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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSIN IN TABLET DOSAGE FORM

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ABSTRACT

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Dutasteride and Tamsulosin in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Acetonitrile 65:35 were set (Buffer P^H 4.25 adjusted with Triethylamine), Kromasil ODS 3V (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 225 nm. As the methanol content was increased Dutasteride and Tamsulosin got eluted with good peak symmetric properties. The retention times for Dutasteride and Tamsulosin was found to be 3.118 min and 6.640 min, respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R^2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Dutasteride and Tamsulosin.

KEY WORDS

Dutasteride and Tamsulosin, RP-HPLC

INTRODUCTION:

Dutasteride is 5-alpha-reductase inhibitors. Chemically Dutasteride, (5α, 17β)-N-{2, 5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide.

DUTASTERIDE block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone. DHT is mainly responsible for enlargement of prostate gland[(1)].

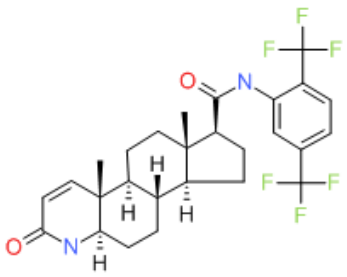

Figure 1: Structure of Dutasteride

Tamsulosin is Antineoplastic Agents, Adrenergic alpha-Antagonists. Tamsulosin, chemically it is found (5α, 17β)-N- {2,bis(triflouromethyl) phenyl}-3-oxo-4-azandrost-1-ene-17 – carboxamide. Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men[2].

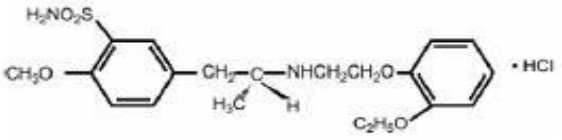

Figure 2: Structure of Tamsulosin[2]

There are very few methods reported in the literature for analysis of Dutasteride and Tamsulosin alone or in combination with other drugs in the pure form and pharmaceuticals formulations by UV-Spectrophotometer, HPLC, HPTLC, LC-MS. In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Dutasteride and Tamsulosin in Tablet dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Dutasteride and Tamsulosin. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Dutasteride and Tamsulosin in Tablet dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Dutasteride and Tamsulosin in Tablet dosage form.

MATERIALS & METHODS

Materials:

Analytically pure samples of Dutasteride & Tamsulosin were procured as gift samples from Dr. Reddy's Laboratories, (Hyderabad, India). VELTAM PLUS Tablet containing, DUTASTERIDE-0.5mg, TAMSULOSIN-0.4mg tablets manufactured by INTAS pharmaceuticals were procured from a local pharmacy. The solvents for the experiment were selected based on the solubility test results of both the drugs. The solubility tests were performed using the common solvents like water, methanol (Sd Fine), Acetonitrile (Merck). The analytical reagents Buffer and Acetonitrile taken in the ratio 65:35 were used to prepare the mobile phase which is filtered through a nylon 0.45µm membrane filter paper.

ASSAY METHOD DEVELOPMENT:

Different mobile phase composition and different chromatographic conditions were used in the trials in order to get well resolved peaks with good peak shapes and higher theoretical plates which pass the USP acceptance criteria of peak parameters were obtained finally.

CHROMATOGRAPHIC CONDITIONS:

Method was developed using a Shimadzu UFLC-20AD chromatographic system (Japan), equipped with isocratic pump, and with SPD-M20A diode array detector attached with data recorder and integrator LC-10 solution software. HPLC with PDA detector (Waters); HPLC column using is Kromasil ODS 3V (250×4.6mm, 5µ) ODS C-18 RP-column; Mobile phase filtration unit was Ultipor Nylon membrane (Pall Life sciences, Mumbai, India).

METHOD VALIDATION: The developed RP-HPLC method for simultaneous estimation of Dutasteride & Tamsulosin formulation was validated as per ICH guidelines.

ASSAY OF FORMULATION:

Preparation of Standard solution:

Accurately Weighed and transferred 0.4mg of Tamsulosin and 0.5mg of Dutasteride working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents(Stock Solution). From stock solution Dutasteride 2.5 ml and Tamsulosin 2.5ml was taken in 10 ml volumetric flask and volume was made up to the mark with diluent (Standard Solution). In this Dutasteride contains 12.5(µg/ml) and Tamsulosin 10(µg/ml).

Sample Preparation:

10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 10 tablets was transferred into a 10 mL volumetric flask, 3mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 2.5ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent. In this sample preparation containing 25 µg/ml.

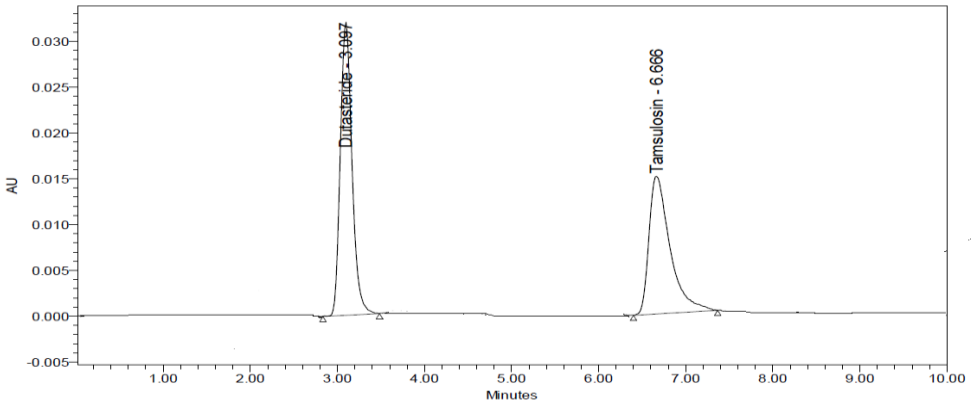

Figure 3: Chromatogram of Standard

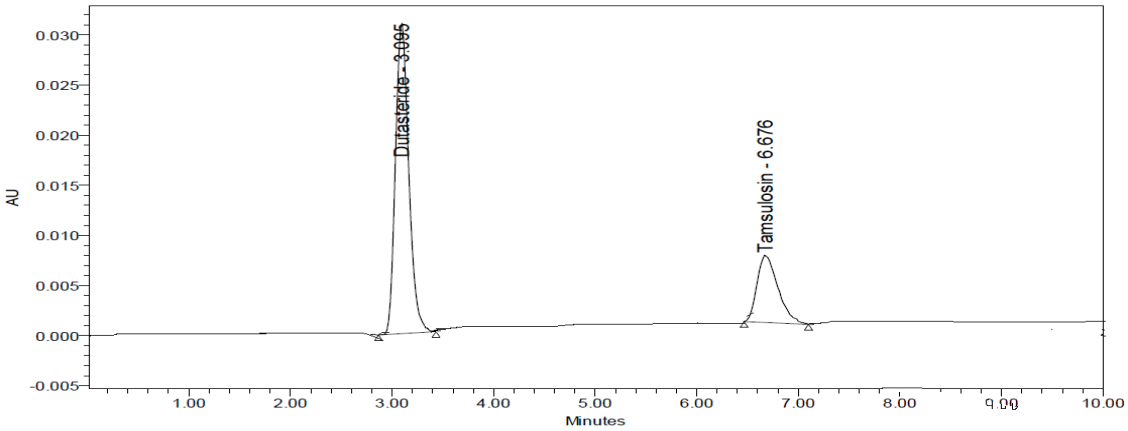

Figure 4- Chromatogram of Test

Table 1: Peak results of Standard & Test Chromatograms for Assay

Parameter	Standard		Test	
	Dutasteride	Tamsulosin	Dutasteride	Tamsulosin
Retention time	3.097	6.666	3.095	6.676
Peak Area	345253	154382	356194	155799
USP Plate Count	2633	4837	2521	4645
Tailing Factor	1.20	1.41	1.21	1.41

Table 2: Results of Assay

Parameters	Dutasteride	Tamsulosin
Standard peak area	345253	154382
Test peak area (mean)	356194	155799
Average Weight	5mg	5mg
Label claim	0.5mg	0.4mg
% Purity of Standard	99.50	99.58
Amt obtained	0.48mg	0.36mg
% Assay	101.5%	100.75%

The % assays of Dutasteride and Tamsulosin were found to be 101.5% and 100.75% respectively. Thus, % Assay results were found to be within the limits i.e., 98-102% for both the drugs. Hence the developed method can be routinely used for the simultaneous estimation of Dutasteride and Tamsulosin in the marketed formulations.

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters. After development of RP-HPLC method for estimation of Dutasteride and Tamsulosin, validation of the method was carried out according to ICH guidelines

The developed method was validated for the following parameters.

A. System suitability
B. Linearity
C. Specificity
D. Precision
E. Accuracy
F. LOD & LOQ
G. Robustness

SYSTEM SUITABILITY TEST (SST)

A Standard solution of Dutasteride and Tamsulosin working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections.

LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Serial dilutions of Dutasteride and Tamsulosin (5-15µg/ml) were injected into the column and detected at a wavelength set at 225nm. The calibration curve was obtained by plotting the concentration vs. peak area. Concentration range 5-15µg/ml was found to be linear with r^2=0.9986.

Table 3: Preparation of Working standard solutions for Linearity

Working standard solutions (Level in %)	Stock solution taken in (ml)	Stock Solution taken in (ml)	Diluted to volume (ml) with diluent	Concentration of Dutasteride (µg/ml)	Concentration of Tamsulosin (µg/ml)
50%	1.25	1.25	10	5	5
75%	1.875	1.875	10	7.5	7.5
100%	2.5	2.5	10	10	10
125%	3.125	3.125	10	12.5	12.5
150%	3.75	3.75	10	15	15

SPECIFICITY:

ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

PRECISION:

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported.

The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported.

METHOD PRECISION:

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions.

The % RSD of peak areas of six samples was calculated. The method precision was performed on Dutasteride and Tamsulosin formulation. The % RSD of the assay value for six determinations should not be more than 2.0%.

SYSTEM PRECISION:

System precision was established to ensure that the optimized analytical method is precise. System precision was performed by injecting six replicate injections of standard solution at 100% concentration and the chromatograms were reviewed for the %RSD of peak areas. % RSD of the assay value for six determinations should not be more than 2.0%.

ACCURACY:

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) *Standerd addition method:* To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
b) *Percentage method:* For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Dutasteride and Tamsulosin at each level should be not less than 95.0% and not more than 105.0%.

The Sensitivity of measurement of Dutasteride and Tamsulosin by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations:

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where, σ is the standard deviation of intercept of calibration plot and S is the average of the slope of the corresponding calibration plot. The LOD and LOQ values for Dutasteride and Tamsulosin were reported in the Table.

Table 4: LOD and LOQ Data of Dutasteride and Tamsulosin

Dutasteride			Tamsulosin		
Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis	Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis
5	154331	S = 91445 c = 70793	5	80933	S = 16099 c =725.8
10	345391	LOD: 2.55µg/ml LOQ: 7.44µg/ml	10	162428	LOD:0.148 µg/ml LOQ: 0.45µg/ml

ROBUSTNESS:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, deliberate change in the Flow rate was made to evaluate the impact on the method.

Effect of variation in flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard and Test solutions of 100% concentration was prepared & injected into the HPLC system by keeping flow rates 0.9 ml/min& 1.1 ml/min. The effect of variation of flow rate was evaluated.

Effect of variation in mobile phase composition: A study was conducted to determine the effect of variation in mobile phase ratio by changing the ratio of organic solvent i.e., Buffer: Acetonitrile by ±2ml. Standard & test solutions of 100% concentration were prepared and injected into the HPLC system and the chromatograms were recorded. The retention times, tailing factors & %RSD values were calculated.

RESULTS AND DISCUSSION

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The mobile phase containing mixture of orthophosphoric acid buffer solution: Acetonitrile (65:35v/v, pH 2.45) with a flow rate of 1.0 ml/min is quite robust.

The optimum wavelength for detection was 225 nm at which better detector response for both the drugs was obtained. The retention times for Dutasteride and Tamsulosin was found to be 3.118 ± 0.005 min and 6.640 ± 0.009 min, respectively. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 5 to 15 µg/ ml and 5 to 15 µg/ml, with regression 0.9979 and 0.9999, Dutasteride and Tamsulosin respectively. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found above 99.3 % for both the drugs.

Robustness of the proposed method was determined by varying various parameters, the %RSD reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

SYSTEM SUITABILITY:

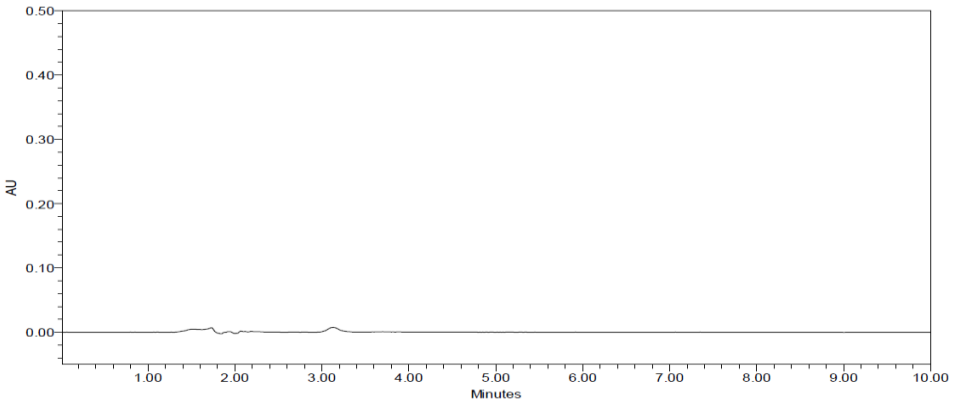

Figure 5: Chromatogram of Blank

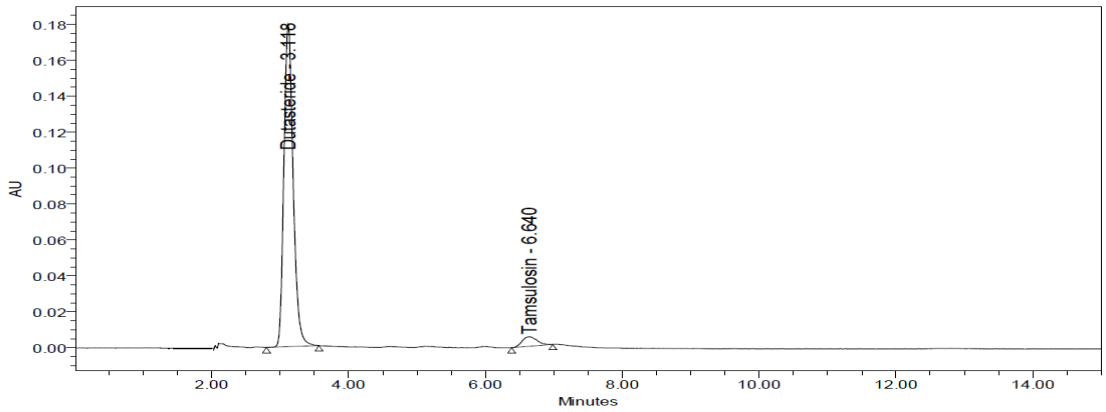

Figure 6: Chromatogram of system suitability

Table 5: Results of System suitability Test for TAMSULOSIN

Injection	Retention time (t_R)	Peak Area	Plate count	Tailingfactor
1	6.666	154507	4891	1.37
2	6.668	154080	4334	1.88
3	6.675	154111	4668	1.41
4	6.709	154801	4253	2.20
5	6.712	154108	4837	1.30
6	6.714	154682	4320	1.99
Mean	-	154382	-	-
SD	-	322.7	-	-
% RSD	-	0.2	-	-

Table 6: Results of System suitability Test for DUTASTERIDE

Injection	Retention time (t_R)	Peak Area	Plate count	Tailing Factor
1	3.100	348066	2607	1.22
2	3.110	346100	2553	1.21
3	3.110	349660	2565	1.19
4	3.112	349065	2577	1.22
5	3.114	346976	2633	1.20
6	3.116	331653	2642	1.21
Mean	-	345253	-	-
SD	-	1462.0	-	-
% RSD	-	0.4	-	-

LINEARITY GRAPH

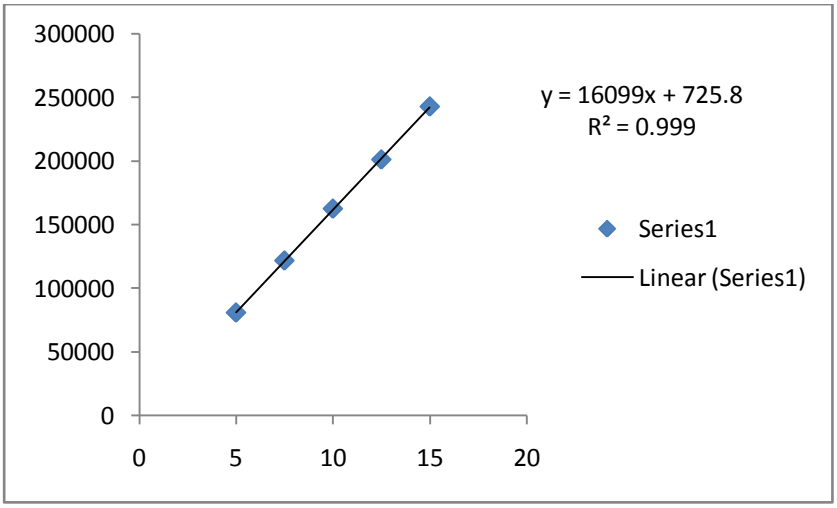

Figure 7: Linearity Graph of TAMSULOSIN

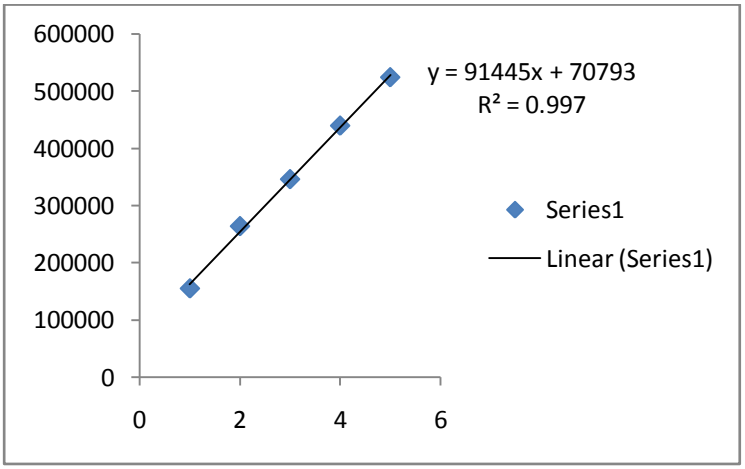

Figure 8: Linearity Graph of DUTASTERIDE

SPECIFICITY

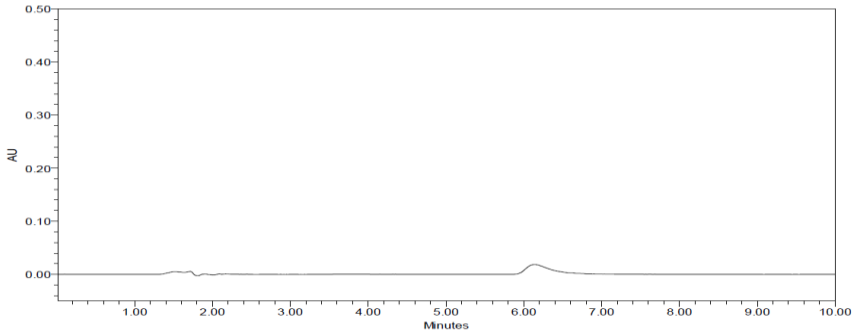

Figure 9:Chromatogram of Blank

METHOD PRECISION

Table 7: Method Precision data for Dutasteride & Tamsulosin

S.No.	Concentration (µg/ml)	Dutasteride		Tamsulosin	
		Retention time(Rt)	PeakArea	Retention time(Rt)	Peak Area
1	10	3.093	343058	6.655	153942
2	10	3.094	342802	6.661	153336
3	10	3.095	343849	6.672	153546
4	10	3.099	343338	6.675	154916
5	10	3.100	344509	6.675	154880
6	10	3.107	347256	6.675	153281
Avg			344135		153984
SD			679.7		745.5
%RSD			0.2		0.4

Table 8: Intermediate Precision data for Dutasteride and Tamsulosin

S.No.	Concentration (µg/ml)	Intermediate Precision			
		Day 1 Dutasteride		Day 1 Tamsulosin	
		Retention time	Peak Area	Retention time	Peak Area
1	10	3.063	343552	6.636	176310
2	10	3.066	338959	6.641	176473
3	10	3.066	341762	6.643	174424
4	10	3.067	339609	6.645	173949
5	10	3.076	334927	6.650	173866
6	10	3.078	338481	6.657	173284
Avg			339548		174718
SD			2959.0		1347.1
%RSD			0.9		0.7

Table 9: Intermediate Precision data for Dutasteride and Tamsulosin

S.No.	Concentration (µg/ml)	Intermediate Precision			
		Day 2 Dutasteride		Day 2 Tamsulosin	
		Retention time	Peak Area	Retention time	Peak Area
1	10	3.043	343342	6.631	176348
2	10	3.052	335959	6.655	176424
3	10	3.061	345712	6.644	174424
4	10	3.066	339562	6.651	173985
5	10	3.071	334986	6.654	173745
6	10	3.076	336794	6.660	173755
Avg			337647		174568
SD			2946.0		1341.6
%RSD			0.9		0.8

Table 10: System Precision data for Dutasteride & Tamsulosin

S.No.	Dutasteride		Tamsulosin	
	Retention time(Rt)	Area	Retention time(Rt)	Area
1	3.062	391568	6.627	174672
2	3.066	387880	6.629	175847
3	3.068	392046	6.652	174040
4	3.069	391263	6.653	178138
5	3.069	386287	6.654	174511
6	3.070	385296	6.659	17588
Avg		389057		175633
SD		294301		1546.2
%RSD		0.8		0.8

ACCURACY

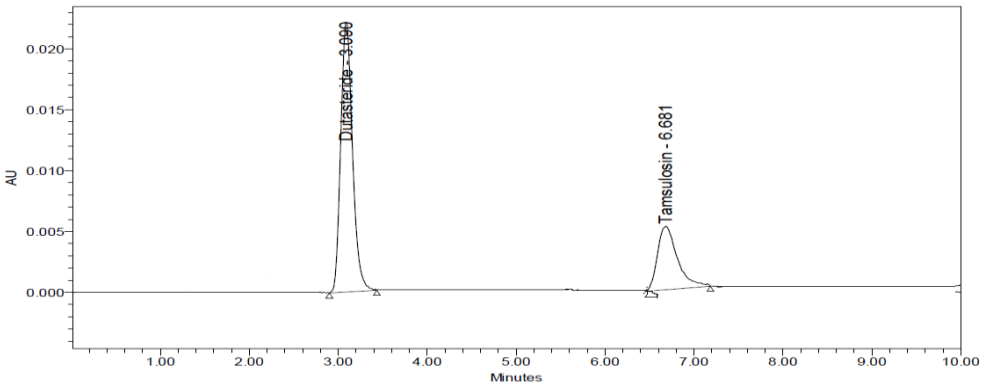

Figure 10: Chromatogram of Accuracy 50%

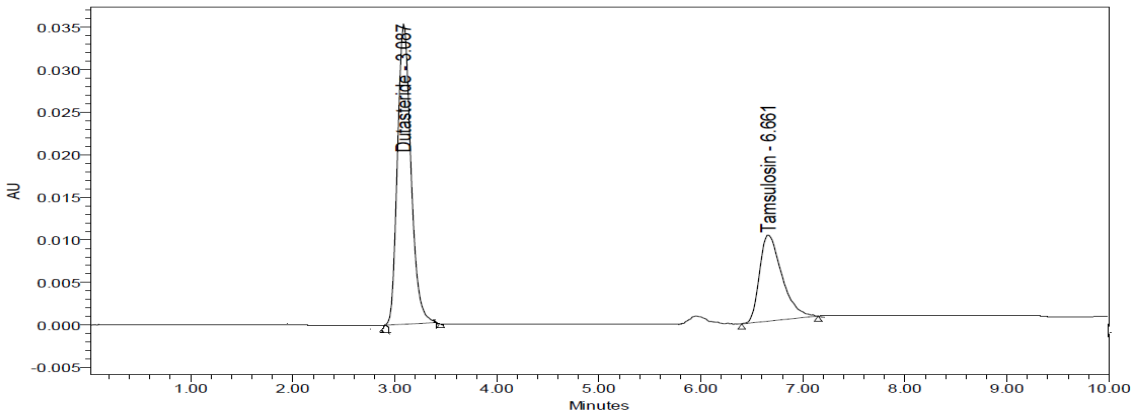

Figure 11: Chromatogram of Accuracy 100%

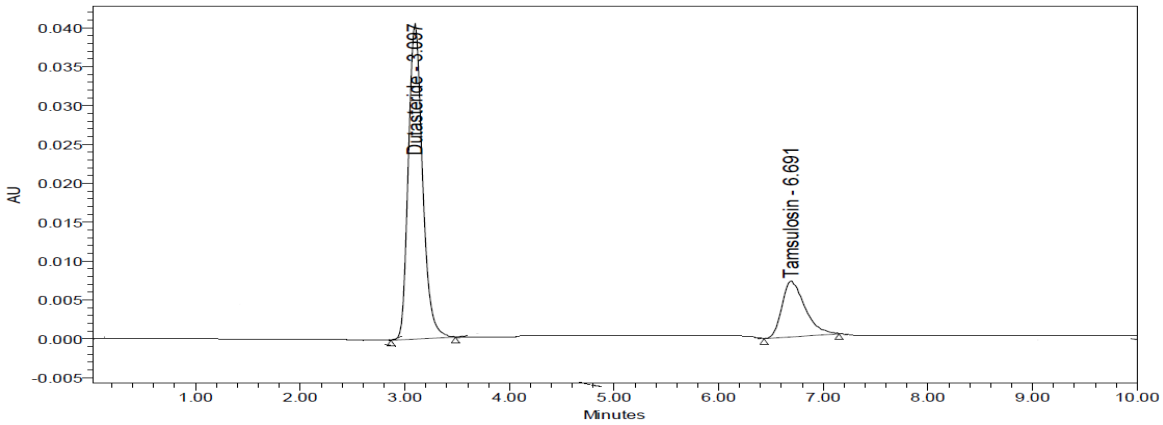

Figure 12:Chromatogram of Accuracy 150%

Table 11: Accuracy Study of Dutasteride

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	4.86	99.4		
50%	5	4.87	99.99	99.6	
50%	5	4.82	99.54		%RSD= 0.293
100%	10	9.68	100.6		
100%	10	9.72	99.25	100.2	
100%	10	9.8	100.75		%RSD=0.84
150%	15	14.7	101.806		
150%	15	14.81	101.313	100.5	
150%	15	14.89	99.605		%RSD=1.14

Table 12: Accuracy Study of Tamsulosin

Conc (µg/ml)	Concn Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
5	4.6	99.308		
5	4.7	99.621		
5	4.5	99.321	99.41	0.18
10	9.3	100.29		
10	9.4	99.460		
10	9.6	99.324	99.69	0.562
15	14.2	100.998	100.52	0.530
15	14.6	100.691		
15	14.9	99.960		

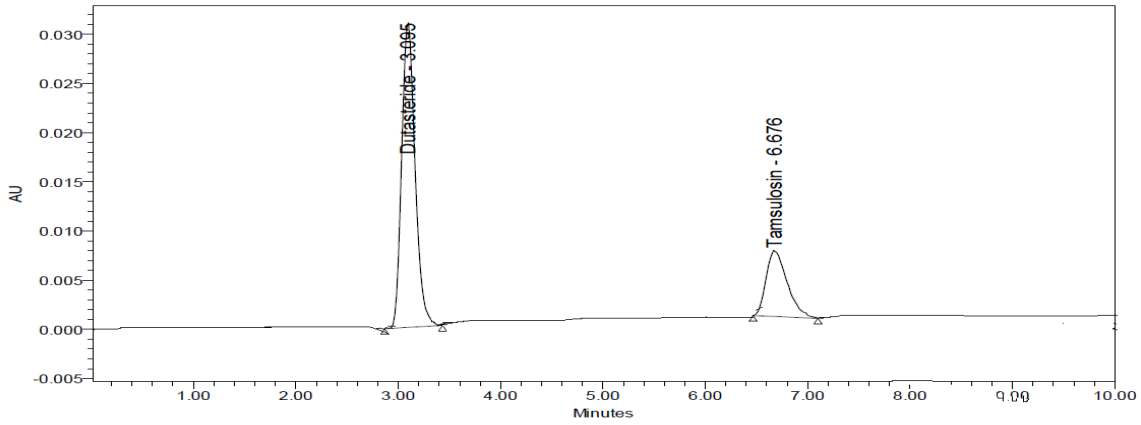

Figure 13: Representative Chromatogram at Flow rate of 0.9 ml/min

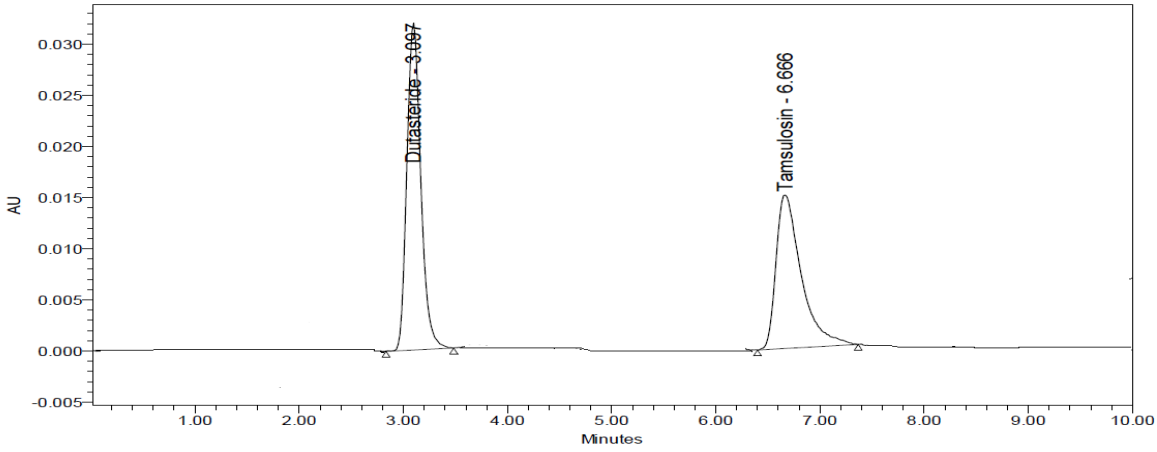

Figure 14: Representative Chromatogram at Flow rate of 1.1 ml/min

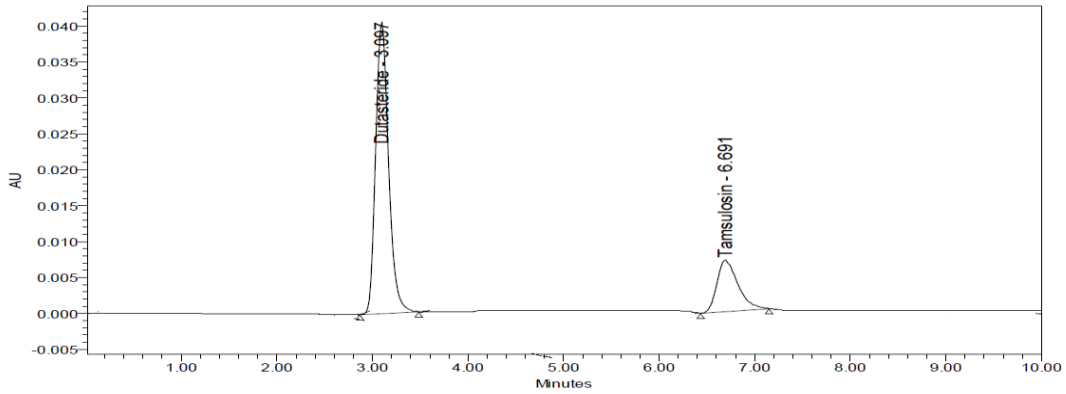

Figure 15: Representative Chromatogram for Mobile phase composition (Buffer: Acetonitrile::67:33)

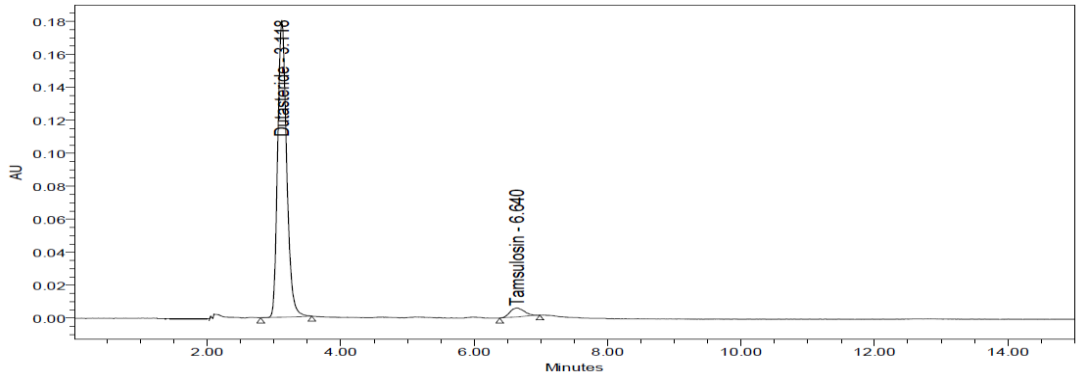

Figure 16: Representative Chromatogram for Mobile phase composition (Buffer: Acetonitrile: 63:37)

Table 13: Robustness data for Dutasteride

Std. Replicate	Variation in flow rate		Variation in Mobile phase composition	
	Flow Rate 0.9ml/min	Flow Rate 1.1ml/min	Buffer: Acetonitrile (67:33)	Buffer: Acetonitrile (63:37)
1	442083	331355	362871	339895
2	443968	334083	369307	338125
Mean	443026	332719	366089	339010
SD	1332.9	1929.0	4551.1	1251.8
%RSD	0.3	0.6	1.2	0.4
Retention time	3.428	3.451	3.691	3.001
Tailing factor	1.17	1.21	1.21	1.21
Theoretical plates	2476	2594	2645	2624

Table 14: Robustness data for Tamsulosin

Parameter	Variation in flow rate		Variation in Mobile phase composition	
Standard	Flow Rate 0.9ml/min	Flow Rate 1.1ml/min	Buffer: Acetonitrile (67:33)	Buffer: Acetonitile (63:37)
1	134400	117576	155130	161083
2	133624	116287	156599	158101
Mean	134012	116932	155865	159592
SD	548.2	911.5	1038.7	2108.4
%RSD	0.4	0.8	0.7	1.3
Retention time	7.437	7.478	6.349	6.397
Tailing factor	1.22	1.19	1.38	1.46
Theoretical plates	4804	4962	4505	4499

CONCLUSION

A simple, specific, precise, accurate, rapid and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Dutasteride and Tamsulosin in tablet dosage form. The method was successfully validated in terms of linearity, precision, accuracy & robustness, LOD, LOQ as per ICH guidelines.

System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R^2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present.

Full length method was not performed; if it is done this method can be used for routine analysis of Dutasteride and Tamsulosin.

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