutasteride pure drugs were kindly supplied as gift samples by NATCO Pharma Ltd., Hyderabad., India, certified to contain > 99% (w/w) on dried basis. Commercially available Urifine-D tablets purchased from local market. Tablets claimed to contain 0.4 mg of Tamsulosin: 0.5 mg of Dutasteride have been utilized in the present work. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

Chromatographic conditions

Separation was performed with Chromosil C18, 250X4.6mm, 5µ UV detector, operated at 234 nm. Spinchrome software was applied for data collecting and processing. The separation was achieved on Chromosil C18, 250X4.6mm, 5µ analytical column. The mobile phase consisted of mixture of Methanol, Acetonitrile and 2% O-phosphoric acid in the ratio of 60:20:20 v/v/v. The flow rate was 1.0 mL/min and UV detection was performed at 234 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. the resulting transparent mobile phase was filtered through a 0.45 µ membrane filter (Millipore, Ireland). The injection volume was 20 µL and all the experiments were performed at ambient temperature.

Preparation of standard solution

Accurately weighed quantity of 40 mg Tamsulosin and 50 mg Dutasteride was transferred to a 100 mL volumetric flask, containing 50 mL of mobile phase, sonicated for 15 min and the volume was made up to 100 mL with mobile phase. From this stock solution pipette out 1ml, 2 mL, 3mL, 4 mL, 5 mL, and 6 mL into 25 mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45 µ filter.

Preparation of sample preparation

Accurately weighed 20 tablets, average weight is taken and powdered. Amount equivalent to 40 mg Tamsulosin and 50 mg of Dutasteride was accurately weighed and taken in a 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered through a 0.45µ filter and solution was made up to mark with mobile phase. From the above solution 2 mL is taken and further diluted in 25 mL volumetric flasks with mobile phase.

Method validation

The developed method was validated according to ICH guidelines (ICH 2005). The system suitability was evaluated by six replicate analysis of Tamsulosin and Dutasteride mixture at concentrations of 50 µg/mL and 100 µg/mL. The acceptance criteria are %RSD of peak areas not more than 2%, theoretical plates numbers (N) at least 3000 per each peak and tailing factors not more than 2.0 for Tamsulosin and Dutasteride.

Linearity

Standard calibration curves were plotted against the concentration ranging from 16-96 µg/mL for TAM and 20-120 µg/mL for DUTA. Different linearity levels was prepared and injected into the HPLC system keeping the injection volume constant.

Recovery

To study the reliability and suitability of developed method, recovery experiments were carried out at three levels 50%, 100% and 150%. Known concentration of commercial tablet was spiked with known amount of TAM and DUTA. At each level of amount six determinations were performed with expected results. The %RSD of individual measurements was also determined.

Precision

Precision of assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for three consecutive days. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD.

Specificity

All chromatograms were examined to determine whether compound of interest coeluted with each other or with any additional excipient peaks. Marketed formulation was analysed to determine the specificity of the optimized method in presence of common tablet excipients.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Robustness

To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate, buffer composition and pH of mobile phase.

Results and discussion

During the optimization of HPLC method, two columns symmetry C-18 and C-8 analytical column (4.6×250 mm; 5 µm) and (4.6×150 mm; 5 µm), two organic solvents (acetonitrile and methanol), two buffers (acetate and phosphate) at two different pH values (3 and 5) were tested.

Initially methanol:acetate buffer, acetonitrile:acetate buffer, methanol:phosphate buffer, acetonitrile:phosphate buffer were tried in different ratios at pH 3-5. TAM and DUTA eluted with tried mobile phases. With acetonitrile:phosphate buffer two drugs eluted and run was 20 min, in order to decrease the run time, symmetry C-18 analytical column (4.6×150 mm; 5 µm) was selected, the mobile phase conditions were optimized so the peak area from the first eluting compound did not interfere with those from the solvent and excipients. Finally mobile phase consisting of mixture of Methanol, Acetonitrile and 2% O-phosphoric acid in the ratio of 60:20:20 v/v/v was selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal to noise ratio with reasonable separation time using a C-18 analytical column (4.6×150 mm; 5 µm), the retention times for TAM and DUTA were observed to be 2.19 and 5.19 min respectively. Total run time was less than 10 min. The chromatogram at 234 nm showed a complete resolution at all peaks (Figure 3). Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability tests. All critical parameters tested meet the acceptance criteria on all days. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks.

Table 1. System suitability parameters of proposed method

S. No.	Parameters	Tamsulosin	Dutasteride
1	Linearity	16-96µg/ml	20-120 µg/ml
2	Theoretical plates	10515	75004
3	Asymmetric factor	1.28	1.06
5	LOD	2.83	3.29 µg/mL
6	LOQ	8.59	9.97 µg/mL

Table 2. Accuracy data for proposed method[a] ([a]n = 6)

Spiked level of drug (%)	Amount of drug added (µg/band)		%Mean recovery (n=6)		%RSD	
	TAM	DUTA	TAM	DUTA	TAM	DUTA
50	16	20	99.39	100.69	0.15	0.52
100	32	40	100.121	100.35	0.196	0.25
150	48	60	99.25	100.233	0.18	0.36

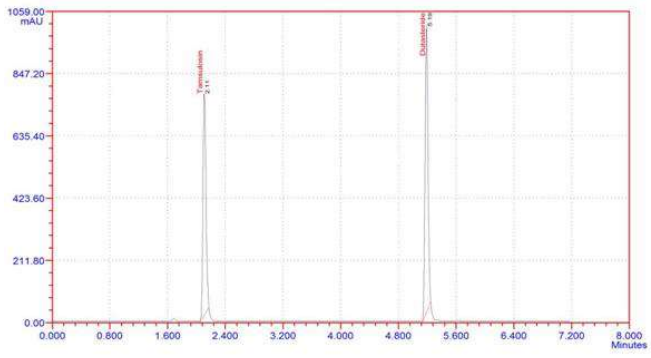

Figure 3. Typical chromatogram of standard for TAM and DUTA

Linearity was obtained for TAM and DUTA in the range of 16-96 µg/mL and 20-120 µg/mL. The correlation coefficient (r^2) was found to be greater than 0.999 in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for TAM and DUTA in tablet dosage form. Results obtained from recovery studies presented in Table 2. Indicate that this assay procedure can be used for routine quality control analysis of binary mixture in tablets. Precision of the analytical method was found to be reliable based on %RSD (<2%) corresponding to peak areas and retention times. As can be seen in Table 3 the %RSD values were less than 2 for intra-day and inter-day precision. Hence, the method was found to be precise for these two drugs.

Table 3. Precision data of proposed method[a]

S. No.	Method Precision		System Precision	
	Area of Tamsulosin	Area of Dutasteride	Area of Tamsulosin	Area of Dutasteride
1.	246121.6	293733	217916.8	260504.5
2.	246668.6	296183.9	219301.9	260838.3
3.	244427.5	290646.3	215898.0	260068.3
4.	242796.7	296494.6	219654.7	265149.3
5.	246460.7	295906.2	219969.6	261070.1
6	241309.8	294075.5	219718.1	260337.3
Mean	244630.8	294506.6	218743.2	261238
SD	2198.087	2209.551	1573.24	1905.525
%RSD	0.89	0.750	0.71	0.72

Table 4. Robustness for Mobile Phase variation of TAM and DUTA

Mobile phase	Peak area of Tamsulosin	Peak area of Dutasteride
MeoH/ACN/OPA 60/18/22	287586	234944
MeoH/ACN/OPA 60/22/18	292277	236558

Table 5. Analysis of marketed formulations by proposed method

Brand name		Label claim (mg)	Amount Found (mg)*	% Label claim
Urifine-D	TAM	0.4	0.39	98
	DUTA	0.5	0.5	100

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and percentage purity calculated was found to be within limits. LOD and LOQ were found to be 2.83µg/mL and 08.59 µg/mL for TAM, 3.29 µg/mL and 9.97 µg/mL for DUTA. In all deliberately varied conditions, the %RSD for replicate injections of DUTA were found to be within the acceptable limit. The tailing factors for two peaks were found to be less than 1.5 and the results are shown in table 4. The validate method was used in analysis of marketed tablet dosage form Urifine-D with a label claim 0.4 mg of TAM and 0.5 mg of DUTA tablet. The results for the drugs assay showed good agreement with label claims and the results are shown in table 5.

Conclusion

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of TAM and DUTA in tablet dosage form. The developed method provides good resolution between TAM and DUTA. It was successfully validated in terms of system suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as or such in combinations.

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