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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TAMULOSIN AND DUTASTERIDE IN A BULK AND TABLET DOSAGE FORM

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Chromatography, Validation, Tamulosin, Dutasteride, Accuracy, Precision

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ABSTRACT: Chromatography is a separation process that is achieved by distributing the substances to be separated between a moving phase and a stationary phase. HPLC is high resolution, high pressure and high-speed liquid chromatography. It has several resolving powers than open column liquid chromatography hence it is used for speedy resolution of complex mixture, separation and determination of species in a variety of organic, inorganic and biological materials. The developed HPLC method was validated for various parameters like accuracy, precision, specificity, LOD, LOQ, linearity, range and robustness as per ICH guidelines. The results obtained were well within the acceptance criteria for all the parameters. The proposed method was applied for simultaneous estimation of Tamsulosin and Dutasteride formulation. The HPLC system used was WATERS Alliance series System with Rheodyne injector 20 µL and the column Inertsil ODS 250 x 4.6 mm, 5 µm. The mobile phase comprised of Sodium phosphate buffer and Acetonitrile in the ratio of 30:70 v/v and flow rate of 1.0 ml/min with UV detection at 233 nm produced peaks of Tamsulosin and Dutasteride in the chromatogram which were well resolved with retention time of 2.939 min and 5.572 min respectively.

INTRODUCTION: Chromatography is unique in the history of analytical methodology and is probably the most powerful and versatile technique available to the modern analyst. In a single procedure it can separate a mixture into its individual components and simultaneously determine quantitatively the amount of each component present [1].

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Principle of Chromatographic Separation: Chromatography is a separation process that is achieved by distributing the substances to be separated between a moving phase and a stationary phase. Those substances distributed preferentially in the moving phase pass through the chromatographic system faster than those that are distributed preferentially in the stationary phase. As a consequence, the substances are eluted from the column in reverse order of their distribution coefficient with respect to the stationary phase. The process is diagrammatically depicted below [2].

Classification of Chromatographic Techniques: All chromatographic separations are carried out using a mobile and a stationary phase.

As a result of this prerequisite, the primary classification of chromatography is based on the physical nature of the mobile phase. Thus, all separation processes that utilize a gas as the mobile phase are classed as gas chromatography. Conversely, all separation processes that utilize a liquid as the mobile phase are classed as liquid chromatography.

A) Adsorption Chromatography

- Gas-solid chromatography
- High performance liquid chromatography
- Thin layer chromatography

B) Partition Chromatography

- Liquid-liquid chromatography
- Paper chromatography
- High performance liquid chromatography

C) Ion exchange chromatography

D) Permeation chromatography

- Size exclusion chromatography

E) Affinity chromatography

- DNA Affinity chromatography

F) Electrophoresis

- Capillary electro chromatography [3, 4].

Fundamental Parameters:

Retention Time: It is the time in between the sample is injected and chromatographic peak is recorded. The total retention time (t_{R1} or t_{R2}) is the time which is needed by sample component to migrate from column inlet (sample injection) to the column end (detector). The net retention time (t'_{R1} or t'_{R2}) is the difference between total retention time and dead time *i.e.* the time the sample component remains in the stationary phase.

Capacity Factor (K′): It is the measure of the position of a sample peak in the chromatogram. It is specific for a given substance. k′ depends on the stationary phase, the mobile phase, the temperature, quality of the packing etc.

Relative Retention (α): Also known as separation factor is the ratio between two capacity factors. Relative retention describes the ability of a system of stationary and mobile phase to discriminate between two compounds. Impurities in the mobile phase (*e.g.* water content) strongly influence relative retention.

Resolution (RS): It is a measure of quality of separation of adjacent bands; obviously overlapping bands have small Rs values. It is calculated from width and retention time of two peaks.

Number of Theoretical Plates (N): Characterize the quality of a column packing and mass transfer phenomena. The larger n, the more complicated sample mixtures can be separated with the column.[5, 6]

High Performance Liquid Chromatography: HPLC is high resolution, high pressure and high-speed liquid chromatography. It has several times resolving power than open column liquid chromatography hence it is used for speedy resolution of complex mixture, separation and determination of species in a variety of organic, inorganic and biological materials [7].

TABLE 1: COMMONLY USED METHODS IN HPLC [7]

	Reverse Phase Chromatography	Normal Phase Chromatography
Mechanism	Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of sample.	Retention by interaction of the stationary phase's polar surface with polar parts of the sample molecules.
Stationary Phase	bonded siloxane with non-polar functional groups like n- octadecyl (C-18) or n- octyl (C-8), ethyl, phenyl, -(CH_2) n-diol, (CH_2) n-CN molecules.	bonded siloxane with polar functional group like SiO_2, Al_2O_3, -NH_2, -CN, -NO_2, - Diol
Mobile Phase	Polar solvents like methanol, acetonitrile, water or buffer (Sometimes with additives of THF or dioxane).	Nonpolar solvents like heptane, hexane, cyclohexane, chloroform, ethyl ether, and dioxane.
Application	Separation of nonionic and ion forming nonpolar to medium polar substances	Separation of nonionic, nonpolar to medium polar substances.
Elution Order	Most polar components are eluted first.	Least polar components are eluted first.

Method Development in HPLC: In developing HPLC method for the quantitative analysis of multicomponent formulation the following general requirements should be fulfilled.

1. The identity of the component to be analyzed should be established.
2. Separation of specific components should be achieved.
3. Sample preparation should be reproducible.
4. Standard of known purity should be available, and accuracy will be directly related to the degree of purity of standards used in determination.
5. A stationary phase that separates the component in a reproducible manner.
6. Sample application or injection should be reproducible.

Analytical Method Validation: Validation is "Action of proving accordance with the principle of good manufacturing practice, that any material, activity or system actually leads to expected result". Method validation is the documented successful evaluation of any analytical method that provides a high level of assurance that such method will consistently yield results that are accurate with in previously established specification [8].

Type of Validation: The following are frequently required to be validated on pharmaceutical process; Equipment, Environment, Materials, Methods, Control, Process, Personnel's Facilities and Operation procedure. Based on these the validation program comprises [8].

Analytical Method Validation: Method validation is a process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical testing of pharmaceutical products is necessary to ensure their purity, stability, safety, and efficacy. Analytical method validation is an integral part of the quality control system. Although a through validation cannot rule out all potential problems, the process of method development and validation should address the most common ones [9].

Steps Involved in Quantitative Analysis:

Sampling and Sample Preparation: The sample should be homogeneous. It should be completely soluble, and the solvent used to dissolve the sample should be initial mobile phase or any solvent miscible with mobile phase.

Chromatographic Separation: After achieving a resolution with an optimized solvent system, obtaining reproducible results following criteria must be satisfied.

- Monitoring flow rate.
- Keeping the solvent composition intact.
- Solvent system must be covered before storage.
- Monitoring column temperature.

Detection: The response obtained from a given detector will vary according to the nature of solute molecules. With a UV detector the response is related to both concentration and molecular extinction coefficient of the component at the wavelength of detection [10].

Measurement and Calibration: The various approaches used for quantitative analysis:

Peak Height Method: Peak height measurements represent a simple, satisfactory method for calculating detector response in the absence of mechanical or electronic signal integrators. In practice, a baseline is drawn from the leading edge of the chromatography peak to the trailing edge. The vertical distance from the peak apex to the predetermined baseline represents the peak height. For quantitative purpose

1. The peak of interest must be symmetrical
2. All parameters that affect peak width must be held constantly.

Peak Area Method: Computing electronic integrators are the simplest and most popular method for the determination of chromatographic peak areas. Quantitative measurements based on peak areas can be performed by several methods such as,

The Internal Standard Method: The highest precision for quantitative chromatography is obtained by use of internal standards because the uncertainties introduced by sample injection are avoided. In this procedure, a carefully measured quantity of an internal standard is added to each standard and sample. And the ration of analysis to internal standard peak areas (Heights) serves as the analytical parameter.

External Standard Method: Separate injections of fixed volume of sample and standard solution are given. Peaks are integrated and concentration of sample calculated by direct comparison [12-15].

METHODOLOGY: A method was developed for simultaneous estimation of Tamsulosin and Dutasteride on HPLC by selecting the appropriate λ max, optimum mobile phase and flow rate which gives peaks with good resolution, HETP, Tailing factor and other related System suitability parameters.

Selection of Solvent: The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. Drug should be stable in the solvent used and should be economical. Various solvents were studied for the solubility of Tamsulosin and Dutasteride. Both the drugs were very soluble in methanol and freely soluble in acetonitrile and slightly soluble in other non-polar solvents. Therefore, methanol was selected as a solvent of choice for both drugs. Both the drugs were stable in methanol [16].

Preparation of Standard Stock Solutions:

For UV: The stock solution of Tamsulosin and Dutasteride was prepared separately by dissolving accurately 20 mg in 20 ml of methanol mix well & sonicate. Take 0.1ml from above stock solution dilute with 10 ml methanol to obtain a final concentration of 10 mg/ml [17].

For HPLC: The stock solution of Tamsulosin was prepared by dissolving accurately weighed 4 mg in 10 ml of methanol mix well & sonicate after that 10ml transferred into the 100ml volumetric flask & make up the volume up to 100ml by methanol to obtain a final concentration of 40µg/ml. The stock solution of Dutasteride was prepared by dissolving accurately weighed 5 mg in 10 ml of methanol mix well & sonicate after that 10ml transferred in to the 100ml volumetric flask & make up the volume up to 100ml by methanol to obtain a final concentration of 50µg/ml. This stock solution is used for system suitability study.

Mixed Stock Solution: The stock solution of Tamsulosin and Dutasteride was prepared by dissolving accurately weighed 4 mg of Tamsulosin & 5 mg of Dutasteride in 10 ml of methanol mix well & sonicate after that 10 ml of standard stock solution of Tamsulosin and Dutasteride was taken and transferred it into a 100ml volumetric flask and diluted to 100 ml with the diluents to get a concentration of 40µg/ ml of Tamsulosin and 50µg/ ml of Dutasteride.

Selection of Wavelength: The wavelength at which Tamsulosin and Dutasteride showed maximum absorption was selected by obtaining overlay spectra of Dutasteride and Tamsulosin of 10 µg/ml concentration in methanol.

Selection of Mobile Phase: Tamsulosin and Dutasteride are marketed as combined dosage formulation. The proposed method for estimation of Tamsulosin and Dutasteride required adequate resolution between the two drug peaks in the chromatogram. Several solvent systems were tried to obtain optimum resolution [18].

Determination of Retention Time: The time at which Tamsulosin and Dutasteride showed peaks with good resolution was determined by injecting 4µg/ml and 5µg/ml solution into a chromatogram at the flow rate of 1.0 ml/min, UV detection at 253 nm.

Determination of Retention Time for Tamsulosin:

Standard Stock Solution of Rosuvastatin: Accurately, 4 mg of Tamsulosin was weighed into a clean and dry 10mL volumetric flask, dissolved with sufficient volume of mobile phase. The volume was then made up to 10 ml with mobile phase to get a concentration of 400µg/ml (Stock I).10 ml of the stock solution was further diluted to 100 ml with mobile phase to get a concentration 40 µg/ml (Stock II).

System Suitability Parameters: The system suitability parameters are an integral part of many analytical procedures. The tests are performed to

ascertain the suitability of the proposed method with mobile phase.

A solution of 4µg/ml of Tamsulosin and 5 µg/ml of Dutasteride was prepared by diluting suitably with mobile phase and was injected into the chromatograph, the peak area, chromatogram and various system suitability parameters such as Theoretical Plates, HETP (Height Equivalent to Theoretical Plate), Peak Asymmetry (As) and Resolution (Rs) [20-22].

System suitability parameters can be calculated using the following equations.

Theoretical Plates: Measurement of the column efficiency. A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry [23].

$$N = 5.54(t_R /W)^2$$

Where, 'W' is the peak width at 50% peak height and t_R is the retention time.

HETP (Height Equivalent to Theoretical Plate)**:** A measure of column's efficiency. For a typical well packed HPLC column with 5µm particles, HETP values are usually between 0.01 and 0.03 mm

$$HETP = L/N$$

Where, L: Length of the column in millimeters. N: Number of Theoretical Plates

Asymmetric Factor: Factor describing the shape of a chromatographic peak. Theory assumes a Gaussian shape and that peaks are symmetrical. A value >1 is a tailing peak, while a value <1 is a fronting peak.

$$Af = b/a \text{ at } 10\% \text{ peak height.}$$

Where, a = peak from at 10% to drop line, b = drop line to peak tail at 10%

Resolution (Rs): Ability of a column to separate chromatographic peaks.

$$RS = (2(t_2-t_1) / (W_1 +W_2)$$

Where, t_1 and t_2 are the retention time of the two components and W1 and W_2 are the width at the 50% of peak height [24-27].

Validation of Analytical Method for the Assay of Tamsulosin and Dutasteride: Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application.

The developed HPLC method for simultaneous estimation of Tamsulosin and Dutasteride using Universal inertsil ODS column Enable 250mm x 4.6mm, 5µm, mobile phase Sodium phosphate buffer: Acetonitrile (30:70), detection wavelength at 233 nm, at a flow rate of 1.0 ml/min was validated for typical analytical parameters like, Accuracy, Precision, Specificity, and Detection [28].

Standard STOCK SOLUTION of Tamsulosin and Dutasteride: Accurately weighed 4mg of Tamsulosin and 5mg of Dutasteride was transferred into a clean and dry 10mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 10mL with mobile phase to obtain concentration of 400 µg/ml and 500 µg/ml .

Standard Stock Solution of Tamsulosin and Dutasteride: Accurately weighed 4mg of Tamsulosin and 5mg of Dutasteride was transferred into a clean and dry 10mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 10 ml with mobile phase to obtain concentration of 400 µg/ml and500 µg/ml .

Working Solution: 10 ml of the stock solution was further diluted to 100 ml with mobile phase to get a concentration 40µg/ml and 50µg/Mr.

Standard Stock Solution of Tamsulosin and Dutasteride: Accurately weighed 4mg of Tamsulosin and 5mg of Dutasteride was transferred into a clean and dry 10mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 10mL with mobile phase to obtain concentration of 400 µg/ml and 500 µg/mL [29].

Working Solution: 10mL of the stock solution was further diluted to 100 ml with mobile phase to get a concentration 40µg/ml and 50µg/Mr.

Procedure: 20µl of mixed working standard solution were injected in different laboratories, by different analysts and chromatograms are recorded.

Acceptance Criteria: the relative standard deviation should be within 2% w/v.

Specificity: Specificity is the ability to assess unequivocally that the analyze in the presence of components which may be expected to be present; typically, these might include impurities, degradation products and matrix components.

Limit of Detection (LOD): Limit of detection is the lowest concentration of the analysis that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions.

Linearity and Range: The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analysis in the sample. Each working standard solution of Tamsulosin and Dutasteride were injected into the chromatograph. The peak areas and chromatograms were recorded. A linearity graph was plotted with concentration variable on x – axis and peak areas on y – axis for Tamsulosin and Dutasteride. The Correlation coefficient and Percentage curve fittings were calculated [30-34].

Standard Stock Solution of Tamsulosin and Dutasteride: Accurately weighed 4mg of Tamsulosin and 5mg of Dutasteride was transferred into a clean and dry 10mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 10 ml with mobile phase to obtain concentration of 400 µg/ml and 500 µg/ml .

Working Standard Solution: Aliquots from standard stock solution were withdrawn in the volumes of 2.5, 5, 7.5, 10, 12.5 and 15mL in separate 100 ml volumetric flasks. The volume was made up with the mobile phase to get concentrations ranging from 10-60 µg/ml for Tamsulosin and 12.5-75 µg/ml for Dutasteride.

Procedure: 20 µl of each working standard solutions were injected into the chromatograph, the peak area and chromatogram obtained were recorded and are presented in **Table 18** and **Fig. 16** & **Fig. 17**. The linearity parameters were calculated and the results obtained are presented in **Table 19**.

Robustness: The robustness of an analytical method 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. The evaluation of robustness should be considered during the development phase and depends on the type of the procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters, like Influence of variations in flow rate, Influence of variations in Buffer Ratio [35].

Standard Stock Solution of Tamsulosin and Dutasteride: Accurately weighed 4mg of Tamsulosin and 5mg of Dutasteride was transferred into a clean and dry 10mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 10mL with mobile phase to obtain concentration of 400 µg/ml and 500 µg/ml .

Working Solution: 10 ml of the stock solution was further diluted to 100 ml with mobile phase to get a concentration 40µg/ml and 50µg/Mr.

Change in Flow Rate: For the method developed, flow rate of 1mL/min was used. For Robustness study, flow rates of 0.9 and 1.1mL/min were selected. Three injections of 20 µl of each working standard solution of Tamsulosin and Dutasteride at flow rate of 0.9 and 1.1 ml/min were injected into the chromatograph, the peak area and chromatogram obtained were recorded and the % assay was calculated.

Change in Buffer Ratio: 20µl of mixed working standard solution were injected in different chromatographic condition of buffer ratio and chromatograms are recorded. The results obtained are presented

TABLE 2: ACCEPTANCE CRITERIA FOR DIFFERENT VALIDATION PARAMETERS

Validation Parameter		Acceptance Criteria
Specificity		No interference by the degraded components at the drug retention time
Precision	System	NMT 2% (% RSD)

	Method Intra day Interday	
Robustness		within 90-110% (%Assay)
Accuracy		Within 90-110% (%Recovery)

Application of the Developed HPLC Method for Simultaneous Estimation of Tamsulosin and Dutasteride in Marketed Formulation: The developed and validated method has been applied for Simultaneous Estimation of Tamsulosin and Dutasteride in marketed formulation [36].

TABLE 3: CHROMATOGRAPHIC CONDITIONS FOR SAMPLE ANALYSIS

Instrument	WATERS alliance series
Injector	Rheodyne
Column	Universal inertsil ODS Column 250mm(length) x 4.6mm(I.D) , 5µm (Particle Size)
Wavelength	233nm
Detector	UV Detector
Flow rate	1.0 ml/min
Injection volume	20µl
Mobile Phase	Sodium phosphate buffer: Acetonitrile (70: 60)

Preparation of Sample Stock Solution: Weighed 20 Tablet; take Avg. wt. of it powdered equivalent to 4 mg of Tamsulosin and 5 mg of Dutasteride was accurately weighed and transferred into clean, dry 10mL volumetric flask. The powder was first dissolved in few ml of mobile phase by sonication, the volume was made up to 100mL and then filtered through a Whatmann filter to obtain the concentration 400µg/ml and 500µg/ml for Tamsulosin and Dutasteride respectively (Stock I) [38].

Working Sample Solutions: From the above stock, volumes of 10mL were transferred into 100mL volumetric flasks and volume made up to 100mL with the mobile phase to get the concentration of 40µg/ml for Tamsulosin and 50µg/ml for Dutasteride respectively.

Procedure: 20 µl solution of the working sample solutions were injected repeatedly into the chromatograph, at a flow rate of 1 ml/min and detection at 233 nm, the chromatograms obtained were recorded.

The amount of drug per Tablet & % assay is calculated by following formula:

Amount /Tablet = (sample peak avg.) / (std peak avg.) × (std wt) / (sample wt) × (std factor) /(sample factor) X avg. wt × (std purity) /100

% Assay = (amount per Tablet) / (label claim) × 100

RESULTS: A method was developed for simultaneous estimation of Tamsulosin and Dutasteride on HPLC by selecting the appropriate λ max, optimum mobile phase and flow rate which gives good and sharp resolution.

Selection of Wavelength: Standard solutions of Tamsulosin and Dutasteride (10 µg/ml) were scanned in the wavelength range of 200-400 nm using Methanol as diluents and UV spectrum obtained is presented below.

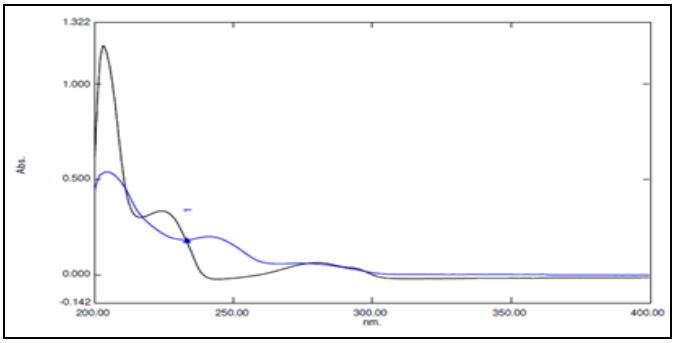
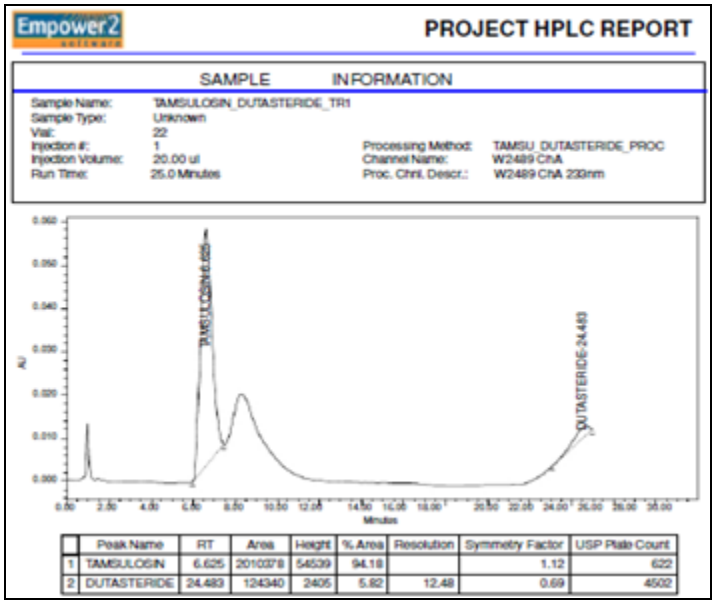


FIG. 1: OVERLAY SPECTRUM OF TAMSULOSIN AND DUTASTERIDE

No.	Wavelength	Absorbance	Description
1	233.20	0.185	Dutasteride&Tamsulosin

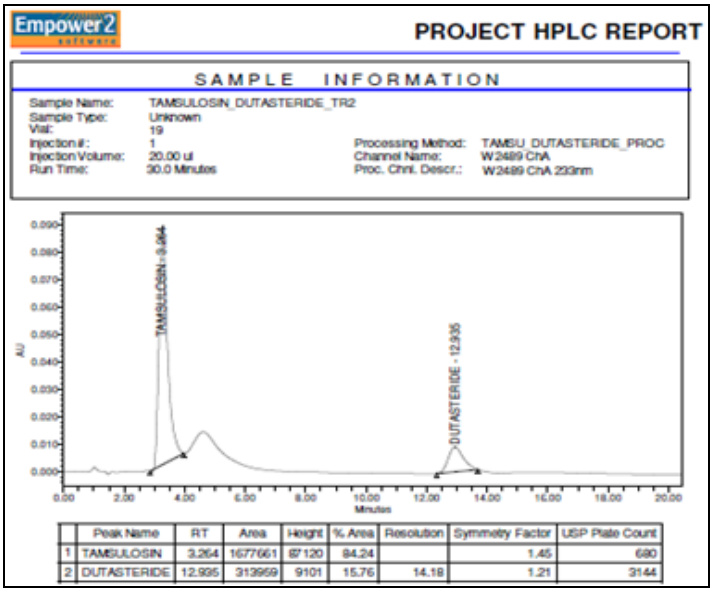
Report: The 10µg/ml of Tamsulosin and Dutasteride solutions showed λ max at 233.20 nm which was selected as wavelength maxima for determination of Tamsulosin and Dutasteride

Selection of Mobile Phase: Several solvent systems were tried to get optimum resolutions of Tamsulosin and Dutasteride in the present method. The observations obtained with various mobile phases in different ratios given in table in methodology chapter are given below.



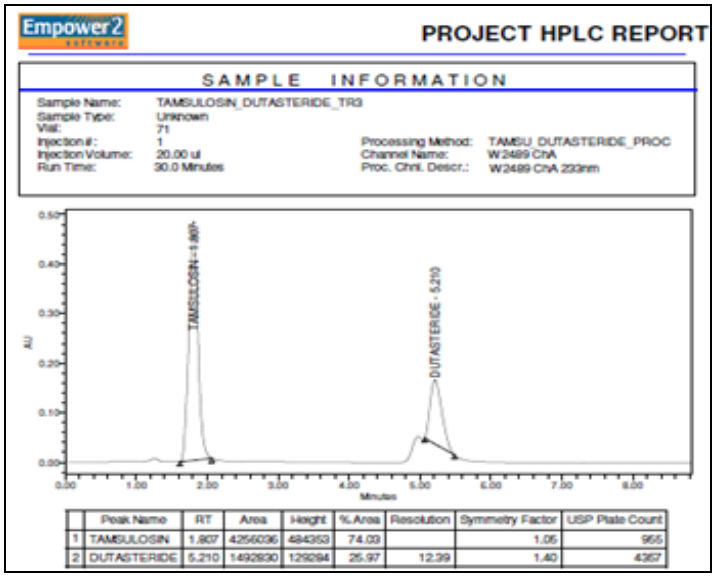
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	6.625	2010378	54539	94.18		1.12	622
2	DUTASTERIDE	24.483	124340	2405	5.82	12.48	0.69	4502

FIG. 2(A): CHROMATOGRAM FOR COMB. F METHANOL AND WATER (70:30)



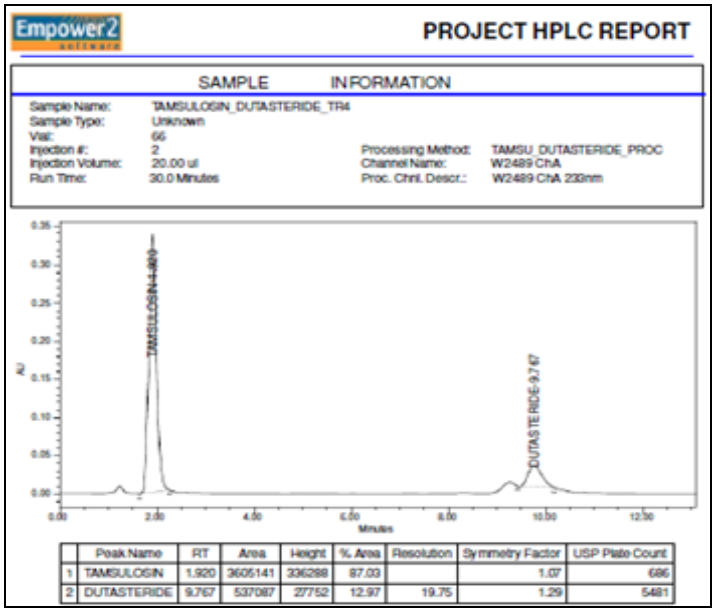
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	3.264	1677661	87120	84.24		1.45	680
2	DUTASTERIDE	12.935	313959	9101	15.76	14.18	1.21	3144

FIG. 2(B): CHROMATOGRAM FOR COMB. OF ACETONITRILE AND WATER (50:50)



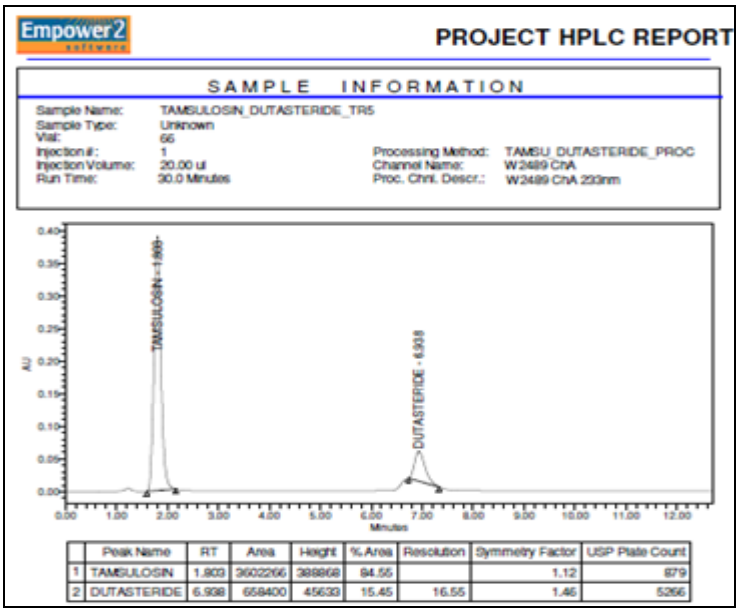
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	1.907	4256036	484353	74.03		1.05	955
2	DUTASTERIDE	5.210	1492930	129294	25.97	12.39	1.40	4357

FIG. 2(C): CHROMATOGRAM FOR COMB. OF ACETONITRILE AND MIX PHOSPHATE BUFFER (70:30)



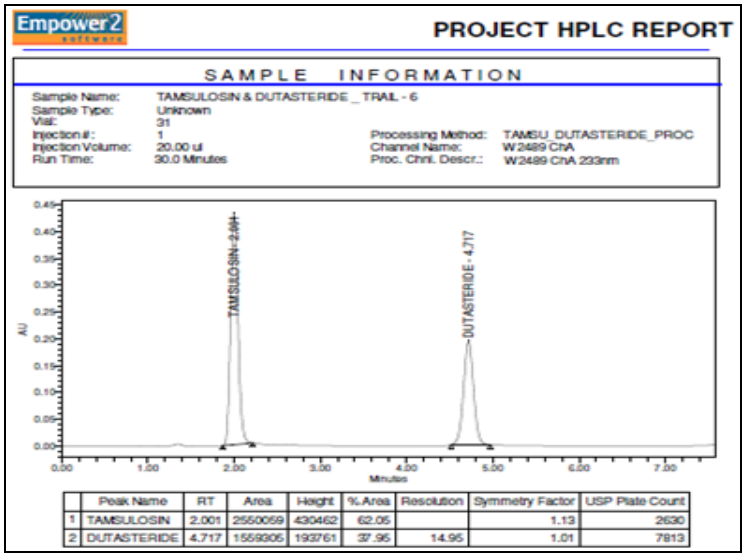
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	1.920	3605141	336288	87.03		1.07	686
2	DUTASTERIDE	9.767	537087	27752	12.97	19.75	1.29	5481

FIG. 2(D): CHROMATOGRAM FOR COMB. OF ACETONITRILE AND MIX PHOSPHATE BUFFER (65:35)



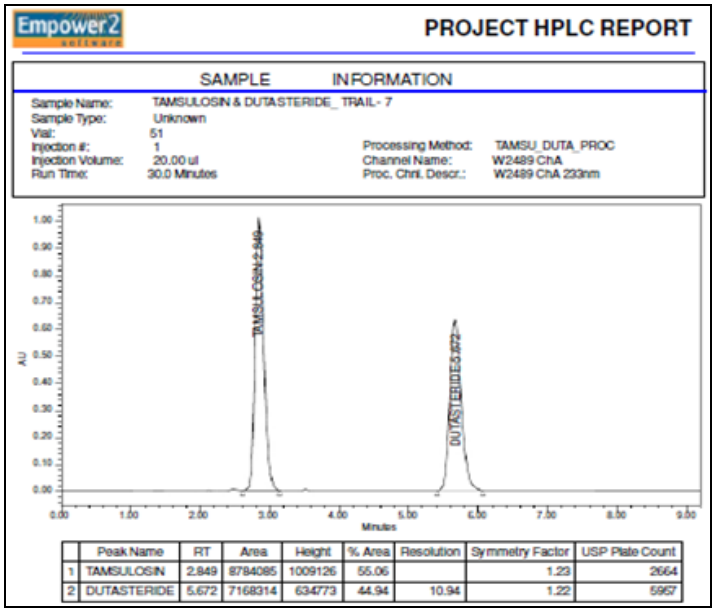
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	1.903	3602266	388868	84.55		1.12	879
2	DUTASTERIDE	6.938	658400	45639	15.45	16.55	1.46	5266

FIG. 2(E): CHROMATOGRAM FOR COMB. OF ACETONITRILE AND MIX PHOSPHATE BUFFER (60:40)



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	2.001	2550059	430462	62.05		1.13	2630
2	DUTASTERIDE	4.717	1559305	193761	37.95	14.95	1.01	7813

FIG. 2(F): CHROMATOGRAM FOR COMB. OF SODIUM PHOSPHATE BUFFER: ACETONITRILE (20:80)



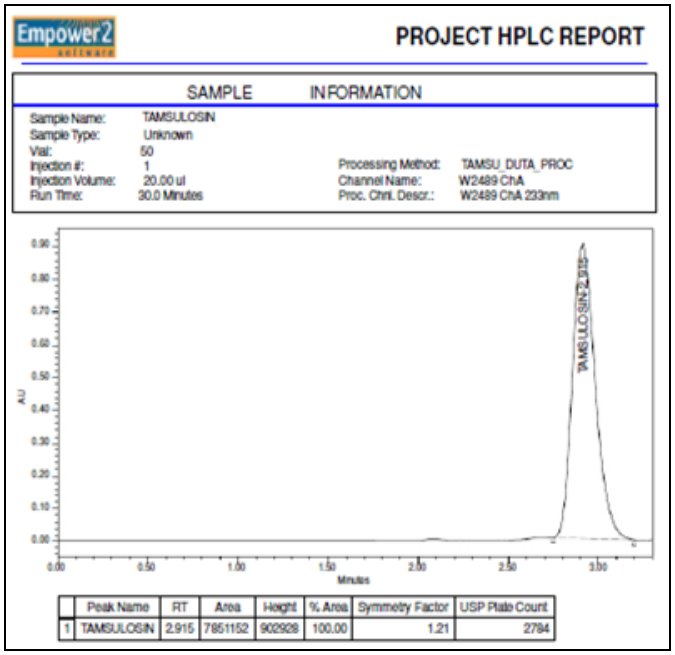
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	2.849	8784085	1009126	55.06		1.23	2664
2	DUTASTERIDE	5.672	7168314	634773	44.94	10.94	1.22	5957

FIG. 2(G): CHROMATOGRAM OF TAMSULOSIN AND DUTASTERIDE PEAKS SHOWING GOOD RESOLUTION WITH MOBILE PHASE SODIUM PHOSPHATE BUFFER: ACETONITRILE (30:70)

Report: In this trail I got proper resolution i.e. 10.94 also proper retention time is 2.949 for Tamsulosin and 5.572 for Dutasteride and plate count are within limit so I finalized this trial as optimized method for Tamsulosin and Dutasteride drug

Determination of Retention Time: The optimum retention time at which Tamsulosin and Dutasteride showed good resolution was selected for the proposed method. The chromatograms obtained individually for Tamsulosin and Dutasteride and in combination are presented below.

(a) Determination of Retention Time for Tamsulosin:



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	TAMSULOSIN	2.915	7851152	902928	100.00	1.21	2784

FIG. 3: CHROMATOGRAM FOR RETENTION TIME OF TAMSULOSIN

Report: The Retention time for Tamsulosin was found to be 2.915 min.

Determination of Retention Time for Tamsulosin and Dutasteride:

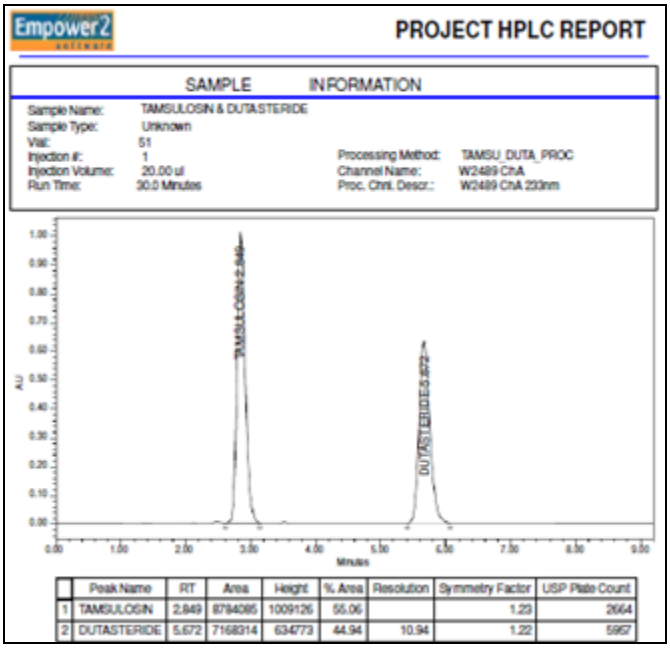


FIG. 4: CHROMATOGRAM FOR RETENTION TIME OF TAMSULOSIN AND DUTASTERIDE IN COMBINATION

Report: The retention time for Tamsulosin was found to 2.949 min and the retention time for Dutasteride were found to be 5.672 min indicating no change in retention time for Tamsulosin and Dutasteride in combination.

System Suitability: This parameter ensures that the analytical system is working properly and can give accurate and precise results.

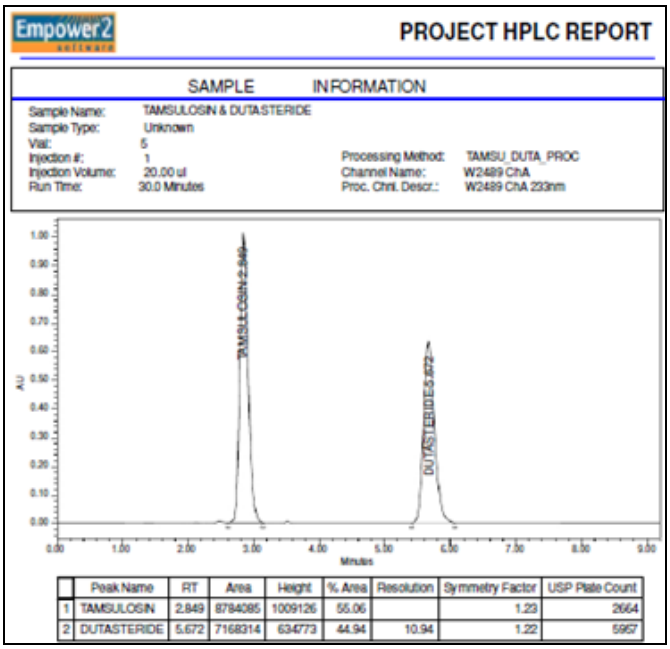


FIG. 5: CHROMATOGRAM OF SYSTEM SUITABILITY PARAMETERS FOR TAMSULOSIN AND DUTASTERIDE

TABLE 4: SYSTEM SUITABILITY DATA OF PARAMETERS FOR TAMSULOSIN AND DUTASTERIDE

System Suitability Factor	Tamsulosin	Dutasteride	Acceptance Criteria
Tailing factor	1.23	1.22	2
HETP(mm)	0.032	0.021	-
Resolution	10.94	-	
Theoretical plates	2664	5957	>1500

Report: The obtained system suitability parameters for Tamsulosin and Dutasteride were found to be within the acceptance criteria.

Validation of analytical method for the assay of Tamsulosin and Dutasteride: The HPLC method developed was validated by performing the various method validation parameters like specificity, LOD, LOQ, linearity, range, precision, robustness, accuracy, and system suitability parameters as per ICH guidelines.

This is to ensure that the performance characteristics of the HPLC method developed meets the requirements for the intended analytical applications.

Accuracy: This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery.

These studies were performed at three different levels (50%, 100% and 150%) and the % recovery of Tamsulosin and Dutasteride was calculated. 20µL of three different solutions of Tamsulosin and Dutasteride were injected repeatedly into chromatograph, the peak area and chromatogram were recorded and are presented below.

TABLE 5: RECOVERY STUDY DATA FOR TAMSULOSIN AND DUTASTERIDE

Standard and Sample	Tamsulosin	Dutasteride
Standard 1 peak area	8696023	7118324
Standard 2 peak area	8697704	7116756
Standard 3 peak area	8706902	7119646
avg. std peak area	8700209.667	7118242
sample peak area	8731772	7144523

TABLE 6: RECOVERY STUDY DATA FOR TAMSULOSIN IN 50%, 100%, 150% SPIKE

Spike	Area Average	Amt. Recovery	% Recovery
50%	13001703.33	49.08	98.16
100%	17352034	99.08	99.08
150%	21616174.67	148.09	98.73

TABLE 7: RECOVERY STUDY DATA FOR DUTASTERIDE IN 50%, 100%, 150% SPIKE

Spike	Area Average	Amt. Recovery	% Recovery
50%	10672883.67	49.57	99.14
100%	14292274	100.41	100.41
150%	17815158.67	149.91	99.94

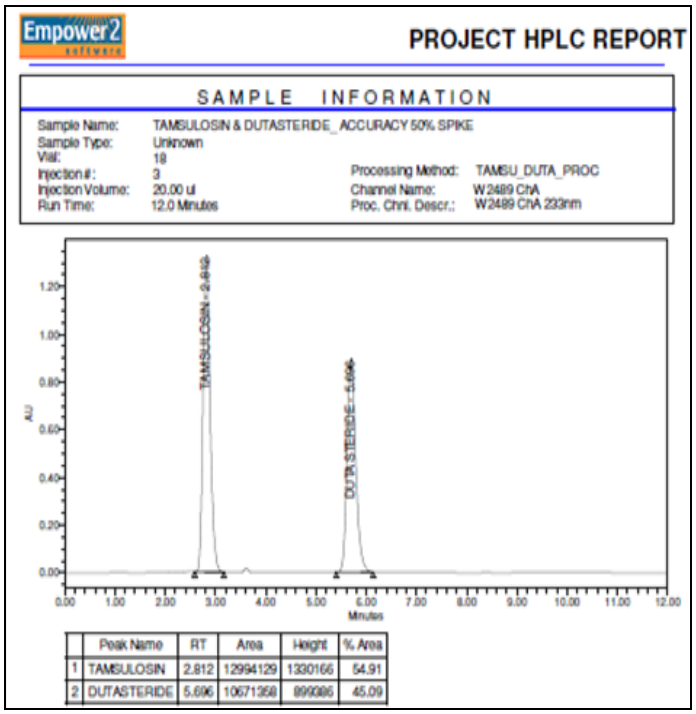


FIG. 6: CHROMATOGRAM FOR RECOVERY STUDIES AT 50% LEVEL

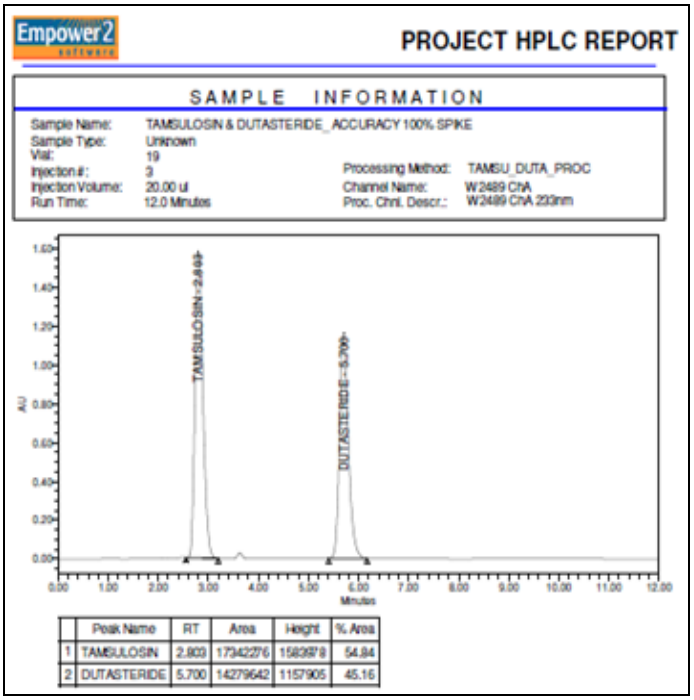


FIG. 7: CHROMATOGRAM FOR RECOVERY STUDIES AT 100% LEVEL

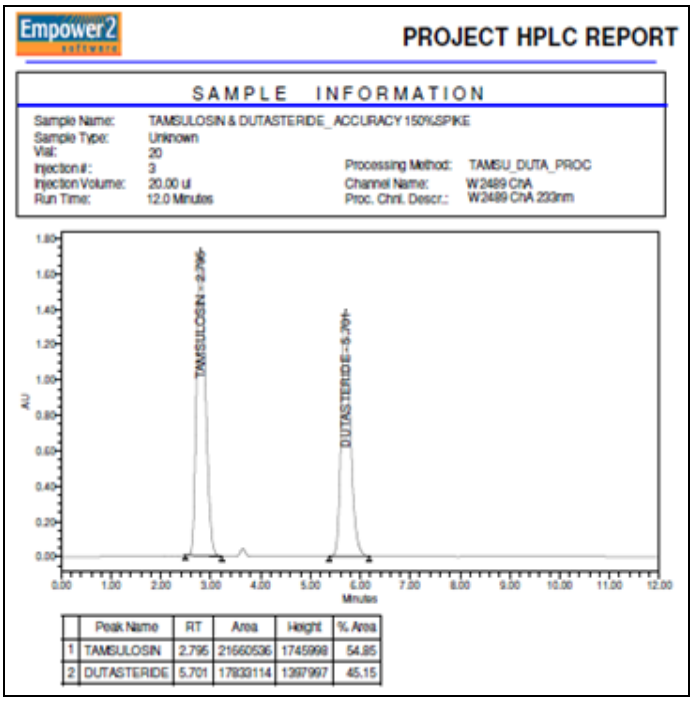


FIG. 8: CHROMATOGRAM FOR RECOVERY STUDIES AT 150% LEVEL

Report: The mean percentage recovery for Tamsulosin and Dutasteride at three different levels was found to be between 98.03-100.11% and 99.29-99.47% respectively, which are well within the limit and hence the method was found to be accurate.

Precision: Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) it is performed to see the closeness of agreement between the series of measurements. It is determined by assaying sufficient number of samples and their relative standard deviation is determined.

System Precision: This method validation parameter was performed to ensure the closeness of results between true value and experimental value. The peak area was recorded and is presented below.

TABLE 8: SYSTEM PRECISION DATA FOR TAMSULOSIN AND DUTASTERIDE

Replicates	Tamsulosin	Dutasteride
	Peak Area	Peak Area
1	8744162	7165098
2	8771446	7174872
3	8795501	7182710
4	8800042	7181358
5	8793223	7182604
6	8834993	7222335
Average	8789895	7184830
Standard Deviation	30368.3	19578.78
%RSD	0.35	0.27

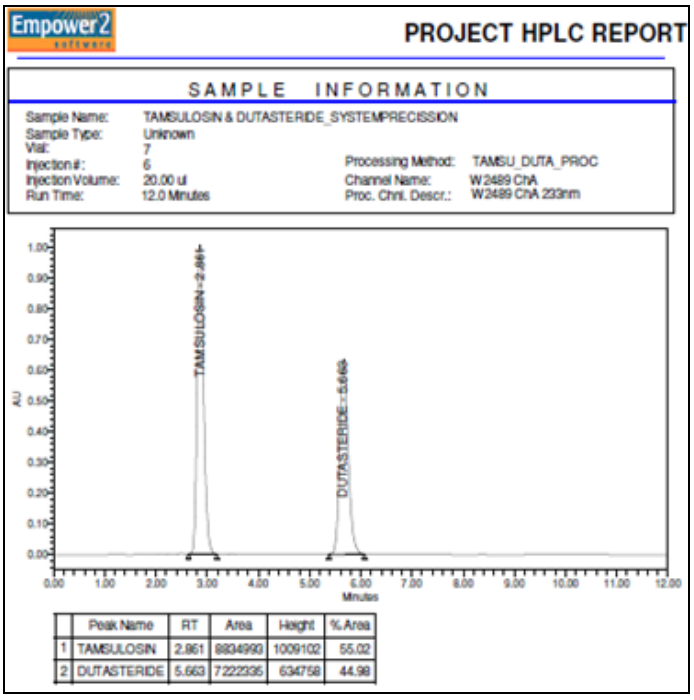


FIG. 9: CHROMATOGRAM OF TAMSULOSIN AND DUTASTERIDE FOR SYSTEM PRECISION

Report: The % RSD values of peak area for six replicate injections of Tamsulosin and Dutasteride were found to be 0.35 and 0.27 respectively which are well within the acceptance criteria limit of NMT 2%.

Method Precision (Repeatability): The method precision was performed to standardize methodology i.e. to check whether the developed method is precise i.e. whether the method is giving consistent results.

The % RSD for peak area and was calculated.

TABLE 9: METHOD PRECISION DATA TAMSULOSIN AND DUTASTERIDE

Replicates	Tamsulosin	Dutasteride
	Peak Area	Peak Area
1	8815237	7199591
2	8829171	7179654
3	8812346	7188695
4	8802160	7178517
5	8785089	7175974
6	8796167	7177897
Average	8806695	7183388
Standard Deviation	15545.38	9093.837
%RSD	0.18	0.13

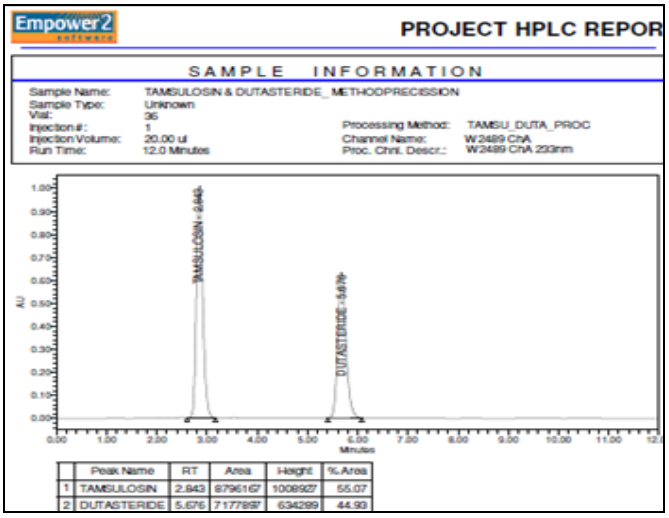


FIG. 10: CHROMATOGRAM OF TAMSULOSIN AND DUTASTERIDE FOR METHOD PRECISION

Report: The % RSD values of concentration for six replicate injections of Tamsulosin and Dutasteride were found to be 0.18 and 0.13 respectively which are well within the acceptance criteria limit of NMT 2%.

Specificity: Specificity was performed to assess and ensure that the impurities, degraded products do not interfere with peaks of analyses.

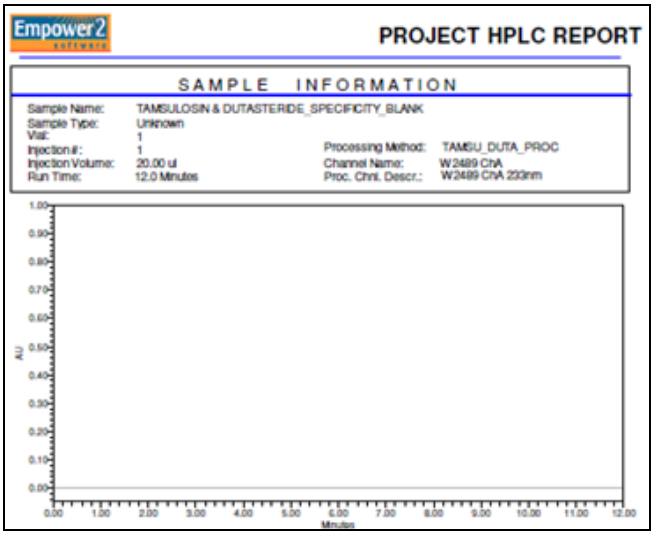


FIG. 11: CHROMATOGRAM FOR SPECIFICITY (BLANK)

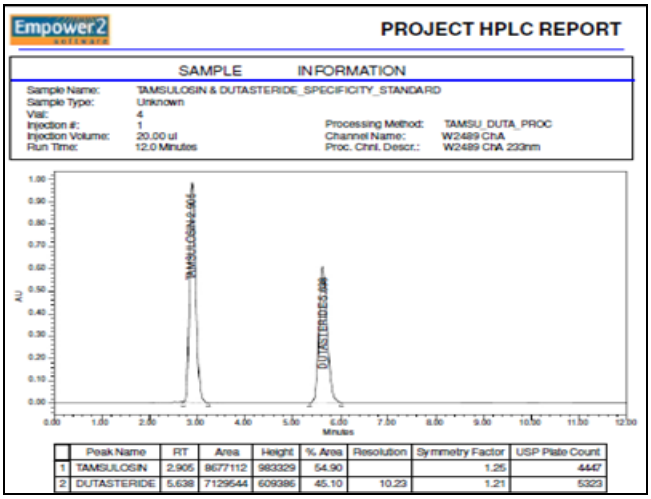


FIG. 12: CHROMATOGRAM FOR SPECIFICITY (STANDARD)

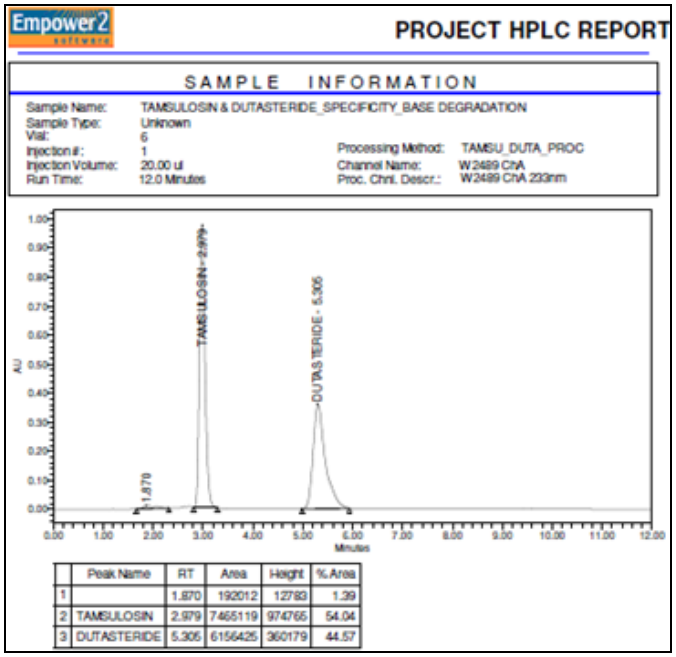


FIG. 13: CHROMATOGRAM FOR SPECIFICITY (ALKALI)

Observation: The analyses do not show any interference with the degraded components.

Report: As there is no interference with the degraded components at retention time of 2.979 (Tamsulosin) and 5.305 min (Dutasteride), hence the proposed method was specific for the detection of Tamsulosin and Dutasteride in combination product.

Limit of Detection (LOD): Limit of detection is the lowest concentration of the analyze that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyze can be detected is determined from the standard deviation of the response and the slope by applying the formula.

Report: The lowest concentration of Tamsulosin that can be detected, was determined from standard curve was 1.151µg/Mr. The lowest concentration of Dutasteride that can be detected was determined from standard curve was 4.498µg/mL.

Limit of Quantification (LOQ): Limit of quantification is the lowest concentration of the analysis in a sample that can be estimated quantitatively. By injecting decreasing amount of drug, with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantification can be obtained from the standard deviation of the response and the slope by applying the following formula.

$$\text{Limit of quantification} = \sigma/S \times 10$$

Report: The lowest concentration at which peak can be quantified is called LOQ, which was found to be 3.593µg/ml for Tamsulosin. The lowest concentration at which peak can be quantified is called LOQ, was found to be 14.041µg/ml for Dutasteride.

Linearity and Range: The linearity was performed to ensure that the test results are directly proportional to the concentration of analyze sample. The results obtained are tabulated below.

TABLE 10: LINEARITY RANGE DATA FOR TAMSULOSIN AND DUTASTERIDE

Volume of stock solution (ml)	Volume made up to (ml)	Concentration (µg/ml)		Tamsulosin Peak Area	Dutasteride Peak Area
		TAM.	DUTA.		
2.5	100	10	12.5	2233029	1776646
5	100	20	25	4381418	3558107
7.5	100	30	37.5	6581161	5368907
10	100	40	50	8770991	7162552
12.5	100	50	62.5	10920404	8939493
15	100	60	75	13104548	10756534

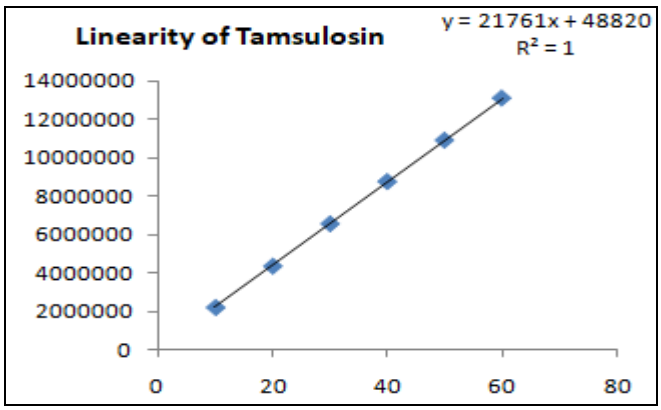


FIG. 14: LINEARITY RANGE GRAPH OF TAMSULOSIN

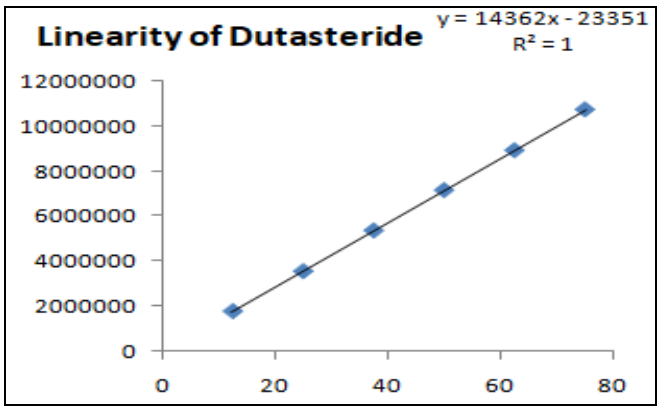


FIG. 15: LINEARITY RANGE GRAPH OF DUTASTERIDE

TABLE 11: LINEARITY REPORT FOR TAMSULOSIN AND DUTASTERIDE

Parameters	Tamsulosin	Dutasteride	Acceptance Criteria
Linearity Range	10-60 µg/ml	12.5-75 µg/ml	-
Regression Equation	y = 217613x+48820	y = 134628x+23351	-
Correlation Coefficient	1	1	0.999
Percentage curve Fitting	100%	100%	99.0%
Intercept	48820	23351	-
Slope	87045	14362	-

Report: The linearity in response for Tamsulosin and Dutasteride was observed in the concentration range of 10 to 60µg/ml and 12.5 to 75µg/ml respectively, with percentage curve fittings found to be well within the limits of acceptance criteria (99%).

Robustness: Robustness was performed to check the ability of the system to give unaffected results for small deliberate changes in system parameters and method parameters.

(a) Change in Flow Rate:

TABLE 12: ROBUSTNESS DATA FOR CHANGE IN FLOW RATE

Drug	Change in flow Rate	Peak Area	% Assay
Dutasteride and Tamsulosin	0.9 ml	9156859	101.37
	1.1 ml	7491021	104.63

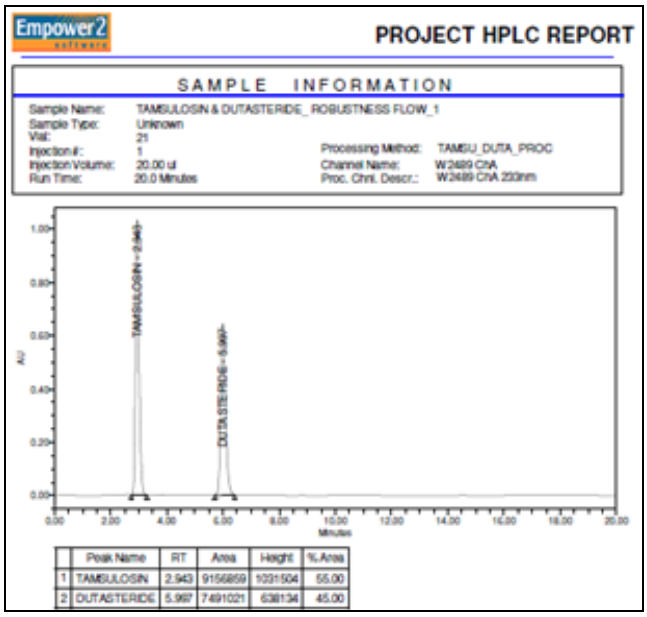


FIG. 16: CHROMATOGRAM FOR CHANGE IN FLOW RATE (0.9ML/MIN)

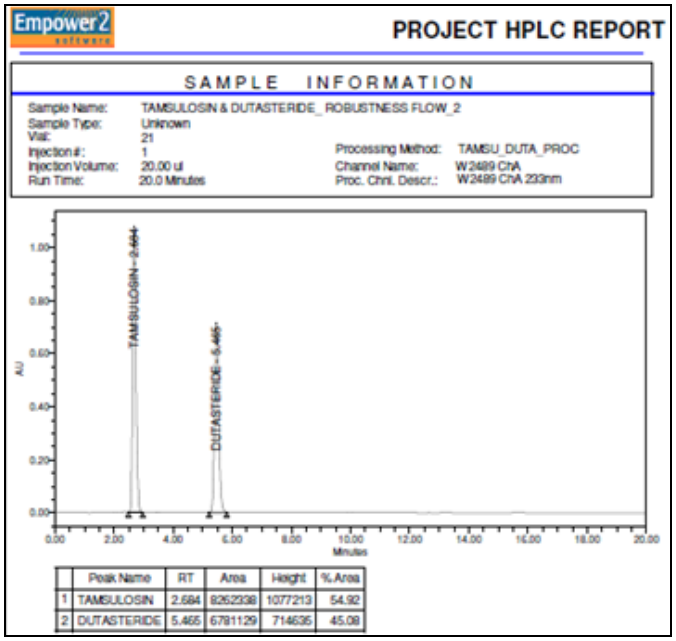


FIG. 17: CHROMATOGRAM FOR CHANGE IN FLOW RATE (1.1ML/MIN)

Report: The % assay were found to be 101.37%, 104.63% for Tamsulosin and Dutasteride when the flow rate was deliberately changed to 0.9 ml and 1.1mL respectively, indicating that the method was found to be robust with deliberate change in flow rate.

CONCLUSION: Stability indicating HPLC were developed for the simultaneous estimation of Tamsulosin and Dutasteride in marketed formulation. The HPLC system used was WATERS Alliance series System with Rheodyne injector 20 µL and the column Inertsil ODS 250 x 4.6 mm, 5 µm. The mobile phase comprised of Sodium phosphate buffer and Acetonitrile in the ratio of 30:70 v/v and flow rate of 1.0 ml/min with UV detection at 233 nm produced peaks of Tamsulosin and Dutasteride in the chromatogram which were well resolved with retention time of 2.939 min and 5.572 min respectively.

The developed HPLC method was validated for various parameters like accuracy, precision, specificity, LOD, LOQ, linearity, range and robustness as per ICH guidelines. The results obtained were well within the acceptance criteria for all the parameters. The proposed method was applied for simultaneous estimation of Tamsulosin and Dutasteride in marketed formulations (Tablet). The assay results conformed to the label claim of the formulation. Hence the proposed method can be used for the routine analysis of Tamsulosin and Dutasteride in their marketed tablet dosage formulations.

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CONFLICT OF INTEREST: Authors declare no conflict of interest.

REFERENCES:

1. Dinnimath BM and Gaonkar SG: Quantitative estimation of drugs for erectile dysfunction and benign prostatic hyperplasia by high performance liquid chromatography: a review. Drug Analytical Research 2024; 8(2): 7-24.
2. Poposka Z, Memeti S, Shishovska M, Mustafa Z, Starkoska K and Arsova-Sarafinovska Z: Identification of counterfeit medicines for erectile dysfunction by validated RP-HPLC method 2023; 5(5): 135-146.
3. Reddy GS, Kumar SA, Debnath MA and Kumar VR: 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. Asian J Pharm Clin Res 2019; 7(2): 105-13.
4. Nagaraju P, Prasad BD and Priyadarshini GI: Development and validation of a Reversed Phase HPLC method for simultaneous determination of Tamsulosin and Dutasteride in tablet dosage form. Advance Pharmaceutical Journal 2019; 2(4): 134-8.
5. Varshini CA, Kumari KS, Sushma S and Prakash K: Development and validation of RP-HPLC method for simultaneous estimation of Alfuzosin Hydrochloride and Dutasteride in bulk and pharmaceutical dosage form. Inventi Rapid: Pharm Analysis & Quality Assurance 2023; 20(4): 191-206.

6. Venkatesh P, Devi DV, Hepcy Kalarani D, Lakshman Kumar D and Purushothaman M: Analytical Method Development and Validation for the Simultaneous Estimation of Tamsulosin and Dutasteride in its Combined Tablet Dosage Form by UV Spectrophotometry and RP-HPLC Methods. Int J Pharm Med Res 2020; 3(4): 30-7.
7. Prasad YR: Determination of dutasteride for analytical method development and validation in bulk as well as in pharmaceutical dosage form by using RP-HPLC: https://doi. org/10.54037/WJPS. 2022.100906. World Journal of Pharmaceutical Sciences 2022; 61-9.
8. Deshmukh AV, Shirode MA and Kadam VJ: Analytical Method Development and Validation for the Quantitative Estimation of Dutasteride in its Tablet Dosage form by RP-HPLC Method.
9. Paljashuva K and Ramarao N: Simultaneous estimation of dutasteride and silodosin in bulk form by RP-HPLC method. World Journal of Current Medical and Pharmaceutical Research 2019; 148-63.
10. Patel D and Patel N: Validated RP-HPLC and TLC methods for simultaneous estimation of tamsulosin hydrochloride and finasteride in combined dosage forms. Acta Pharmaceutica 2021; 60(2): 197-205.
11. Subramanian P and Rajinikanth PS: Development and validation of a new RP-HPLC method for the estimation of dutasteride in bulk and pharmaceutical formulations. J of Applied Pharmaceutical Science 2020; 6(12): 047-55.
12. Ali M and Sharma DK: A New Method Development and Validation of 5a Reductase Inhibitor (Dutasteride) Using R-HPLC in Bulk and Pharmaceutical Dosage Form (Dexamethasone Use as an Internal Standard). Journal of Advanced Zoology 2023; 44(5).
13. Sweetman SC: editor. Martindale, the Complete Drug Reference. 34th ed. London: The Pharmaceutical Press 2005; 1549.
14. Budavari S: editor. The Merck Index. 13th ed. Whitehousestation, NJ: Merck & Co Inc 2001; 3504.
15. Ramakrishna NV, Vishwottam KN, Puran S, Koteshwara M, Manoj S and Santosh M: Selective and rapid liquid chromatography-tandem mass spectrometry assay of dutasteride in human plasma. J Chromatogr B 2022; 809: 117–24.
16. Kamat SS, Choudhari VB, Vele VT and Prabhune SS: Determination of dutasteride by LC: validation and application of the method. Chromatographia. 2023; 67: 911–6.
17. Subba Rao DV and Radhakrishnanand P: Stress degradation studies on dutasteride and development of a stability-indicating HPLC assay method for bulk drug and pharmaceutical dosage form. Chromatographia 2022; 67: 9–10.
18. Agarwal S, Gowda KV, Sarkar AK, Ghosh D, Uttam B and Chattaraj T: Simultaneous determination of tamsulosin and dutasteride inhuman plasma by LC-MS-MS. Chromatographia 2024; 67: 11–2.
19. Ch. Amrutha Varshini, K Shantha Kumari, S Sushma and K Prakash: Development and Validation of RP-HPLC Method for Simultaneous Estimation of Alfuzosin Hydrochloride and Dutasteride in Bulk and Pharmaceutical Dosage Form. Inventi Rapid: Pharm Analysis & Quality Assurance 2019(4): 1-4, 2012.
20. Dipti B. Patel, Patel NJ, Prajapati AM and Patel SA: RP-HPLC method for the estimation of dutasteride in pharmaceutical dosage form. Journal of Planar Chromatography 2022; 24(3): 218-221.
21. Patel DB and Patel NJ: Validated reverse phase high performance liquid chromatographic and high performance thin layer chromatographic method for simultaneous analysis of tamsulosin hydrochloride and dutasteride in pharmaceutical dosage form. Acta Chromatographia 2018; 22(3): 419-431.
22. Shivprasad S. Deshmukh, Vaishali V. Musole, Vidhya K. Bhusari, Sunil R. Dhaneshwar. Validated HPTLC method for simultaneous analysis of Alfuzosin Hydrochloride 2019; 5(9): 561-575.
23. Dipti B. Patel, Natubhai J. Patel, Sejal K. Patel and Paresh Patel: Validated stability indicating HPTLC method for the determination of dutasteride in pharmaceutical dosage form. Chromatography Research International, Article ID 278923, 2011; 10(8): 625-637.
24. Vishnu P .Choudhari and Anna Pratima Nikalije: Stability indicating TLC method for the determination of dutasteride in pharmaceutical dosage form. Chromatographia 2019; 70(1-2): 309-313.
25. Ramakrishna NVS, Vishwottam KN, Puran S, Koteshwara M, Manoj S and Santosh M: Selective and Rapid liquid chromatographytandem mass spectrometry assay of Dutasteride in human plasma. JCB 2024; 8(5): 117-114.
26. Noel A. Gomes, Ashutosh Pudage, Santosh S. Joshi, Vikas V. Vaidya, Sagar A. Parekh and Amod V, Tamhankar: Rapid and Sensitive LC-MS-MS method for the Simultaneous Estimation of Alfuzosin and Dutasteride in Human Plasma. Chromatographia 2022; 6(4): 9-18.
27. Md.Ruhul, Amin, Moynul, Hasan and Abdullah, AlMasu: Validated UV spectrophotometric estimation of dutasteride in tablet dosage form. International journal of comprehensive Pharmacy 2018; 2(4): 22-31.
28. Shivprasad S. Desmukh, Shweta S. Havele, Vaishali V. Musale and Sunil R. Dhaneshwar: Development and validation of RP-HPLC method for simultaneous estimation of alfuzosin hydrochloride and dutasteride in pharmaceutical dosage form. Der Pharmacia Lettre 2021; 2(6): 342-349.
29. Chandrasekhar K and Manikandan A: Novel RP-HPLC method development and validation of Tamsulosin HCl and dutasteride in tablets by ratio's method. Rasayan J Chem 2021; 14(2): 665-71.
30. Skoog, Holler and Nieman: Principals of Instrumental Analysis, 5thEdition, Harcourt Publishers International Company 2011; 543-554.
31. William Kemp: Organic Spectroscopy, Palgrave, New York 2005; 7-10: 328-330.
32. P.D. Sethi. HPLC: Quantitative Analysis Pharmaceutical Formulations, CBS Publishers and distributors, New Delhi (India) 2011; 3-137.
33. Michael E, Schartz IS and Krull: Analytical method development and Validation 2014; 25-46.
34. Snyder R, Kirkland J and Glajch L: Practical HPLC method development, II Ed, A Wiley International publication 2017; 235: 266-268,351-353.653-600.686-695.
35. Berry RI and Nash AR: Pharmaceutical Process Validation, Analytical method validation, Marcel Dekker Inc. New work 2008; 57: 411-28.
36. Anthony C. Moffat, M. David Osselton and Brian Widdop: Clarke's Analysis of Drugs and Poisons, Pharmaceutical Press, London 2019; 1109-1110: 1601-1602.
37. Ravichandiran V: Rapid and Simple RPHPLC Method for the Estimation Dutasteride and Tamsulosin HCl of in Tablet dosage form. Indian J Pharm Sci 2020; 70(2): 198–202.
38. Mousumi Kar: HPLC method for estimation Dutasteride of in formulated microspheres and tablet dosage form. Indian Journal of Pharmaceutical Sciences 2019; 71(3): 318-320.

39. Bhoomaiah: Development and validation of RP-HPLC method for simultaneous determination tamsulosinhcl and finasteride in bulk and pharmaceutical formulation. Int J Pharm Pharm Sci 2022; 6(6): 135-141.
40. Sadhana B. Todkar: The development and validation of liquid chromatography method for simultaneous estimation of dutasteride and tamsulosinhcl in bulk and tablet dosage form with the pre-column derivatization of voglibose. Indo American Journal of Pharmaceutical Research 2021; 3(8): 6555-6561.
41. Kumar SA: Development of stability indicating RP- HPLC method for simultaneous estimation of Dutasteride And TamsulosinHcl phosphate monohydrate in bulk as well as in pharmaceutical formulation, Pelagia Research Library Der Pharmacia Sinica 2019; 4(4): 47-61.
42. Dipti B. Patel, N. J. Patel, A. M Prajapati and S. A. Patel: RP-HPLC method for the estimation of Dutasteride in tablet dosage form. Indian J Pharm Sci 2022; 72(1): 113-116.
43. Priyadarshani SB, Chetan SC, Preeti G, Chatrapal S, Ravindra K, Mahendra SR and Sujata SB: Analytical method development and validation of dutasteride with tamsulosin in pharmaceutical capsule dosage form by RP-HPLC method. International Journal of Pharmacy 2021; 5: 493-499.
44. Sindhura M, Raghavi K, Prashanthi R and Nalluri BN: Simultaneous Estimation of Finasteride and Tamsulosin Hydrochloride in Combined Dosage Forms by RP-HPLC-PDA Method, Journal of Applied Pharmaceutical Science, 2023; 02(06): 203-209.
45. Khaled Bin Sayeed, Sh. Rizwan and Hanifa Begum: Development and validation of stability indicating method for the simultaneous determination of tamsulosin and dutasteride in bulk drugs and pharmaceutical dosage forms using UV-spectrophotometric method. International Journal of Research and Development in Pharmacy and Life Sciences 2022; 4(2): 1434-1446.

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