Development and Validation of A Stability Indicating Liquid Chromatographic Method for Simultaneous Estimation of *Dutasteride* and *Tamsulosin* in Combined Dosage Form

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ABSTRACT

A novel stability indicating isocratic, reversed phase-liquid chromatographic method has been developed and validated for simultaneous quantitative determination of Dutasteride (DTS) and Tamsulosin (TAM) in combined pharmaceutical dosage form. An ACE C18 (250*4.6*5µ) column with mobile phase containing pH 5.2 (Potassiumdihydrogenphosphate) Buffer: Methanol in the ratio of 600:400 (v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored by using a photodiode array detector at 247 nm. The retention times of Dutasteride and Tamsulosin were found to be 2.419 min and 5.119 min, respectively. Correlation co-efficient for Dutasteride and Tamsulosin were found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Dutasteride and Tamsulosin in formulations was found to be in a range of 97-103% and 97-103% respectively and this confirms the non-interferences of the excipients in the formulation. Dutasteride and Tamsulosin were exposed to stress conditions like acidic hydrolysis, basic hydrolysis, oxidative, photolytic, humidity and thermal conditions. Due to its simplicity, rapidness and high precision, this method was successfully applied to the estimation of Dutasteride and Tamsulosin in combined dosage form.

Key words: Dutasteride, Tamsulosin, Liquid chromatography, Method validation.

INTRODUCTION

Dutasteride (DTS) is chemically (5α,17β)-N-{2,5-bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide with an empirical formula $C_{27}H_{30}F_6N_2O_2$ and molecular weight of 528.5 g/mol. It is a selective inhibitor of both type 1 and type 2 isoforms of 5α-reductase enzyme that converts testosterone to 5α-dihydrotestosterone which is

responsible for enlargement of prostate. Dutasteride is used in treatment of benign prostatic hyperplasia, frequently occurring in men over the age of 50 years. Tamsulosin hydrochloride (TAM) is chemically 5-[(2R)-2-[2-(2-ethoxy phenoxy)ethylamino]propyl]-2-methoxy benzenesulfonamide with an empirical formula $C_{20}H_{28}N_2O_5S$. It is a selective antagonist of alpha-1A and alpha-1B adrenoreceptors at the prostate. It works by blockingÙ–receptor that is found

in the muscle of the prostate gland, which causes the muscle in the prostate to relax. Tamsulosin is given by mouth as hydrochloride salt. Dutasteride and Tamsulosin hydrochloride Capsules (JALYN) contains the following: One dutasteride oblong, opaque, dull-yellow soft gelatin capsule, containing 0.5 mg of dutasteride dissolved in a mixture of butylated hydroxytoluene and mono-di-glycerides of caprylic/caproic acid. The inactive ingredients in the soft-gelatin capsule shell are ferric oxide (yellow), gelatin, glycerin, and titanium dioxide. Tamsulosin hydrochloride white to off-white pellets, containing 0.4 mg tamsulosin hydrochloride and the inactive ingredients: methacrylic acid copolymer dispersion, microcrystalline cellulose, talc, and triethyl citrate.

EXPERIMENTAL

Instrumentation

The separation was carried out on Waters 2695 alliance HPLC system with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and an ACE C18 (250*4.6*5µ) column.

Chemicals and Reagents

Dutasteride and Tamsulosin was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai Potassium dihydrogen phosphate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions

The mobile phase consisting of water (pH 5.2 adjusted with Potassium dihydrogen phosphate) and methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 600:400 v/v was pumped into the column at a flow rate of 1.0 ml/min. The column temperature was 30°C. The detection was monitored at 247nm and the run time was 8 minutes. The volume of injection loop was 10 µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

Preparation of standard solution
Dutasteride

Accurately weighed quantity, 5.0 mg of Dutasteride was transferred into 25 ml of volumetric

flask and adds 10 ml of mobile phase and sonicate for 15 minutes make up the volume with mobile phase. 5 ml of above solution was into 10 ml volumetric flask and diluted to the mark with mobile phase.

Tamsulosin

Accurately weighed quantity, 4.0 mg of Tamsulosin was transferred into 25 ml of volumetric flask and adds 10 ml of mobile phase and sonicate for 15 minutes make up the volume with mobile phase. 5 ml of above solution was transferred into 10 ml volumetric flask and diluted to the mark with mobile phase.

Preparation of sample (drugs from marketed formulations) solution

Accurately weighed quantities, 2874 mg of sample powder was transferred into 25 ml of volumetric flask and added 10 ml of mobile phase and sonicate for 30 minutes and make up the volume with mobile phase, filtered through the 0.45 µm filter paper. 5 ml of above solution was transferred into 10 ml volumetric flask and make up the volume with mobile phase.

Statistics

Results are presented as the mean±SD and results were analyzed using Excel® 10, WinStat®v2003.1 and Chromeleon®. A p value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

The analytical procedure for the estimation of Dutasteride and Tamsulosin in marketed formulation was optimized with a view to develop a precise and accurate assay method. Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase containing pH 5.2 (Potassium dihydrogen phosphate) Buffer: Methanol in the ratio of 600:400 (v/v) gave a better resolution. Using UV-visible PDA detector at 247nm carried out the detection. Amongst the several flow rates tested, the flow rate of 1 ml/min was the best for all the drugs with respect to location and resolution of peaks. The retention time of Dutasteride and Tamsulosin was found to be 2.419 min and 5.119 min respectively. The chromatograms of standard and sample solution of Dutasteride and Tamsulosin were shown in figure

01 and 02 .The asymmetry factor of Dutasteride and Tamsulosin was 1.026 and 1.273 found to be respectively, which indicates symmetrical nature of the peak

Method validation
System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 01). The values obtained demonstrated the

Table 1: System Suitability Parameters

Parameter	Dutasteride	Tamsulosin
Correlation Coefficient	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	0.0317	0.038
LOQ	0.1055	0.127
Theoretical plates	9478	6276
Tailing	1.026	1.273

Table 2: Precision Studies

S. No	Sample Wt	Area(DTS)	Area(TAM)	% Assay(DTS)	% Assay(TAM)
1	2874.0	6618179	5574128	97	99
2	2874.0	6619102	5574598	97	99
3	2874.0	6616779	5579191	97	99
4	2874.0	6615049	5579645	97	99
5	2874.0	6614357	5577874	97	99
6	2874.0	6619153	5576521	97	99

Table 3: Accuracy for Dutasteride

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	Mean
50%	1437.00	3300071	50.000	48.41	97	97
50%	1437.00	3303227	50.000	48.45	97	
50%	1437.00	3304769	50.000	48.48	97	
50%	1437.00	3303682	50.000	48.46	97	
50%	1437.00	3303614	50.000	48.46	97	
50%	1437.00	3305620	50.000	48.49	97	
100%	2874.00	6615564	100.000	97.04	97	97
100%	2874.00	6617871	100.000	97.08	97	
100%	2874.00	6614497	100.000	97.03	97	
150%	4311.00	9929697	150.000	145.66	97	97
150%	4311.00	9922247	150.000	145.55	97	
150%	4311.00	9924747	150.000	145.58	97	
150%	4311.00	9921280	150.000	145.53	97	
150%	4311.00	9929772	150.000	145.66	97	
150%	4311.00	9929813	150.000	145.66	97	

Table 4: Accuracy for Tamsulosin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	Mean
50%	1437.00	2781589	39.600	39.44	100	100
50%	1437.00	2787723	39.600	39.53	100	
50%	1437.00	2785789	39.600	39.50	100	
50%	1437.00	2789625	39.600	39.56	100	
50%	1437.00	2788722	39.600	39.54	100	
50%	1437.00	2787905	39.600	39.53	100	
100%	2874.00	5579623	79.200	79.12	100	100
100%	2874.00	5572967	79.200	79.03	100	
100%	2874.00	5572842	79.200	79.02	100	
150%	4311.00	8364829	118.800	118.61	100	100
150%	4311.00	8369515	118.800	118.68	100	
150%	4311.00	8369962	118.800	118.69	100	
150%	4311.00	8368102	118.800	118.66	100	
150%	4311.00	8366113	118.800	118.63	100	
150%	4311.00	8365010	118.800	118.62	100	

Table 5: LOD and LOQ FOR Dutasteride and Tamsulosin

S. No	Sample Name	Inj.	Name	Rt	Area	USP Tailing	USP Plate Count
1	LOD	1	DUT	5.175	418524	1.104	9394
2	LOQ	1	DUT	5.169	979109	1.162	8556
1	LOD	1	TAM	2.416	213319	1.338	4642

Table 6: Robustness for Dutasteride

S. No	Sample Name	Inj.	Rt	Area	USP Tailing	USP Plate count	S/N
1	TEMP-1	1	3.711	6612970	1.009	9833	528.949
2	TEMP-2	1	3.658	6525595	0.984	1026	628.305
3	FLOW-1	1	4.626	8170846	1.003	10883	639.708
4	FLOW-2	1	3.109	5525199	0.969	8962	689.078

Table 7: Robustness for Tamsulosin

S. No	Sample Name	Inj.	Rt	Area	USP Tailing	USP Plate count	S/N
1	TEMP-1	1	2.337	5621057	1.259	6317	573.514
2	TEMP-2	1	2.331	5561350	1.231	6419	659.453
3	FLOW-1	1	2.912	6940733	1.226	6731	668.252
4	FLOW-2	1	1.954	4701336	1.145	5801	754.142

suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table 03&04). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. Chromatograms of three different levels are shown in

Fig 3, 4 & 5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-02).

Linearity and Range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616 x (R2=0.99) for Tamsulosin and y = 19288 x (R2=0.99) for Dutasteride. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Dutasteride and Tamsulosin shown in Fig 6 and the results for calibration curves are given in Figure 07 & 08.

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Dutasteride and Tamsulosin. Relative standard deviation (ó) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels and the results are tabulated in Table 05.
(a) LOQ = 10 σ / S
(b) LOD = 3.3 σ / S

Table 8: Degradation studies for Tamsulosin

| Stress condition	Sample weight	Area	% Assay	% Deg.
Acid	2874	4626589	82	17
Base	2874	4626296	82	17
Peroxide	2874	4626201	82	17
Heat	2874	4628511	82	17
Light	2874	4624255	82	17

Table 9: Degradation studies for Dutasteride

Stress condition	Sample weight	Area	% Assay	% Deg.
Acid	2874	5516555	81	19
Base	2874	5517208	81	19
Peroxide	2874	5519402	81	19
Heat	2874	5510088	81	19
Light	2874	5513641	81	19
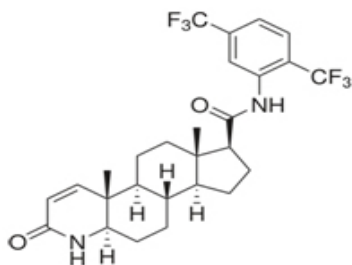
Structure of Dutasteride
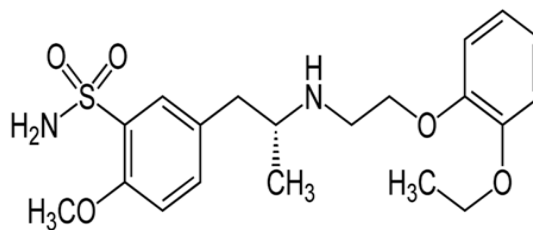
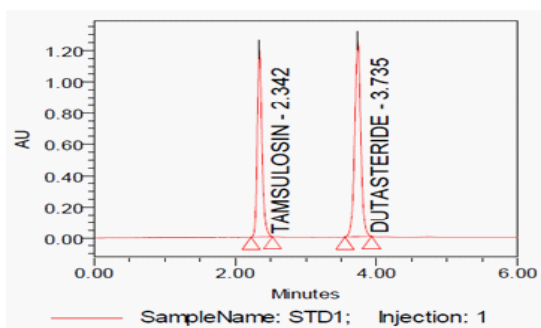**Structure of Tamsulosin**

Fig. 1: Standard chromatogram for Dutasteride and Tamsulosin
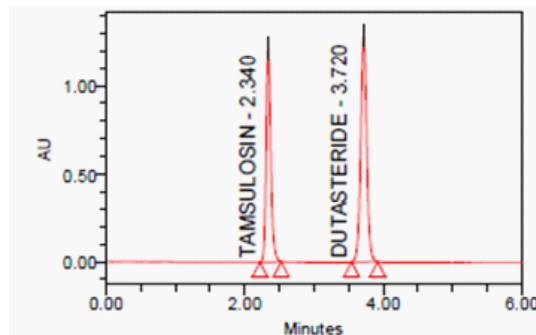

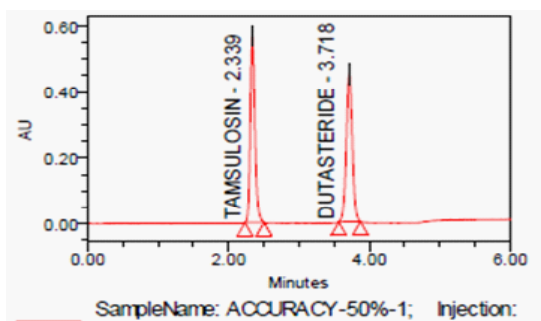**Fig. 2: Formulation chromatogram for Dutasteride and Tamsulosin**

Fig. 3: Accuracy Chromatograms-50% of Dutasteride and Tamsulosin
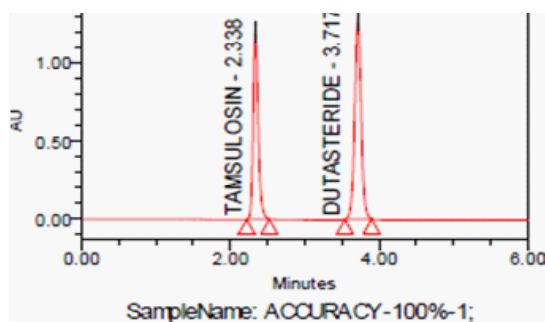

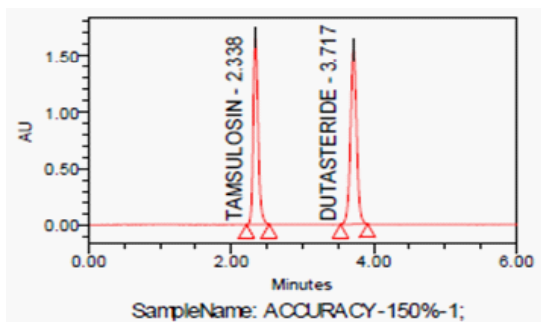**Fig. 4: Accuracy Chromatograms-100% of Dutasteride and Tamsulosin**

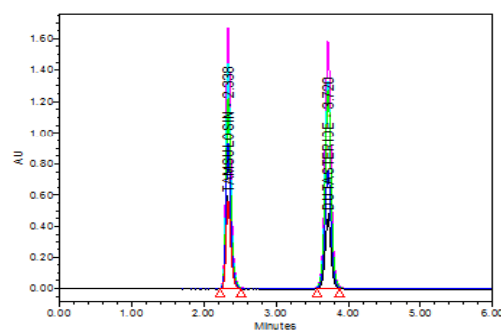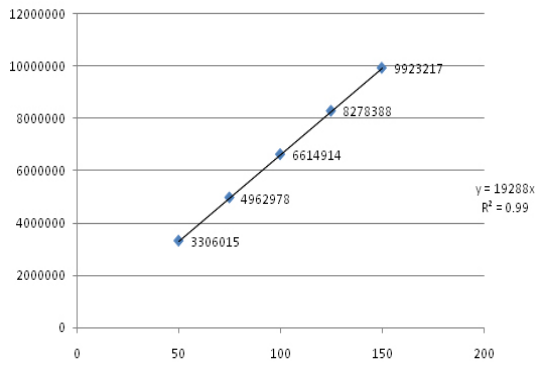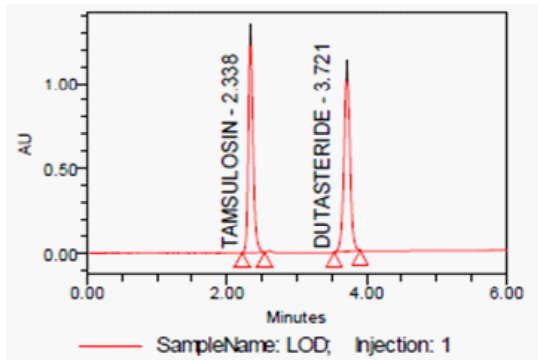**Fig. 5: Accuracy Chromatograms-150% of Dutasteride and Tamsulosin**

Fig. 6: Overlay chromatograms of Linearity for Dutasteride and Tamsulosin

Fig. 7: Linearity Curve for Dutasteride
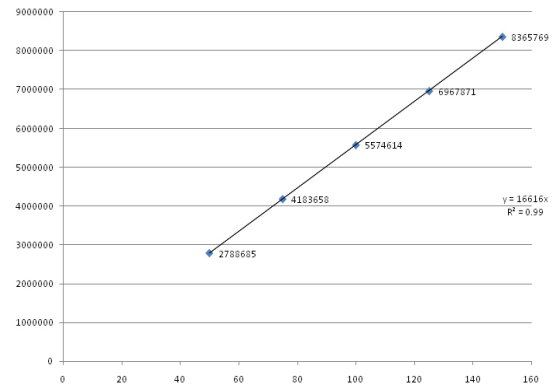
Fig. 8: Linearity Curve for Tamsulosin

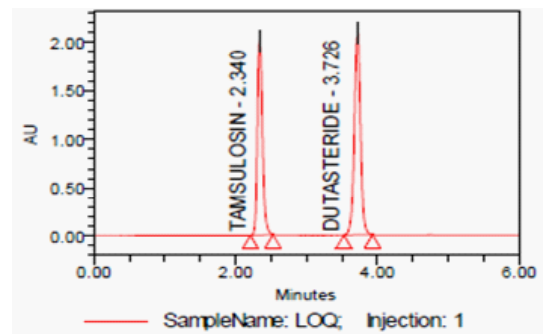
Fig. 9: LOD chromatograms for Dutasteride and Tamsulosin
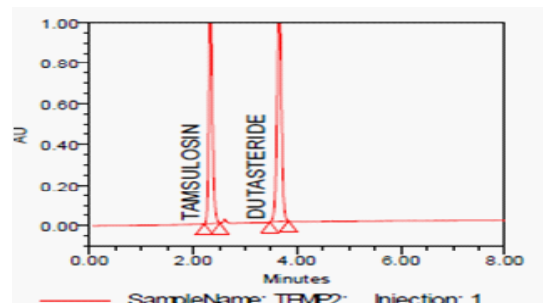

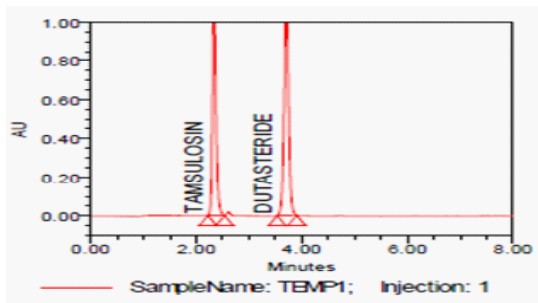
Fig. 10: LOQ chromatograms for Dutasteride and Tamsulosin
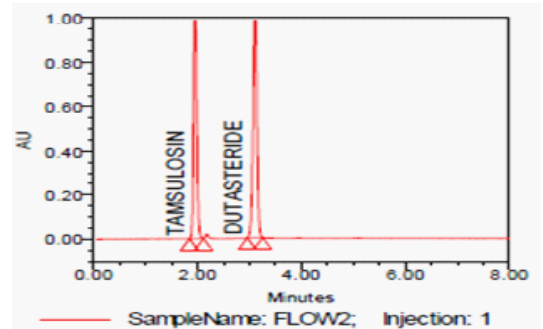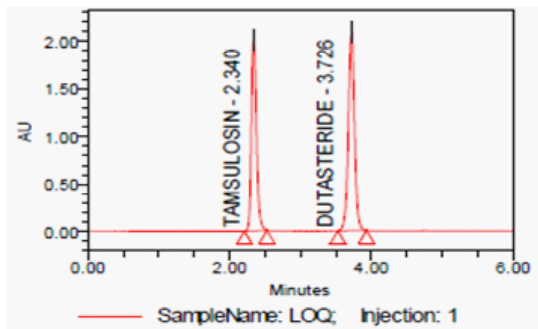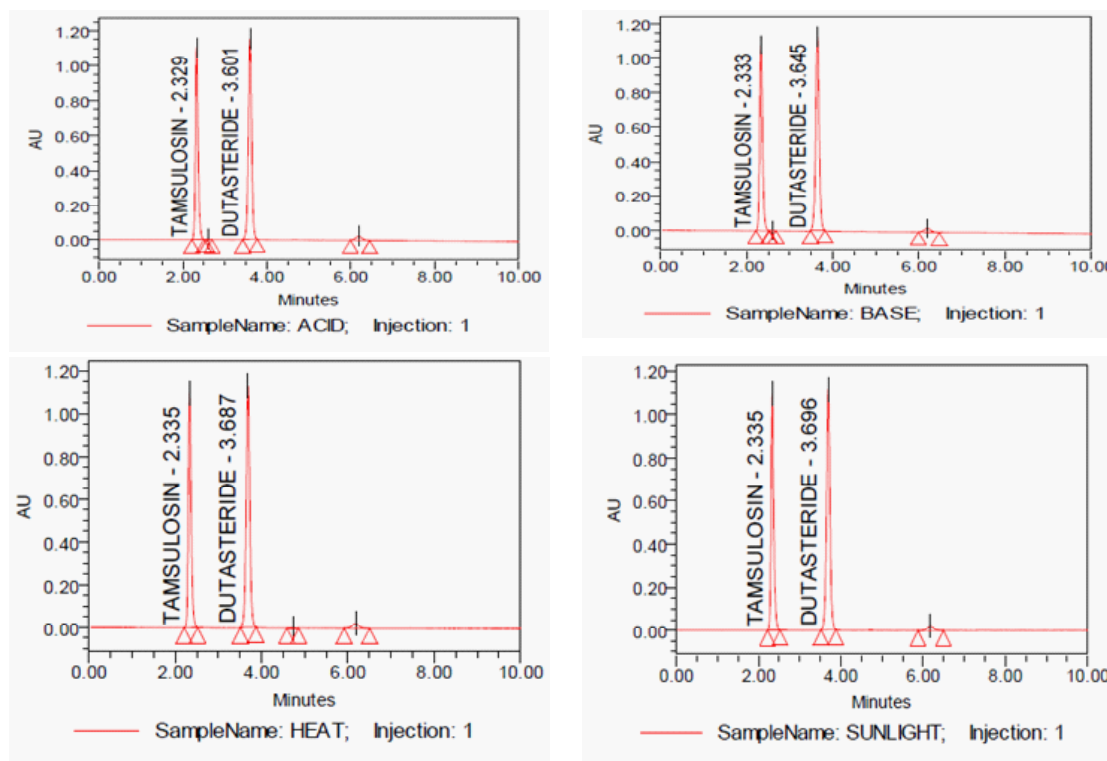

Fig. 11: Robustness chromatograms for Dutasteride and Tamsulosin

Fig. 12: chromatograms of forced degradation studies on Dutasteride and Tamsulosin

Where

σ = residual standard deviation of response

S = slope of the calibration curve.

Robustness

    Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust (Table 07 & 08).

Forced degradation studies

    The stability studies were determined by applying the physical stress (acid, base, peroxide, heat and light) to the product. It was observed that there were marked degradation in the chromatograms, and the data given in table 09 & 10).

    System suitability results were given in table-1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1,

so we can say system is suitable for analysis and method specificity was concluded by fig:1 and fig:2 Dutasteride and Tamsulosin standard chromatogram and other one is formulation they were not observed in placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 03 says that the method precision passed for both Dutasteride and Tamsulosin studies. The method accuracy was evaluated by recovery studies. Dutasteride and Tamsulosin recovery was found to be 97% and 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate as shown in table 3 & 4. Linearity calibration curve was given in fig: 7 & 8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be Y= 16616 for Tamsulosin and y = 19288 for Dutasteride Method robustness results were given by table 06&07, LOQ and LOD Results were given by table 05. Degradation studies are given in table 08 & 09.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Dutasteride and Tamsulosin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Dutasteride and Tamsulosin in pure and its pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

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