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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS DETERMINATION OF DUTASTERIDE AND TAMSULOSIN IN BULK AS WELL AS IN PHARMACEUTICAL DOSAGE FORM BY USING PDA DETECTOR

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ABSTRACT

Objective: The present work was undertaken with the aim to develop and validate a rapid and consistent stability indicating RP-HPLC in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the dosage form, bulk drug as well as for routine analysis in Quality control.

Method: Reversed-phase high-performance liquid chromatography (RP-HPLC) methods was developed and validated for simultaneous estimation of Tamsulosin hydrochloride and Dutasteride in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) under an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (20%) whose pH was adjusted to 2.5 by using Orthophosporic Acid & Acetonitrile (80%) [HPLC Grade]. The flow rate was monitored at 0.8 ml per min. The wavelength was selected for the detection was 274 nm.

Result: The run time was 7min. The retention time found for the drugs Dutasteride & Tamsulosin were 2.003 min. & 5.067 min. respectively. The linearity was established in the range of 25 to 125µg/ml. The proposed method was adequate sensitive, reproducible, and specific for the determination of Dutasteride and Tamsulosin hydrochloride in bulk as well as in Pharmaceutical dosage form. The validation of method was carried out utilizing ICH-guidelines.

Conclusion: The described RP-HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. The drug was exposed to Thermal, Hydrolytic and Oxidative stress conditions and the stressed samples were analyzed by the proposed method. The peak homogeneity data for the drugs Dutasteride and Tamsulosin hydrochloride were obtained by using Photodiode Array Detector in the stressed sample chromatograms which demonstrated the specificity of the method for the estimation in the presence of degradants. Overall the proposed method was found to be suitable and Accurate for the Quantitative determination and stability study of the drug in Pharmaceutical dosage form.. The method was effectively separated the drug from its degradation product and it was employed as a stability-indicating one. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Dutasteride and Tamsulosin hydrochloride in bulk drug and in combined dosage forms.

Keywords: Tamsulosin, Dutasteride, ICH Guideline, RP-HPLC, LOD, LOQ

INTRODUCTION

Dutasteride, selective inhibitor of both, type 1 and type 2 isoforms of 5α-reductase (5-AR) enzyme that converts testosterone to 5α-dihydrotestosterone (DHT) which is responsible for enlargement of prostate, is used in treatment of benign prostatic hyperplasia, frequently occurring in men over the age of 50 years [1]. Chemically, DTS is (5α,17β)-N-{2,5 bis (trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide with an empirical formula $C_{27}H_{30}F_6N_2O_2$, representing a molecular weight of 528.5 g/mol[2]. Literature survey revealed LC-MS and HPLC methods for estimation of DTS in human plasma and pharmaceutical dosage forms [3–5]. A LC-MS-MS method is reported for the simultaneous determination of Tamsulosin and Dutasteride in human plasma [6]. The intracellular enzyme that converts Testosterone to Dutasteride is used for the treatment of patients with symptomatic benign prostatic hyperplasia. Literature survey reveals that several analytical and Bioanalytical methods was reported for the analysis of Dutasteride. For Dutasteride, the methods reported were alone or in combination with other drugs. These include, HPLC [7-9] and HPTLC [10] methods in bulk and pharmaceutical dosage form, stability indicating LC methods [11- 12], LC-MS [13- 14] methods, spectrophotometric analysis of Dutasteride in tablets [15] were reported.

Tamsulosin hydrochloride [TAM] is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. Tamsulosin acts by relaxing the muscle around prostrate there by allowing the free flow of urine and decreases the symptoms leading to disease [17]. Tamsulosin hydrochloride is extensively metabolized by cytochrome P450 enzymes in the liver; however, the pharmacokinetic profile of the metabolites in humans was not established. Chemically it is 5-[(2R)-2-[2-(2-ethoxyphenoxy) ethylamino] propyl]-2 methoxy benzene sulfonamide [18]. This drug is not official in any pharmacopoeia; hence no official method was available for the estimation of this drug in the pharmaceutical formulations. Literature survey revealed several Bioanalytical methods for its estimation which include Reversed Phase-High Performance Liquid Chromatography [RP-HPLC] with fluorescence detection, HPLC - electrospray tandem mass spectrometry, LC-MS, liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry and RP-HPLC method[19-20]. The developed method was unique advantage over the above mentioned methods, as it is simple, economical, faster, precise, accurate and specific for quantitative determination of Tamsulosin hydrochloride in pharmaceutical dosage form.

Till date, there was no single RP-HPLC method was developed for the simultaneous estimation of Tamsulosin and Dutasteride in bulk drug as well as in pharmaceutical dosage forms. The present work deals with development and validation of stability indicating RP-HPLC method for the quantitative analysis of Tamsulosin and

Dutasteride and its stress degradation products. The aim of the present work was to develop an economic, accurate, specific, reproducible, and stability-indicating RP-HPLC method using PDA detection for the determination of Tamsulosin and Dutasteride in the presence of its degradation products, either in bulk form or in tablets. The chemical structures of the drugs were represented in fig. no. 1 & 2.

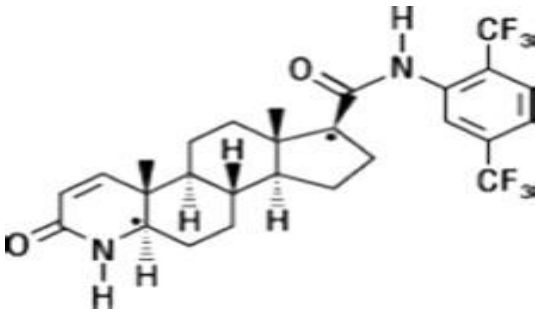

Fig. 1: Chemical structure of Dutasteride.

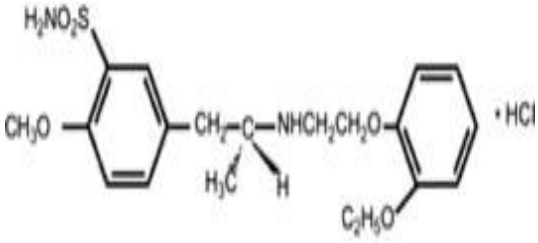

Fig. 2: Chemical structure of Tamsulosin HCl.

MATERIALS & METHOD

Chemicals and Reagents Used

The following chemicals were procured for the process: Water [HPLC Grade], Methanol [HPLC Grade], Acetonitrile [HPLC Grade], Tamsulosin and Dutasteride [Working standards] & KH_2PO_4 all the chemicals were procured from STANDARD SOLUTIONS, HCL **[**LR Grade**]** procured from FINAR CHEMICAL LIMITED, NaOH [L R Grade] procured from S D FINE- CHEM LIMITED & H_2O_2 procured from ALPHA PHARMA LIMITED and the tablets were collected from the Local market.

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

Equipment

High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.

UV/VIS spectrophotometer: LAB INDIA UV 3000+
pH meter: Adwa – AD 1020
Weighing machine: Afcoset ER-200A
Temperature: Ambient
Column: Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent

Phosphate Buffer

6.8 grams of Potassium Dihydrogen Ortho Phosphate in 1000 ml Water [HPLC Grade] pH adjusted with Orthophosporic acid.

pH: 2.5

Mobile phase: Phosphate Buffer: Acetonitrile (20: 80 v/v)

Flow rate: 0.8 ml per min

Wavelength: 274 nm

Injection volume: 20 µl

Run time: 7min.

Preparation of Phosphate buffer [21]

The buffer solution was prepared by dissolving accurately weighed 6.8 grams of potassium dihydrogen ortho phosphate and transferred into a clean and dry 1000ml volumetric flask, dissolved and diluted with 1000ml water [HPLC Grade]. The final pH of the buffer was adjusted to 2.5 by using Orthophosporic acid.

Preparation of mobile phase

The Mobile Phase was prepared by mixing 200 ml (20%) of the above buffer and 800 ml of Acetonitrile [HPLC Grade] (80%) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as Diluent.

PREPARATION OF THE TAMSULOSIN AND DUTASTERIDE STANDARD & SAMPLE SOLUTION

Preparation of Stock solution

The stock solution was prepared by weighing accurately 50mg Dutasteride and 50 mg Tamsulosin and transferred into a clean and dry 100 ml volumetric flask. About 70 ml of diluent was added and sonicated. The volume was made upto the mark with the same diluent. From the above prepared Stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10ml volumetric flask, the diluent was added upto the mark to get final concentration of 75µg/ml.

Preparation of Sample Solution

The sample solution was prepared by weighing equivalently 50 mg of Dutasteride and Tamsulosin hydrochloride and transferred into a 100 ml clean and dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration of 75µg/ml. The standard and sample solutions of 75µg/ml were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

System Suitability [22-24]

The Tailing factor for the peaks due to Dutasteride and Tamsulosin hydrochloride in Standard solution should not be more than 1.5. The Theoretical plates for the Dutasteride and Tamsulosin hydrochloride peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Dutasteride and Tamsulosin hydrochloride standard. The parameters of system suitability were checked.

Table 1: It shows the results for system suitability parameters for the drugs Dutasteride and Tamsulosin

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Dutasteride	2.003	920101	116666	11.0	1.6	2711.8
2	Tamsulosin	5.067	552058	41531		1.3	3428.2

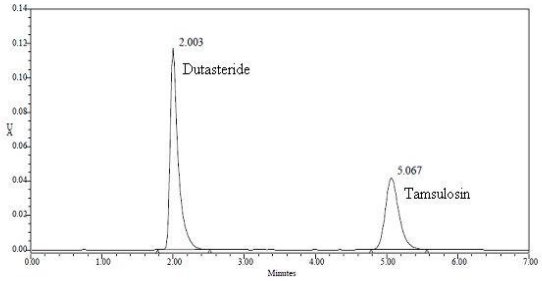

Fig. 3: It shows the Chromatogram for System Suitability.

Acceptance criteria

The Resolution between two drugs should not be less than 2. The Theoretical plates should not be less than 2000. The Tailing factor should not be less than 0.9 and not more than 2. It was found from above data; that all the system suitability parameters for developed method were within the limit.

VALIDATION DEVELOPMENT [25-32]

Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. (Table no.2 & 3). The chromatogram was represented in Fig. no.6.

Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. (Table no. 4 & 5). The chromatogram was represented in fig no. 7.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solution with Accuracy -50%, Accuracy -100% and Accuracy -150% were injected into chromatographic system and calculated the amount found and amount added for Dutasteride & Tamsulosin and further calculated the individual recovery and mean recovery values. (Table no. 6). The chromatograms were represented in fig. no. 8, 9 &10.

Linearity

It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. It is determined by series of three to six injections of five of more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. (Table no. 7). The chromatograms were represented in fig. no. 11 & 12.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

Limit of Detection for the drugs Dutasteride & Tamsulosin

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics) The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

Limit of detection (LOD) = $\frac{\sigma}{S} \times 3.3$

Where S – slope of the calibration curve

σ – Residual standard deviation

The chromatograms were represented in Fig. No. 15 & 16. (Table no. 9)

Limit of quantification

It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio.

Limit of quantification for the drugs Dutasteride and Tamsulosin

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. The chromatograms were represented in Fig. no. 13 & 14. (Table no. 10). Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from linearity curve by applying the following formula

Limit of Quantification (LOQ) = $\frac{\sigma}{S} \times 10$

Where S – slope of the calibration curve

σ – Residual standard deviation

The data were represented in Table No. 9, 10 & 11 and the chromatograms were represented in fig. no. 13, 14, 15 & 16.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The standard and samples of Dutasteride and Tamsulosin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The flow rate was varied at 0.7 ml/min to 0.9ml/min.

The Standard solution of Dutasteride & Tamsulosin was prepared and analysed using the varied flow rates along with method developed flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it was indicated that the method was robust even by change in the flow rate.

The Organic composition in the Mobile phase was varied from 70% to 90%.

The Standard solution for the drug Dutasteride & Tamsulosin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition. On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase does not affected the method significantly. Hence it was indicated that the method was robust even by change in the Mobile phase ±10. The system suitability parameters were within the limits and shown in Table No. 11 & 12 and chromatograms were represented in Fig. no. 17, 18, 19 & 20.

Stability indicating studies [33]

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Dutasteride

and Tamsulosin using the proposed method. The chromatograms were represented in Fig. No. 21, 22, 23 & 24. (Table No. 13)

Acid degradation

From the prepared stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration of 75µg/ml, then 3 ml of 0.1N HCl was added. The volumetric flask was kept at normal condition for 90 minutes and further it was neutralized with 0.1 N NaOH and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

Alkaline degradation: From the prepared stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration of 75µg/ml, then 3 ml of 0.1N NaOH was added. The volumetric flask was kept at normal condition for 90 minutes and further it was neutralized with 0.1 N HCl and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

Thermal degradation: From the prepared stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration of 75µg/ml, then 3 ml of diluent was added. Then, the volumetric flask was kept under heat at 105^0 C for 24 hours and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

Peroxide degradation: From the prepared stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration of 75µg/ml, then 1 ml of 3 % w/v of hydrogen peroxide was added. Then the volumetric flask was kept at room temperature for 15 min and the final volume was made up to the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

RESULTS & DISCUSSION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage is important in pharmacokinetic, toxicological biological studies. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form.

The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity,

precision and accuracy in estimation of drugs. The present work was undertaken with the aim to develop and validate a rapid and consistent stability indicating RP-HPLC in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the Pharmaceutical dosage form, bulk drug as well as for routine analysis in Quality control. Overall the proposed method was found to be suitable and accurate for the Quantitative determination and stability study of the drug in Pharmaceutical dosage form. The method was effectively separated the drug from its degradation product and it was employed as a stability- indicating one. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Dutasteride and Tamsulosin hydrochloride in bulk drug and in combined dosage forms. The Reversed-phase high-performance liquid chromatography (RP-HPLC) methods was developed and validated for simultaneous estimation of Tamsulosin hydrochloride and Dutasteride in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (20%) whose pH was adjusted to 2.5 by using Orthophosporic Acid & Acetonitrile (80%) [HPLC Grade]. The flow rate was monitored at 0.8 ml per min. The wavelength was selected for the detection was 274 nm. The run time was 7min. The retention time found for the drugs Dutasteride & Tamsulosin were 2.003 min. & 5.067 min. respectively. It was represented in fig. no. 4 & 5.

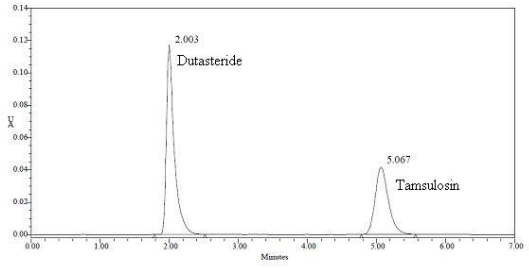

Fig 4: It shows the Chromatogram for the optimized method development.

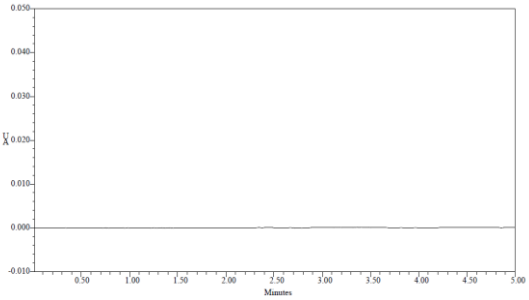

Fig 5: It shows the Chromatogram for the Blank.

The Precision data for the drugs Dutasteride & Tamsulosin were represented in Table no. 2 & 3 and the chromatograph was represented in Fig. No. 6.

Table 2: Precision result for the drug Dutasteride.

Sr. No.	Sample area	Standard area	Percentage purity
	983375	971536	
1	985049	973007	101.04
2	982956	975717	101.03
3	985219	978909	100.54
4	994145	981422	100.44
5			101.09
Average			**100.84**
%RSD			**0.304**

Table 3: Precision result for the drug Tamsulosin.

Sr. No.	Sample area	Standard area	Percentage purity
	592403	577531	
1	592352	580381	101.36
2	592357	577723	101.85
3	592323	582190	102.32
4	596525	583378	101.44
5			101.09
Average			**101.24**
%RSD			**0.46**

Acceptance criteria

The %RSD for sample should be NMT 2. The %RSD for the standard solution is below 2, which is within the limits hence the method was precise.

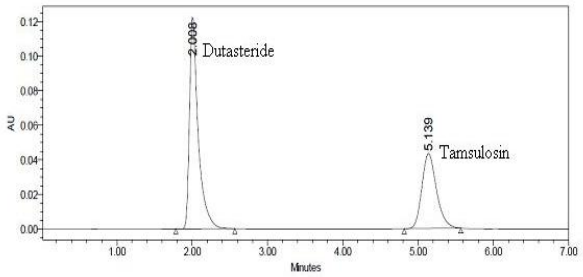

Fig 6: Chromatogram for Precision.

When the drugs Dutasteride & Tamsulosin were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed it was represented in Table no. 4 & 5 and the chromatogram was represented in Fig. no.7 which shows that the developed RP-HPLC method was highly precise.

Table 4: Ruggedness result for the drug Dutasteride

Sr. No	Sample area	Standard area	Percentage purity
	979556	984395	
1	982467	984039	99.30
2	979717	983976	99.64
3	978909	984278	99.36
4	981432	973915	99.28
5			100.57
Average			**99.63**
%RSD			**0.54**

Table 5: Ruggedness result for the drug Tamsulosin

S. No	Sample area	Standard area	Percentage purity
	583416	593403	
1	583657	594352	99.12
2	584731	593357	99.01
3	583594	592673	99.52
4	597649	593671	99.61
5			99.12
Average			**99.27**
%RSD			**0.27**

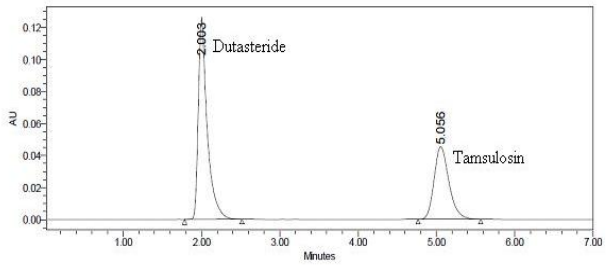

Fig 7: Chromatogram for Intermediate Precision

Acceptance criteria: The %RSD of five different sample solutions should not be more than 2. The %RSD obtained is within the limit, hence the method was rugged.

The standard solution with Accuracy -50%, Accuracy -100% and Accuracy -150% were injected into chromatographic system and calculated the amount found and amount added for Dutasteride & Tamsulosin and further calculated the individual recovery and mean recovery values. (Table no. 6). The chromatograms were represented in fig. no. 8, 9 &10.

Table 6: Accuracy results for the drug Dutasteride & Tamsulosin

Sample Concentration	Sample set No.	Sample area Dutasteride	Tamsulosin	Assay Dutasteride	Tamsulosin	% Recovery Dutasteride	Tamsulosin
50%	**1**	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					**99.7%**	**99.7%**
100%	**1**	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					**99.6%**	**99.8%**
150%	**1**	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					**99.7%**	**99.8%**

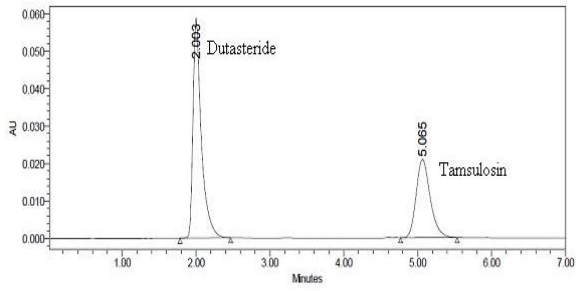

Fig 8: Chromatogram for Accuracy (50%).

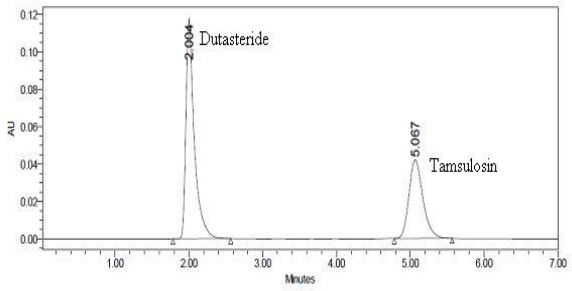

Fig 9: Chromatogram for Accuracy (100%).

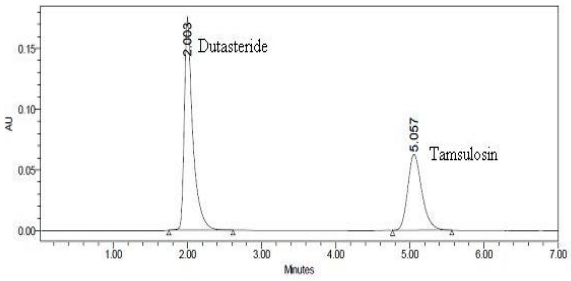

Fig 10: Chromatogram represents for Accuracy (150%)

Acceptance criteria

The percentage recovery at each level should be between (97-103%). The results obtained for recovery at 50%, 100%, 150% were within the limits. Hence the method was accurate.

In order to test the linearity of the method, five dilutions of the working standard solutions for the drugs Dutasteride & Tamsulosin in the range of 25 to 125µg/ml were prepared. The data were represented in Table no. 7. Each of the dilution was injected into the column and the Linearity Curve was represented in Fig. no.11 & 12.

Table 7: Linearity results for the drug Dutasteride & Tamsulosin

Concentration (µg/ml)	Peak area of Dutasteride	Peak area of Tamsulosin
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

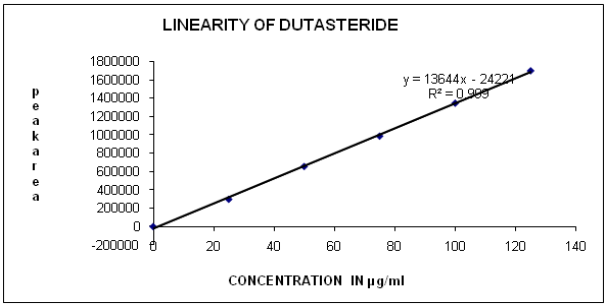

Fig 11. Linearity curve for the drug Dutasteride

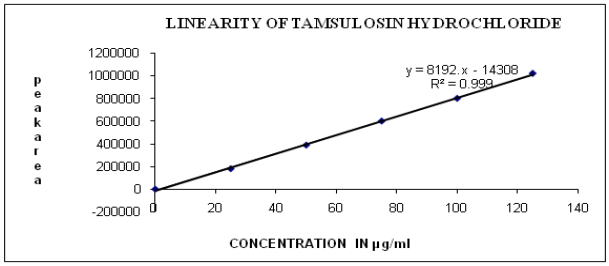

Fig 12. Linearity curve for the drug Tamsulosin Hydrochloride

Table 8: Analytical performance parameters for the drugs Dutasteride and Tamsulosin

Parameters	Dutasteride	Tamsulosin
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria

The Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which was in the acceptance limit. The linearity was established in the range of 25 to 150µg/ml.

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The data were represented in Table No. 9, 10 & 11 and the chromatograms were represented in Fig. No. 13, 14, 15 & 16. The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution**.** The results obtained were within the limit.

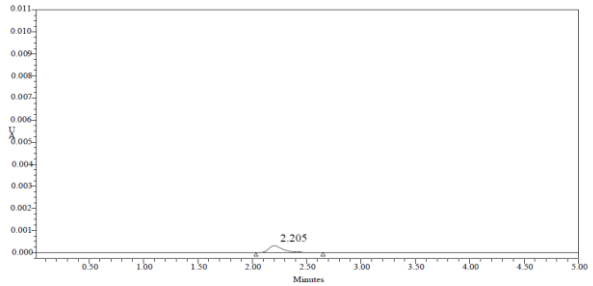

Fig.13: Chromatogram for the drug Dutasteride (LOD)

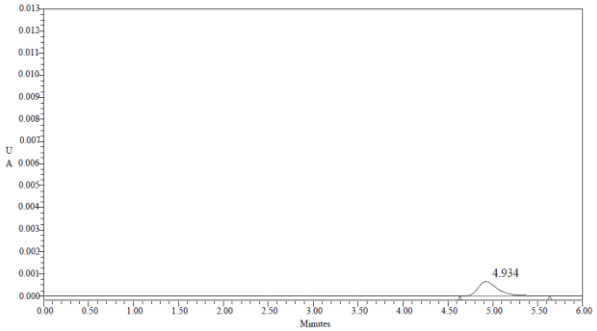

Fig. 14: Chromatogram for the drug Tamsulosin (LOD)

Table 9: LOD results for the drugs Dutasteride & Tamsulosin

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Dutasteride	56	176	3.14
Tamsulosin	56	154	2.75

Acceptance criteria

The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit.

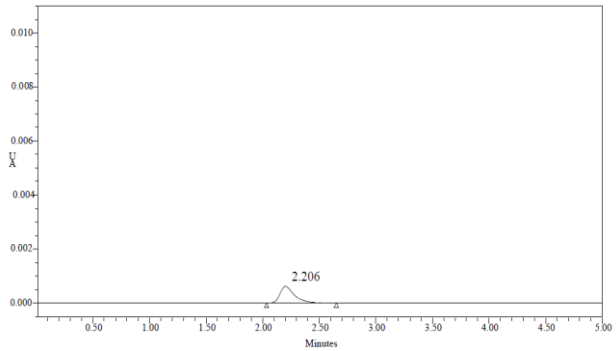

Fig 15: Chromatogram for the drug Dutasteride (LOQ)

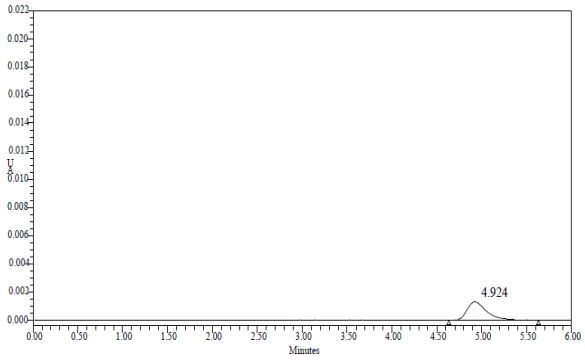

Fig.16: Chromatogram for the drug Tamsulosin (LOQ)

Table 10: LOQ results for the drugs Dutasteride & Tamsulosin

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Dutasteride	56	563	10.05
Tamsulosin	56	558	9.96

Acceptance criteria

The Signal to noise ratio should be 10 for LOQ solution**.** The results obtained were within the limit.

The Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate Buffer and Acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table No. 11 & 12 and chromatograms were represented in Fig. no. 17, 18, 19 & 20.

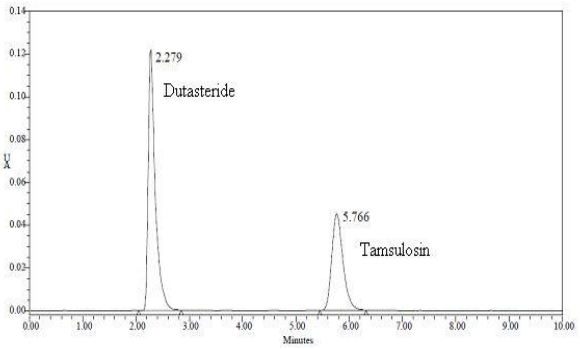

Fig 17: Chromatogram for Less Flow Rate (0.7ml/min.)

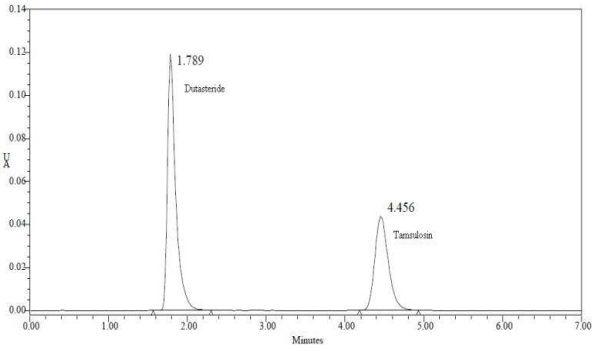

Fig 18: Chromatogram for More Flow Rate. (0.9ml/min.)

Table 11: It shows the result for effect of variation in flow rate

S. No	Peak Area for Less flow rate (0.7 ml/min)		Peak Area for More flow rate (0.9 ml/min)	
	Dutasteride	Tamsulosin	Dutasteride	Tamsulosin
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

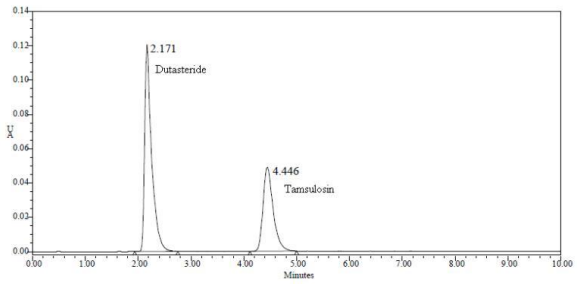

Fig 19: Chromatogram for Less Organic Composition (70%)

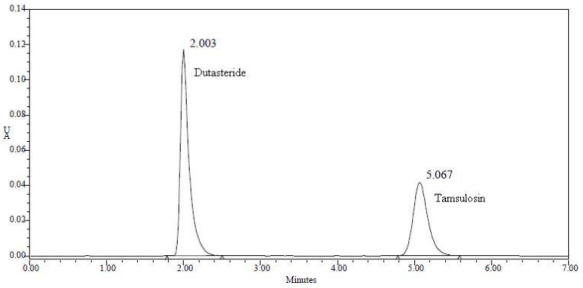

Fig 20: Chromatogram for More Organic Composition (90%)

Table 12: Result for effect of variation in mobile phase composition (Organic Phase)

S. No	Peak area for Less organic phase (70%)		Peak area for More organic phase (90%)	
	Dutasteride	Tamsulosin	Dutasteride	Tamsulosin
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	**987286**	**583658**	**986641**	**592877**
%RSD	**0.45**	**0.90**	**0.51**	**0.57**

Acceptance criteria

The Percentage RSD should not be more than 2. The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 2, which was within the acceptance criteria. Hence the method was robust.

The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms. It was concluded that the proposed RP-HPLC method was sufficiently sensitive and reproducible for the analysis of Dutasteride & Tamsulosin. In order to evaluate the stability of Dutasteride & Tamsulosin and ability of the method to separate Dutasteride &

Tamsulosin from its degradation products, the drug was subjected to various stress conditions such as Hydrolytic degradation under acidic condition (using 0.1N HCl & 0.1 N NaOH), Hydrolytic degradation under alkaline condition (using0.1N NaOH & 0.1N HCL), Thermal induced degradation (at 105° C for 24 hrs.), Oxidative degradation (by using 3 % w/v of hydrogen peroxide). The following chromatograph represents the degradation studies for the drug Dutasteride & Tamsulosin which were represented in table no. 13 and Fig. no. 21, 22, 23 & 24.

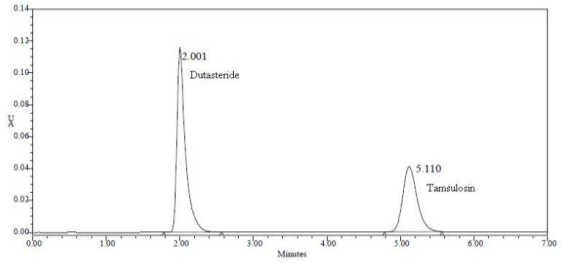

Fig 21: Chromatogram for Acid Degradation study

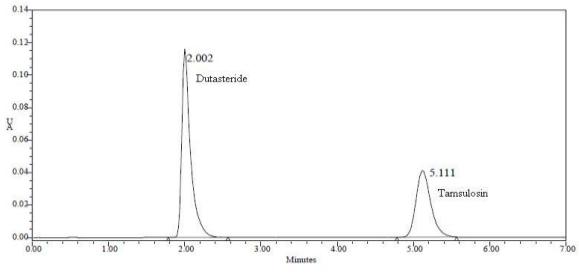

Fig 22: Chromatogram for Alkaline Degradation study

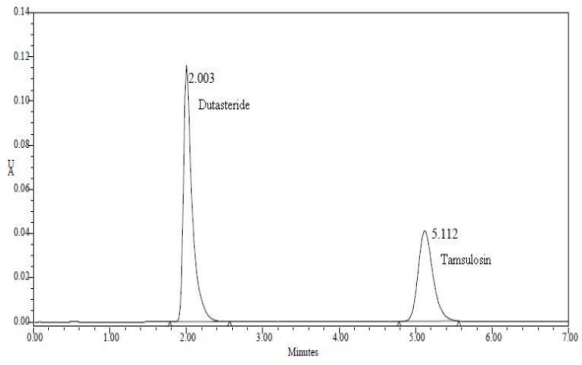

Fig 23: Chromatogram for Thermal Degradation study

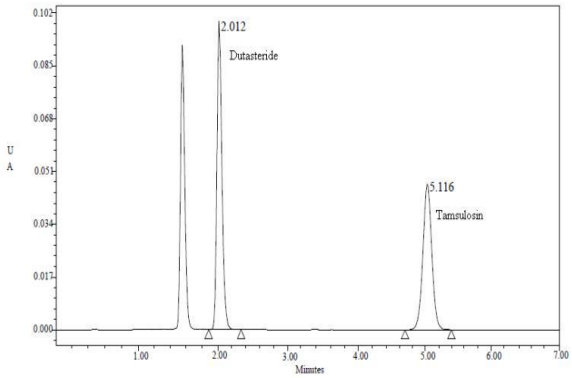

Fig 24: Chromatogram for Peroxide Degradation study

Table 13: Result for Forced degradation studies for the drugs Dutasteride & Tamsulosin

Sr. No.	Type of Degradation	Wt. of Sample (ppm)	Area of sample		Assay content (% w/w)	
			Dutasteride	Tamsulosin	Dutasteride	Tamsulosin
1	Acid	75	848682	505192	91.87	91.37
2	Base	75	830431	494327	89.89	89.41
3	Peroxide	75	784529	439981	84.92	79.58
4	Thermal	75	793929	472599	85.94	85.48

CONCLUSION

It was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Dutasteride & Tamsulosin in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Dutasteride & Tamsulosin depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Dutasteride & Tamsulosin. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Dutasteride & Tamsulosin from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of drug in bulk and pharmaceutical dosage forms.

FUTURE ASPECT

The proposed method can be use in future for the clinical, biological and spharmacokinetic studies of Dutasteride & Tamsulosin.

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