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Simultaneous Estimation Of Dutasteride And Silodosin In Bulk Form By RP-HPLC Method

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ABSTRACT

Dutasteride and Silodosin both are approved drugs by USFDA (Food & Drug Administration). On literature survey, it was found that few method have been reported for simultaneous estimation of Dutasteride and Silodosin. Therefore, it was thought of interest to develop a simple, accurate, precise, sensitive and economic analytical method and to validate as per ICH guidelines. So RP-HPLC method was developed and validated for simultaneous estimation of Dutasteride and Silodosin in multiunit system. Separation was achieved on Shimadzu HPLC; Agilent C18 Column (250×4.6mm,5µm) by using a mobile phase containing Methanol : Water in 50:50 v/v ratio. Analysis was done at the filow rate of 1.0 ml/mint and PDA detection was carried out by wavelength at 280 nm. The retention time of Silodosin and Dutasteride was found to be 2.050 min & 2.623 min respectively. The method was validated in terms of linearity, precision, accuracy, specificity, robustness etc. The linearity was found to be in the range of 10-50 µg/ml for both Silodosin and Dutasteride with correlation coefficient of 0.999 for Silodosin and 0.999 for Dutasteride. %RSD of method precision was found to be less than 2%. This indicates that the method is precise.

Key words:
Method development,
RP-HPLC,
Validation.

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1. INTRODUCTION

1.1. ANALYTICAL CHEMISTRY

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter.

- **Qualitative:** Deals with the identification of the substance.
- **Quantitative:** Deals with the determination on how much of the constituent is present.

The methods used for the analysis of substance may be done by two methods

I. Classical methods

II. Instrumental methods

I.CLASSICAL METHODS

a. Precipitation

b. Extraction

c. Distillation

II. INSTRUMENTAL METHODS[1-3].

In instrumental analysis, a physical property of a drug is utilized to determine its chemical composition. A study of the physical properties of drug molecules is a prerequisite for product formulation and often leads to a better understanding of the inter-relationship between molecular structure and drug action. In the instrumental analysis some physical properties of molecules such as absorption of radiation, scattering of radiation, Raman Effect, emission of radiation, rotation of the plane of the polarized light and diffraction phenomenon are involving interaction with the radiant energy.

Physical properties encompass specific relations between the molecules and well defined forms of energy e.g. Half-cell potential, current voltage, electric conductivity, dielectric Constant, heat of reaction, thermal conductivity or other yardsticks of measurements. By carefully associating specific physical properties with the chemical nature of closely related molecules conclusions can be drawn that;

- ❖ Describe the spatial arrangement of drug molecules
- ❖ Provide evidence for the relative chemical or physical behaviour of a molecule and
- ❖ Suggest methods for quantitative and qualitative analysis of a particular pharmaceutical agent.

Analytical methods, in a broad sense, can be classified into chemical methods and instrumental methods. Chemical methods are defined as those that depend on chemical operations in combination with the manipulation of simple instruments. In general, the measurement of mass, i.e. gravimetric and of volume, i.e., volumetric analysis falls in this class.

An instrumental method encompasses the use of more complicated instrumentation based on analytical methods. Although in recent years, spectro photometric methods are extensively used, but it would be wrong to conclude that instrumental methods have totally replaced chemical methods. In fact, chemical steps are often an integral part of an instrumental method. The sampling, dissolution, change in oxidation state, removal of excess reagent, pH adjustment, addition of complexing agent, precipitation, concentration and the removal of interferences are the various chemical steps which are part of an instrumental method.

In recent years HPLC (High Performance Liquid Chromatography) is extensively used, because HPLC is not limited by sample volatility or thermal stability. HPLC is able to separate macromolecules and ionic species, labile natural products, polymeric material and a wide variety of other high molecular weight poly-functional group because of the

relatively high pressure necessary to perform this type of chromatography; a more elaborate experimental setup is required.

Because of the high cost of the instrument and costly analytical process. The variation of the colour of a system with change in concentration of some component forms the basis of what the chemists commonly term as colorimetric analysis.

1.2. ANALYTICAL METHOD DEVELOPMENT & VALIDATION STEPS OF METHOD DEVELOPMENT[1-7]

Documentation starts at the very beginning of the development process, a system for full documentation of the development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

- **Analyte standard characterization**

  a) All known information about the analyte and its structure is collected i.e., physical and chemical properties, toxicity, purity, hygroscopic nature, solubility and stability.

  b) The standard analyte (100% purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, desiccators, and freezer).

  c) When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.

  d) Only those methods (MS, GC, HPLC etc.,) that are compatible with sample stability are considered.

- **Method requirements**

  The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectivity, linearity, range, accuracy and precision are defined.

- **Literature search and prior methodology**

  The literature for all types of information related to the analyte is surveyed, for synthesis, physical and chemical properties, solubility and relevant analytical methods. Books, periodicals, chemical manufacturers and regulatory agency compendia such as USP / NF, Association of Official Analytical Chemists (AOAC) and American Society for Testing and Materials (ASTM) publications are reviewed. Chemical Abstracts Service (CAS) automated computerized literature searches are convenient.

- **Choosing a method**

  a) Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analysis and samples.

  b) If there is no prior method for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest.

- **Instrumental setup and initial studies**

  a) The required instrumentation is setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified.

  b) Always new consumables (e.g. solvents, filters and gases) are used, for example, method development is never started on a HPLC column that has been used earlier.

  c) The analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g., bulk drug), then it is possible to start work with the actual sample.

  d) Analysis is done using analytical conditions described in the existing literature.

- **Optimization**

  During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan and every step is documented (in a lab notebook) in case of dead ends.

- **Documentation of analytical figures of merit**

  The originally determined analytical figures of merit Limit of quantitation (LOQ), Limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc., are documented.

- **Evaluation of method development with actual samples**

  The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

- **Determination of percent recovery of actual sample and demonstration of quantitative sample analysis**

  Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average ± standard deviation) from sample to sample and whether recovery has been optimized has been shown. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainty. The validity of analytical method can be verified only by laboratory studies. Therefore documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications.

2.0 AIM:

The present work is aimed to develop a new, simple, fast, rapid, accurate, efficient, reproducible, RP-HPLC method for the method development and validation analysis of simultaneous estimation of Silodosin and Dutasteride capsules as per ICH guidelines.

3.0 OBJECTIVE:

The analytical method for the method development and validation estimation of Silodosin and Dutasteride capsules will be developed by RP-HPLC method by optimizing the chromatographic conditions. Develop a new, simple, rapid, sensitive, accurate, reproducible, and economical analytical method for the determination of Silodosin and Dutasteride capsules by RP-HPLC method. The developed method is

validated according to ICH guidelines for various parameters specified in ICH guidelines.

4.0 PLAN OF WORK

- Selection of the drug.
- Drug profile.
- Literature review.
- Study of solubility.
- Selection of the method.
- Initial set up of the Chromatographic conditions.
- Method development.
- Optimization of the developed method.
- Validation of the developed method.
- Evaluation of the results.

5.0 DRUG PROFILE

Generic Name: Silodosin (sye-LOE-doe-sin)
Brand Name : Rapaflo
Silodosin is a medication for the symptomatic treatment of benign prostatic hyperplasia. It act as α_1-adrenoreceptor antagonist with high uroselectivity (Selectivity for the prostate)

Silodosin Trade Names:

Rapaflo : USA

Silodyx : Europe and South Africa

Rapilif & Silodal : India

History of Silodosin:

- Silodosin received its first marketing approval in Japan in May 2006 under the trade name Urief, which is jointly marketed by Kissei Pharmaceutical Co., Ltd. And Daiichi Sankyo Pharmaceutical Co., Ltd.
- FDA approved silodosin on October 9, 2008 Silodosin is marketed under the trade names Rapaflo in the US and Silodyx in Europe.

Silodosin Chemical Structure:

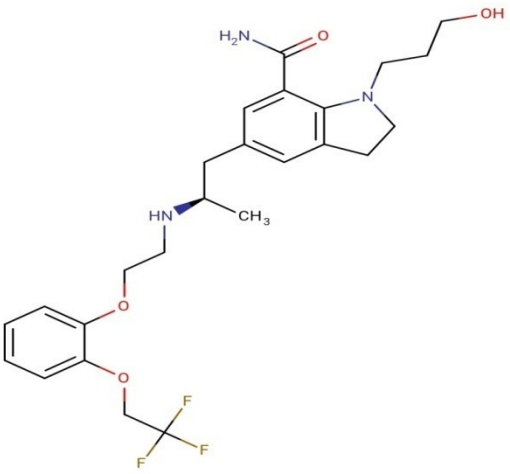


Fig 01: Structure of Silodosin
IUPAC Name: 1-(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino)propyl]indoline-7-carboxamide
Category : Alpha adrenoreceptor antagonists
Molecular Formula: $C_{25}H_{32}F_3N_3O_4$
Molecular Weight: 495.534 g/mol
Solubility : Water Solubility (Very slightly soluble)
PHARMACOKINETIC DATA:
Routes of administration : Oral
Bioavailability : 32%
Protein binding : 97%
Metabolism : Hepatic glucuronidation (UGT2B7-mediated) also minor CYP3A4 involvement.
Biological half life : 13±8 hours
Excretion : Renal and Fecal

Mechanism of action:
Silodosin has high affinity for the α_{1A} adrenergic receptor, it causes practically no orthostatic hypotension (in contrast to other α_1 blockers). On the other side, the high selectivity seems to be the cause of silodosin's typical side effect of loss of seminal emission.
As α_{1A} adrenoceptor antagonists are being investigated as a means to male birth control due to their ability to inhibit ejaculation but not orgasm, a trial with 15 male volunteers was conducted. While silodosin was completely efficacious in preventing the release of semen in all subjects, 12 out of the 15 patients reported mild discomfort upon orgasm. The men also reported the psychosexual side effect of being strongly dissatisfied by their lack of ejaculation.
Major Side Effects:
If any of the following side effects occur while taking silodosin, check with your doctor immediately:

Less common:

- Chills
- Cold seats
- Confusion
- Dizziness,faintness

Incidence not known:

- Abdomnal or stomach pain
- Clay-colored stools
- Dark urine
- Fever
- Headache
- Itching

Minor Side Effects:
More common:

- Change or problem with discharge of semen

Less common:

- Diarrea
- Muscle aches
- Sore throat
- Stuffy or runny nose

Uses:
Silodosin is a prescription medication used for the treatment of the signs and symptoms of benign prostatic hyperplasia (BPH) including:

- Silodosin is an alpha-blocker. It works by relaxing muscles in the prostate and bladder, which helps to improve urine flow and reduce symptoms of BPH.
- Difficulty urinating (hesitation, dribbling, weak stream, and incomplete bladder emptying)
- Painful urination.
- Urinary frequency and urgency.

This medication may be prescribed for other uses. Ask your doctor or pharmacist for more information.

Silodosin Usage

- Take silodosin exactly as prescribed.
- Silodosin comes in capsule form and is taken once daily, with food.
- If you miss a dose, take the missed dose as soon as you remember. If it is almost time for the next dose, skip the missed dose and take your next dose at the regular time. Do not take two doses of silodosin at the same time.

Silodosin Dosage

- Take this medication exactly as prescribed by your doctor. Follow the directions on your prescription label carefully.
- The recommended dose of silodosin is 8mg once daily, with a meal.
- The recommended dose of silodosin for those with moderate renal impairment is 4mg once daily, with a meal.

Silodosin Overdose

If you take too much silodosin, call your healthcare provider or local Poison Control Center, or seek emergency medical attention right away.

Other Requirements

- Store silodosin at room temperature
- Keep this and all medicines out of the reach of children.

DRUG PROFILE OF DUTASTERIDE

- **Generic Name:** Dutasteride (doo-TAS-ter-ide)
- **Brand Name** : Avodart
- **Dutasteride** is a medication used to treat benign prostatic hyperplasia (enlarged prostate) and androgenetic alopecia (pattern hair loss).

It was developed by GlaxoSmithKline and is a 5α-reductase inhibitor which prevents the conversion of the androgen sex hormone testosterone into the more potent dihydrotestosterone (DHT). The drug has been licensed for the treatment of androgenetic alopecia in South Korea since 2009, but has not been approved for this specific indication in the United States, though it is commonly used off-label.

History of Dutasteride:

Dutasteride was patented in 1996 and was first described in the scientific literature in 1997. It was approved by the FDA for the treatment of BPH in November 2001 and was introduced into the U.S. market the following year under the brand name Avodart. Dutasteride has been introduced in many other countries as well, including throughout Europe and South Americ. The patent protection of dutasteride expired in November 2015 and the drug has since become available in the U.S.in a variety of low-cost generic formulations.

Silodosin Chemical Structure:

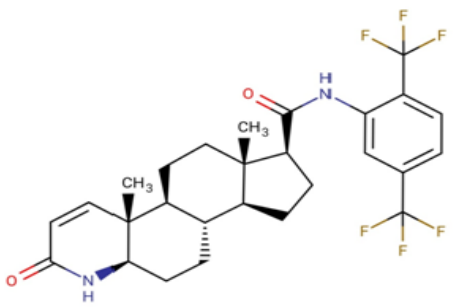


Fig 02: Structure of Dutasteride

IUPAC Name: (5α, 17β)-*N*-{2,5-Bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide

Category : Alpha adrenoreceptor antagonists

Molecular Formula: $C_{27}H_{30}F_6N_2O_2$

Molecular Weight : 528.53 g/mol

Solubility : Soluble in **ethanol** (44 **mg**/ml), methanol (64 **mg**/ml) , **polyethylene glycol** 400 (3 **mg**/ml), and **DMSO** (62 **mg**/ml at 25° C 117mM).Insoluble in water.

Pharmacokinetic Data:

Routes of administration : Oral

Bioavailability : 60%

Protein binding : 99%

Metabolism : Liver (CYP3A4-mediated)

Biological half life : 4 to 5 weeks

Excretion : Feces

Mechanism of action:

Dutasteride belongs to a class of drugs called 5α-reductase inhibitors, which block the action of the 5α-reductase enzyme that convert testosterone into DHT. It is an irreversible inhibitor of all three isoforms of 5α-reductase, types I, II, and III. This is in contrast to finasteride, which is similarly an irreversible inhibitor of 5α-reductase but only inhibits the type II and III isoenzymes. As a result of this difference, dutasteride is able to achieve a reduction in circulating DHT levels of as much as 98%, whereas finasteride is only able to achieve a reduction of 65 to 70%. In spite of the differential reduction in circulating DHT levels, the two drugs decrease levels of DHT to a similar extent of approximately 85 to 90% in the prostate gland, where the type II isoform of 5α-reductase predominates.

Major Side Effects:

Rare:

- Chest pain or discomfort
- Dilated neck veins
- Extreme fatigue
- Irregular breathing
- Shortness of breath

Incidence not known:

- Blistering, flaking, or peeling of the skin
- Cough
- Difficulty with swallowing
- Dizziness
- Fast heartbeat

Minor Side Effects:

- Abnormal ejaculation, Impotence
- Decreased interest in sexual intercourse, performance or desire
- Loss in sexual ability or performance

Uses:

- Dutasteride is occasionally used for treating benign prostatic hyperplasia (BPH); colloquially known as an "enlarged prostate".
- In those who are being regularly screened, 5α-reductase inhibitors such as finasteride and dutasteride reduce the overall risk of being diagnosed with prostate cancer,& treat for androgenetic alopecia in South Korea at a dosage of 0.5 mg/day.
- There is insufficient data to determine if they have an effect on the risk of death and may increase the chance of more serious cases.
- Dutasteride has also been used off-label in the treatment of female pattern hair loss.

6.0 MATERIALS AND METHODS

INSTRUMENTS USED

S. No	Instrument	Model
1	HPLC	WATERS e 2695 separation module. PDA WATERS 2998 detector. software: EMPOWER
3	Digital pH meter	LAB INDIA
4	Weighing machine	SHIMADZU ATX 224
5	Pipettes	Borosil
6	Beakers	Borosil
7	Vacuum filter	Vacuum PR, Pump (MERCK) 4BAR 220v/50Hz
8	Ultrasonic bath	LOBA LIFE

Table 01: Representation of various Instruments Used

CHEMICALS USED

S. No	Chemical	Brand
1	Silodosin and Dutasteride	Rapaflo and Avodart
2	Potassium hydrogen phosphate	MERCKS
3	Methanol for HPLC	MERCKS
4	Acetonitrile for HPLC	MERCKS
5	Orthophosphoric acid	LOBAL CHEMICALS
6	Water	Fisher Scientific

Table 02: Representation of various Chemicals Used

7.0 METHOD DEVELEPMENT

METHOD OPTIMIZATION

Mobile Phase Optimization

Initially the mobile phase tried was Phosphate buffer:Acetonitrile: Methanol (20:40:40 v/v). Then tried with 0.1% Orthophaspharicacid : Acetonitrile in varying proportions and then with 0.1% Orthophaspharicacid: Methanol (60:40 v/v) and later with Methanol: 0.1% Orthophaspharic acid (60:40 v/v) with various combinations as varying proportions. Finally, the mobile phase was tried with Methanol: 0.1% Orthophaspharic acid (75:25 v/v) respectively and then it was optimized.

Optimization of Column

The method was performed with various columns like Zorbax SB, cosmicsil, Zodiac columns. Agilent C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

TRAIL-1:

PREPARATION OF MOBILE PHASE

Preparation of Phosphate buffer pH 3.00

Accurately weighed 1.8918 grams of Dipotassium Hydrogen Phosphate was taken in a 1000ml volumetric flask & add 500ml of HPLC grade water (Milli-Q water) & sonicate for 10 min. Filter through 0.45 µ filter under vacuum filtration unit & makeup to 1000ml with HPLC grade water (Milli-Q water) and then the pH was adjusted to 3.00 with Orthophosphoric acid solution.

Preparation of mobile phase

Accurately measured 200 ml (20%) of above buffer and 800 ml (80%) of organic mixture containing Acetonitrile, Methanol of each 400 ml. were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of diluents

Accurately measured 200 ml (20%) of above buffer and 800 ml (80%) of organic mixture containing Acetonitrile, Methanol of each 400 ml. were mixed and sonication in an ultrasonic water bath for 10 minutes.

Instrument	Waters e HPLC 2695 with PDA 2998 detector & EMPOWER
	Software
Temperature	Column temperature 40^0C, Sample temperature 25^0C
Column	Agilent C18 (4.6 X250mm, 5µm)
PH	3.00
Buffer	1.8918 grams of Dipotassium Hydrogen Phosphate in 1000 ml
	water: PH 3.00 was adjusted with Orthophosphoric acid
Mobile phase	20% Buffer, 40% Methanol, 40% Acetonitrile.
Flow rate	1.5 ml per min
Wavelength	260 nm for Silodosin & Dutasteride
Injection volume	10 µl
Run time	7min

Table 03 Optimized Chromatographic Conditions for Trial-1

Preparation of blank

- Diluent is used as blank solution

Preparation of Standard

Preparation of Dutasteride Standard Stock Solution

Accurately weighed 6.318 mg of Dutasteride working standard was taken in a 100ml volumetric flask. Initially add 30 ml of methanol for dissolved Dutasteride API and sonication in an ultrasonic water bath for 20 minutes. Make up to 100 ml volumetric flask with methanol and mixed well.

Preparation of Silodosin & Dutasteride Standard

Accurately weighed 80.4 mg of Silodosin working standard was taken in a 100ml volumetric flask. Initially add 30 ml of methanol for dissolved Silodosin API and sonication in an ultrasonic water bath for 5 minutes and add 1 ml of Dutasteride standard stock solution. Make up to 100 ml volumetric flask with methanol and mixed well.

Further, Pippet out 5 ml of above solution in 50 ml volumetric flask and make up with diluent up to 50 ml mark and mixed well.

PROCEDURE

The system suitability is an inject the one injection blank solution and inject five injections of standard solution.

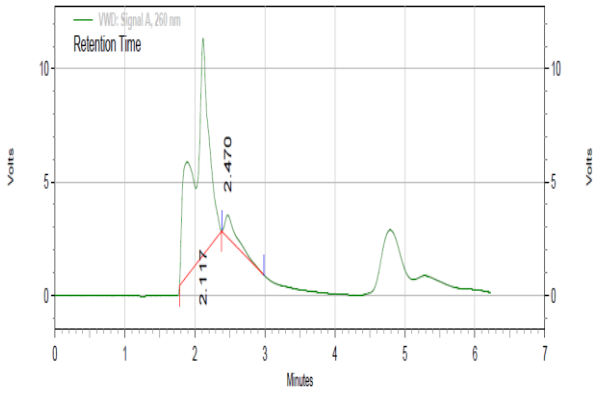


Figure No: 03 Chromatogram for Silodosin and Dutasteride Trial-1

Result: Uniform resolution was not observed.

7.0 RESULTS AND DISCUSSION:

A simple isocratic high-performance liquid chromatographic method was developed for the determination of Silodosin and Dutasteride in pure form and in laboratory prepared capsule formulations using analytical column C18 (250×4.6mm,5µm) equilibrated with mobile phase containing combination of Methanol & 0.1%orthophosphoric acid in ratio of 75:25v/v at flow rate of 1.0 ml/min and eluent was monitored at 270 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 5 min. Experimental conditions such as ratio of mobile phase, flow rate, selection of wavelength, etc. were critically studied and the optimum conditions were selected.

SYSTEM SUITABILITY

System suitability solution (Silodosin 50µg/ml and Dutasteride 100µg/ml) was prepared as per the method and analysed six times. The following table shows the peak area for Silodosin and Dutasteride.

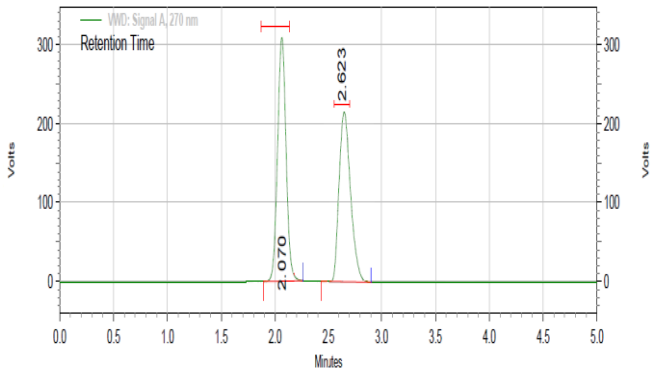
Table No: 04 Standard results of Silodosin and Dutasteride:

S.No	Silodosin peak area	Dutasteride peak area
1	390771	1482789
2	391458	1482876
3	392512	1482564
4	390123	1482654
5	391122	1488765
6	391542	1482675
Average	391254.7	1483721
SD	804.6111	2473.695
%RSD	0.206	0.167

Acceptance criteria:

The %RSD of peak area of all peaks for the six replicate injections should be not more than 2.0.

Figure No: 04 Chromatogram for system suitability injection-1



Acceptance criteria: The %RSD of peak area of all peaks for the six replicate injections should be not more than 2.0.

Figure No: 05 Chromatogram for system suitability injection-1

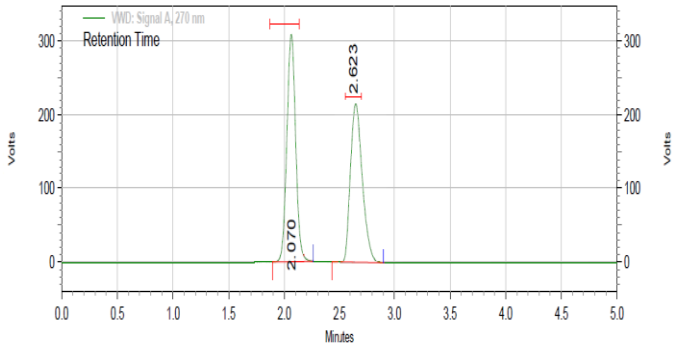


Figure No: 06 Chromatogram for system suitability injection-2

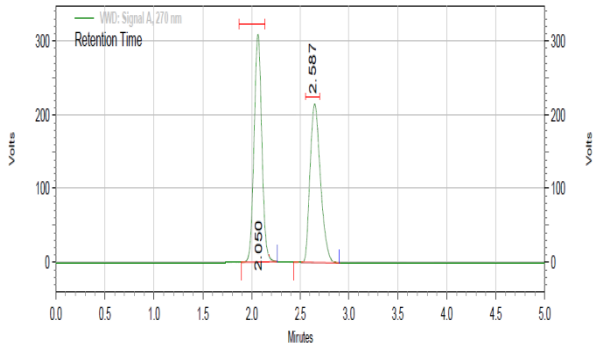


Figure No: 07 Chromatogram for system suitability injection-3

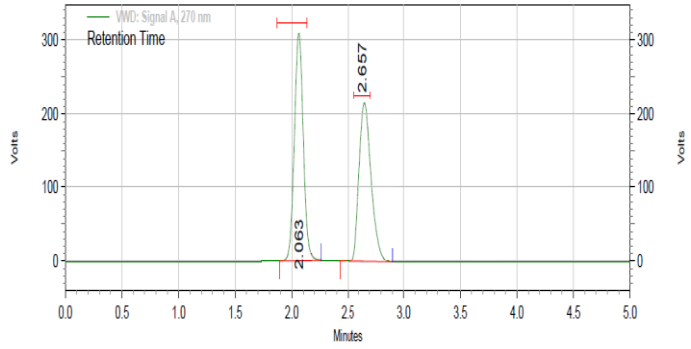


Figure No: 08 Chromatogram for system suitability injection-4

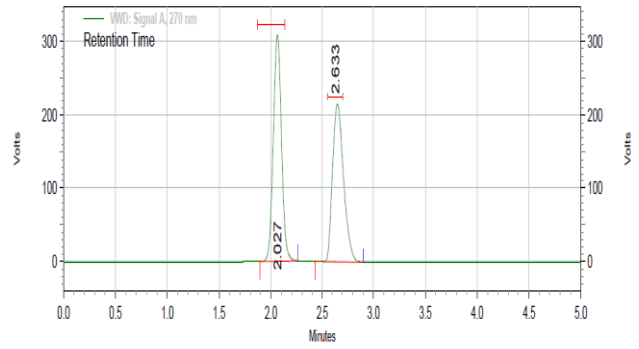


Figure No: 09Chromatogram for system suitability injection-5

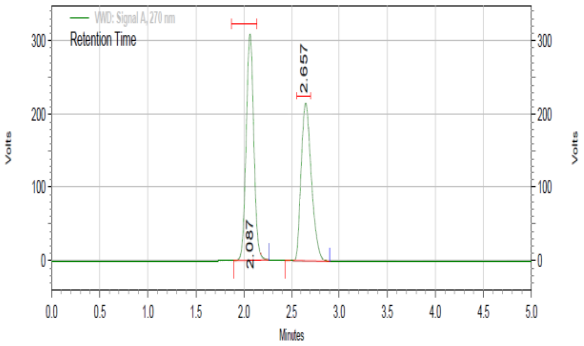
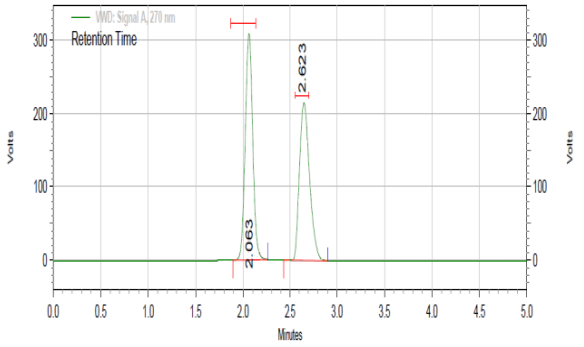


Figure No: 10 Chromatogram for system suitability injection-6



RESULT:

System suitability studies are summarized in the above table. Six consecutive Results of injections of the standard solution showed uniform retention time and also pass the %RSD of all six replicate injections.

SPECIFICITY

Silodosin and Dutasteride solutions were prepared individually at a concentration of about 20 µg/ml and 20µg/ml samples were also prepared. All the solution were analysed as per the HPLC method. Chromatogram of blank, standard and sample was attached.

Acceptance criteria:

All the peaks are well separated from each other.

Figure No: 11 Chromatogram for blank injection

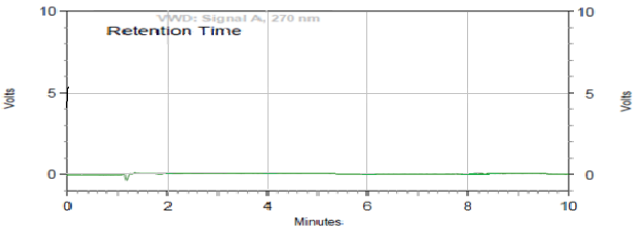
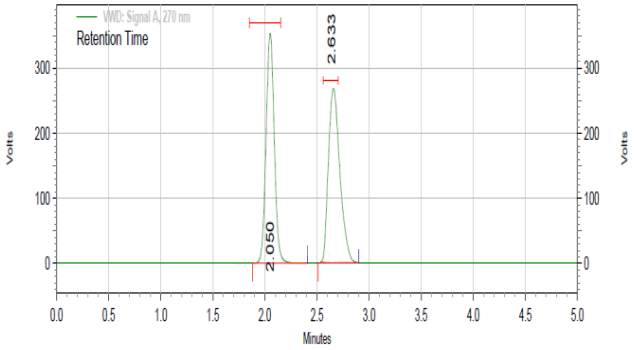


Figure No: 12 Chromatogram for standard injection



Results: All peaks are well separated from each other.

LINEARITY:

The linearity of the HPLC method was demonstrated by analysing the solution ranging from 10 µg/ml- 50 µg/ml of both Silodosin and Dutasteride was prepared.

The result shows the line of best fit for concentration versus peak area of Silodosin and Dutasteride. The corresponding chromatograms are attached.

Table No: 05 standard results of Silodosin and Dutasteride

Silodosin		Dutasteride	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
10	141756	10	258094
20	390771	20	1482876
30	537051	30	2383418
40	724129	40	3094636
50	923152	50	3824462
R^2 = 0.996		R^2 = 0.983	

Figure No: 13 Linearity of Silodosin

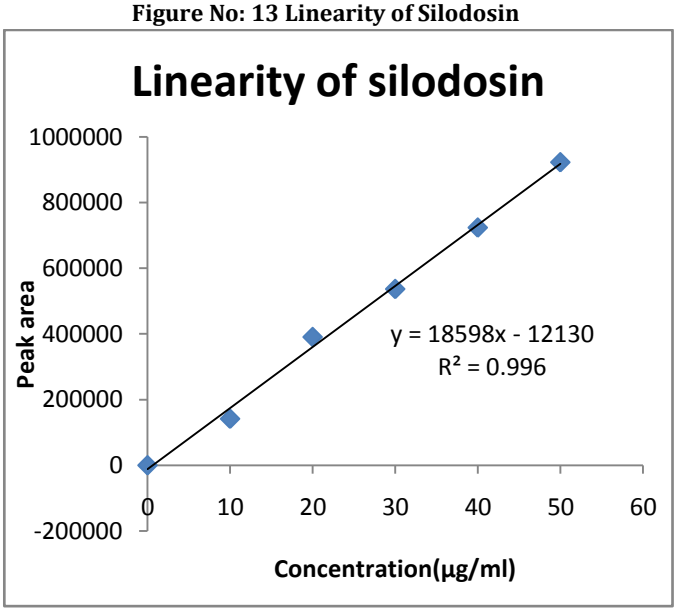


Figure No: 14 Linearity of Dutasteride

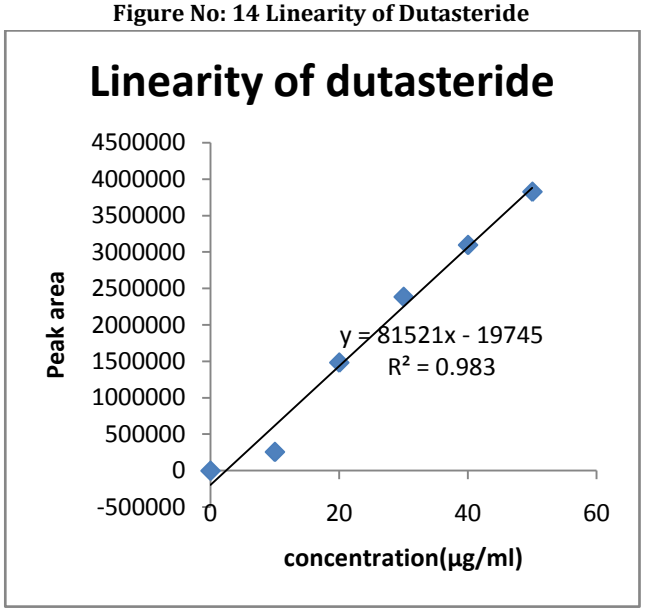


Figure No: 15 Chromatogram for linearity level injection-1

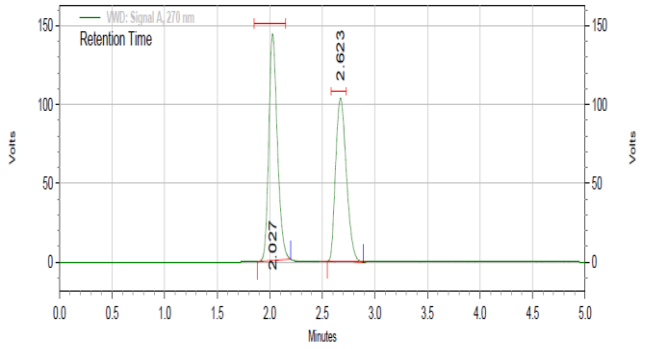


Figure No: 16 Chromatogram for linearity level injection-2

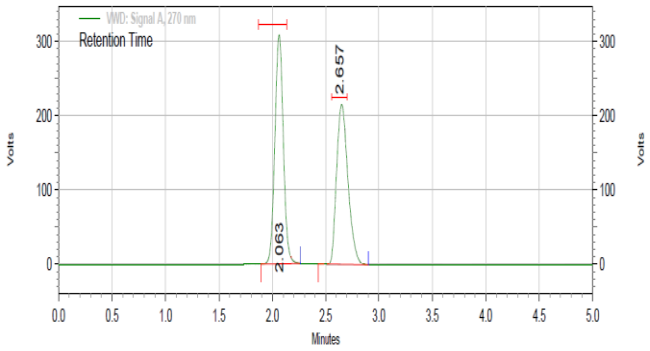


Figure No: 17 Chromatogram for linearity level injection-3

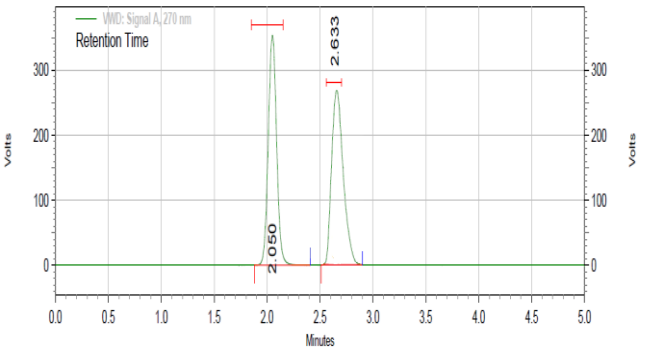


Figure No: 18 Chromatogram for linearity level injection-4

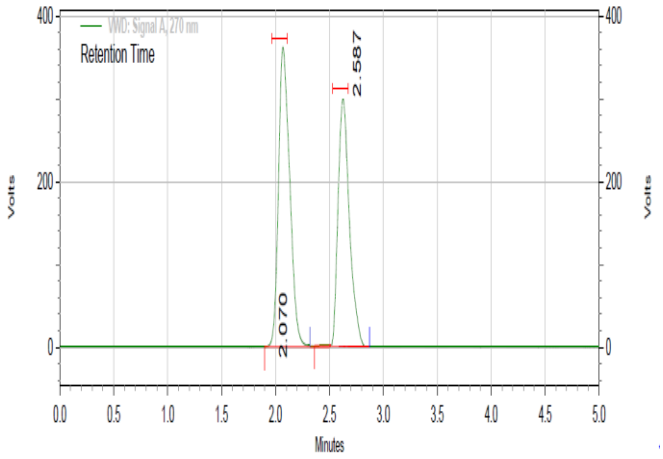
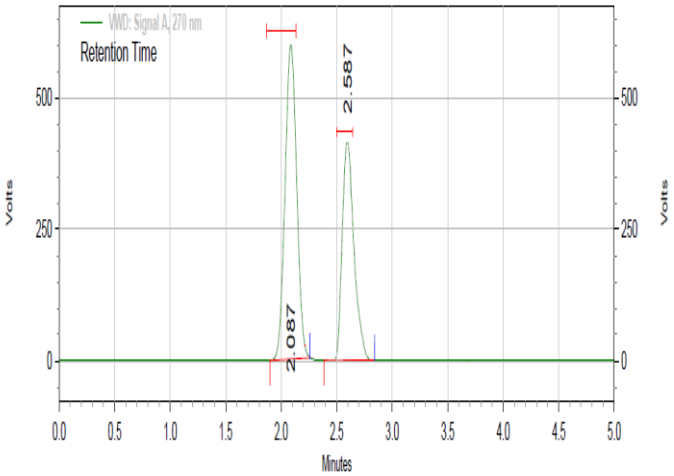


Figure No: 19 Chromatogram for linearity level injection-5



RESULTS:

A linear relationship between peak areas versus concentration (µg/ml) was observed for Silodosin (10 µg/ml- 50µg/ml) and Dutasteride (10µg/ml- 50µg/ml). The correlation coefficient ('r') value was found to be 0.996 for Silodosin and 0.983 for Dutasteride. Hence it is prove that the method is linear.

Acceptance criteria:

The correlation coefficient ('r') value should more than or equal to 0.980

ACCURARCY

The accuracy of the method was determined by analysing solutions containing Silodosin and Dutasteride at approximately 50%, 100% and 150% of the working strength of Silodosin and Dutasteride. Each solution was prepared individually in triplicate and analysed. The percentage recovery values obtained are listed in table. The corresponding chromatograms are attached.

Table No: 06 Accuracy of Silodosin

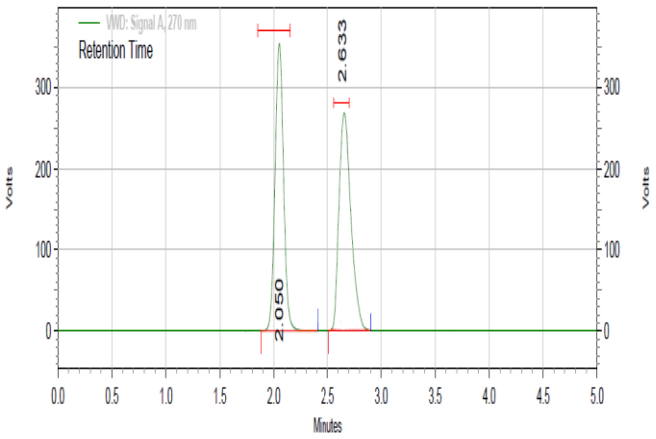
%Concentration (at specification Level)	Area			Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
	Sample Area	Average	Standard Area				
50 %	537051	537154	360771	30	29.78	99.26	100.66
	537253						
	537158						
100 %	724129	724207		40	40.15	100.37	
	724250						
	724242						
150 %	923152	923208		50	51.18	102.36	
	923241						
	923230						

Table No: 07 Accuracy of Dutasteride

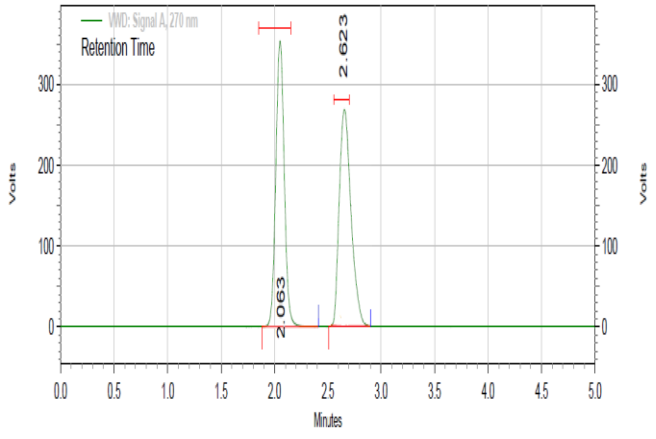
%Concentration (at specification Level)	Area			Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
	Sample Area	Average	Standard Area				
50 %	2360418	2331372	1482876	150	31.44	104.81	104.12
	2312176						
	2321523						
100 %	3094236	3094331		200	41.73	104.34	
	3094639						
	3094118						
150 %	3824548	3825741		250	51.60	103.20	
	3828212						
	3824462						

Figure No: 20 Chromatograms for accuracy (50% Level)

Accuracy 50% Level injection-1



Accuracy 50% Level injection-2



Accuracy 50% Level injection-3

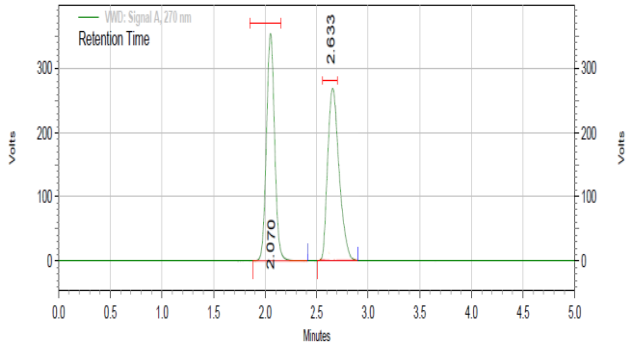
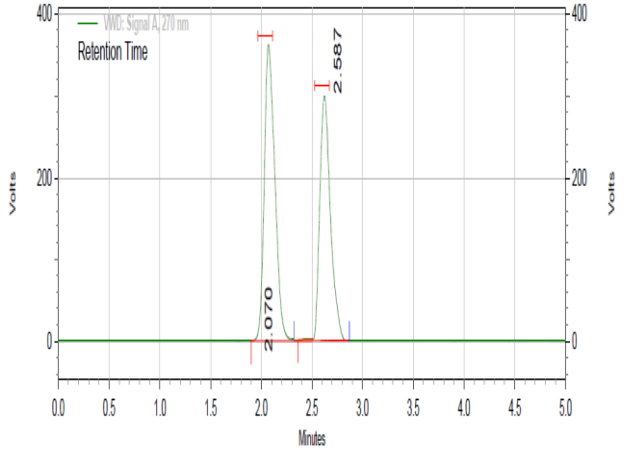
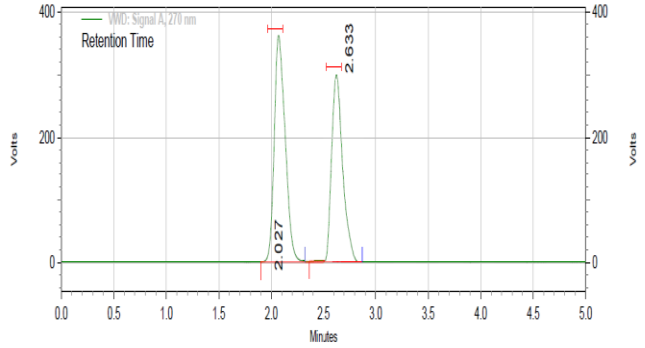


Figure No: 21 Chromatograms for accuracy (100% Level)

Accuracy 100% Level injection-1



Accuracy 100% Level injection-2



Accuracy 100% Level injection-3

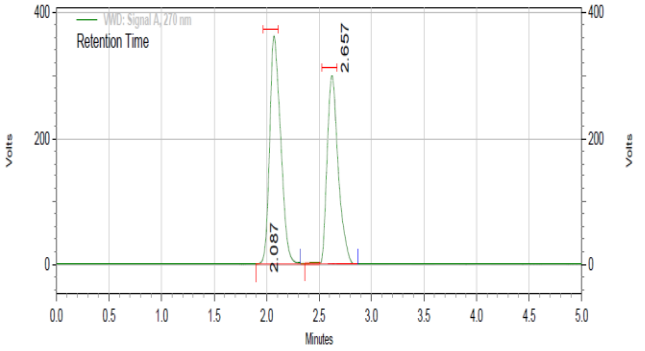
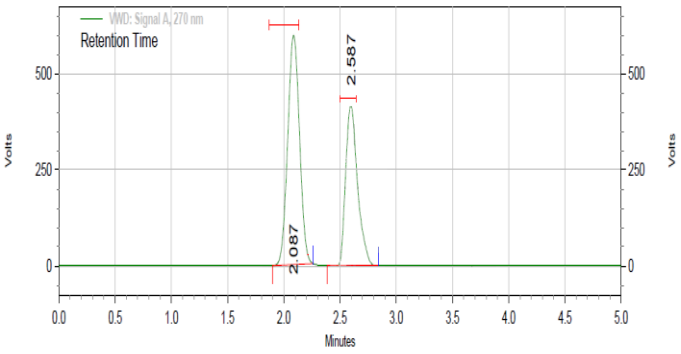
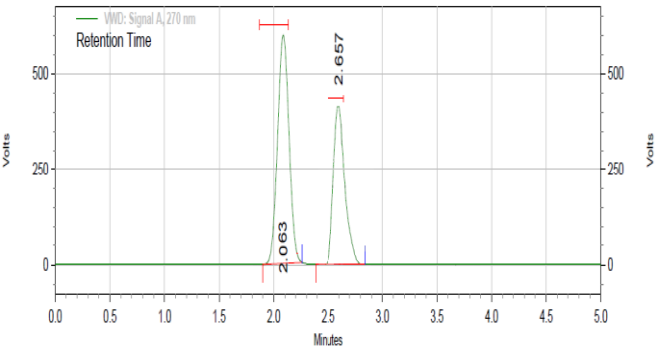


Figure No: 21 Chromatograms for accuracy (150% Level)

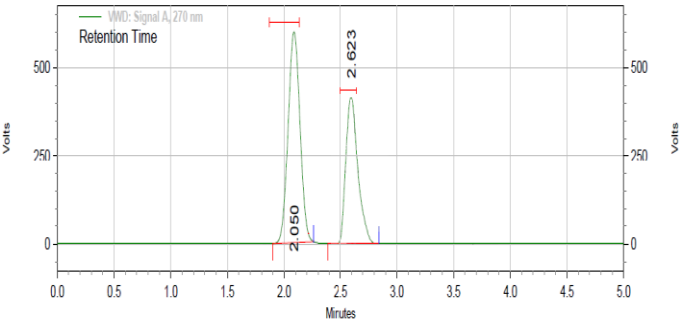
Accuracy 150% Level injection-1



Accuracy 150% Level injection-2



Accuracy 150% Level injection-3



RESULTS:

Results of accuracy study were presented in the above table. The measured values were obtained by recovery test. The mean percentage recovery values were obtained as 100.66 for Silodosin and 104.12 for Dutasteride.

All the results indicate that the method is highly accurate.

Acceptance criteria: The recovery values should be in the range of **95.0%-105%.**

PRECISION

System precision:

System precision was performed by injecting a standard solution of Silodosin and Dutasteride at working concentration of six times. Results of peak area of the Silodosin and Dutasteride are summarized in table. The corresponding chromatograms are attached.

Table No: 08 Summary of System precision:

S.No	Silodosin peak area	Dutasteride peak area
1	360771	1482102
2	360561	1482456
3	360521	1482248
4	360489	1482963
5	360251	1482004
6	360165	1482218
Average	360459.7	1482332
SD	220.086	344.6949
%RSD	0.0611	0.0233

The percentage relative standard deviation for the peak area of standard solution of Silodosin and Dutasteride were **0.0611** and **0.0233** at the working concentration.

Acceptance criteria:

The percentage relative standard deviation for the peak area of Silodosin and Dutasteride should be not more than 2.0.

Method precision:

The method precision was performed by analysing a sample of Silodosin and Dutasteride at working concentration six times (six individual sample preparation).The following table shows the percentage relative standard deviation values. The corresponding chromatograms are attached

Table No: 09 Summary of Method precision:

S.No	Silodosin assay	Dutasteride assay
1	360841	1481569
2	360021	1482254
3	360968	1482567
4	360276	1482014
5	360106	1482258
6	360124	1482314
Average	360389.3	1482163
SD	409.4023	340.1057
%RSD	0.1136	0.0229

The percentage relative standard deviation for the peak area of sample solution of Silodosin and Dutasteride were **0.1136** and **0.0229** at the working concentration.

Acceptance criteria:

The percentage relative standard deviation for the assay values should not be more than 2.0.

Figure No: 22 Chromatograms for precision injection-1

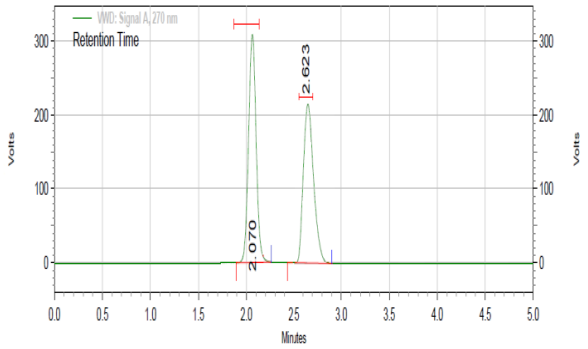


Figure No: 23 Chromatograms for precision injection-2

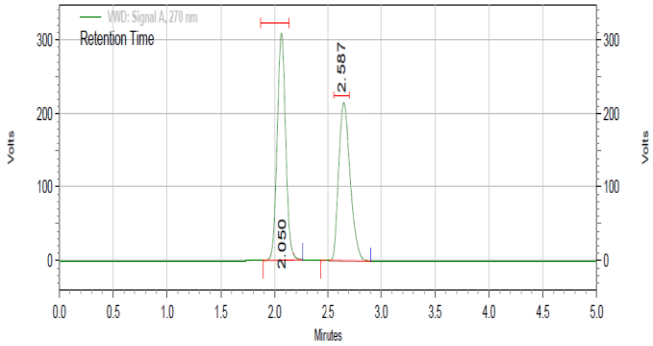


Figure No: 24 Chromatograms for precision injection-3

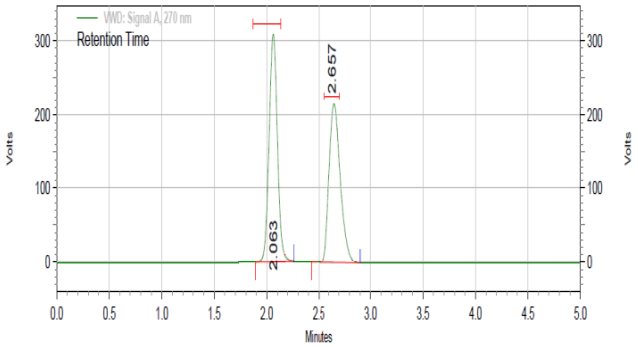


Figure No: 25 Chromatograms for precision injection-4

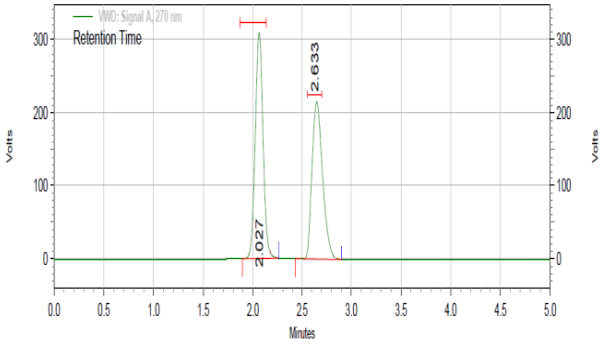


Figure No: 25 Chromatograms for precision injection-5

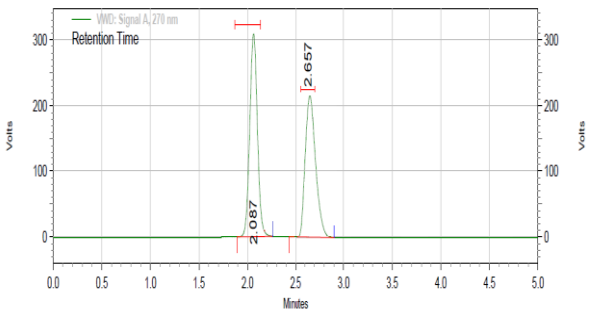
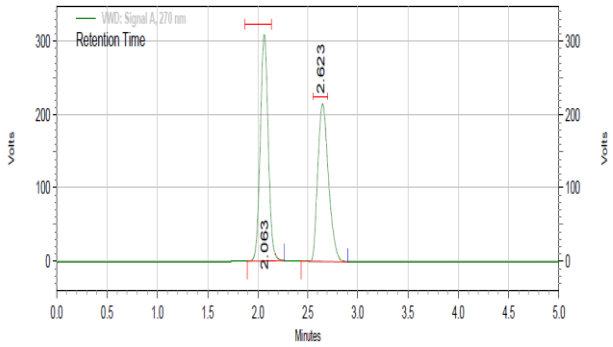


Figure No: 26 Chromatograms for precision injection-6



RESULT:

Results of variability were summarized in the above table. %RSD of peak area was calculated and it's found to be less than 2% which proves that method is precise.

Ruggedness (Intermediate precision)

The intermediate precision was performed by analysing a sample of Silodosin and Dutasteride at working concentration six times (six individual sample preparation) by different days, different regents and different columns assessed the method ruggedness. The following table shows the percentage relative standard deviation values.

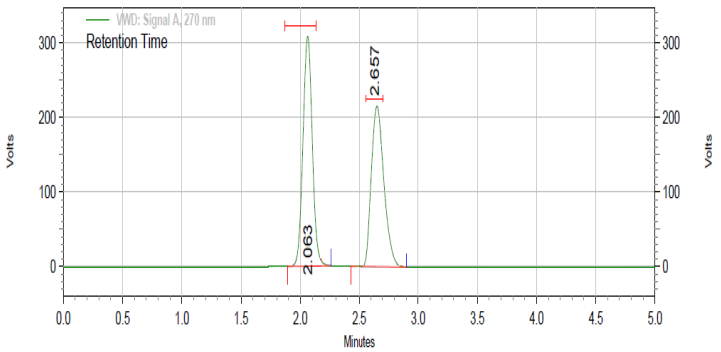
Table No: 10 summary of intermediate precision (Ruggedness)

S.No	Silodosin peak area	Dutasteride peak area
1	361212	1482546
2	362546	1482789
3	362145	1481483
4	361456	1481056
5	361023	1482142
6	364561	1484189
Average	362157.2	1482368
SD	1311.569	1102.779
%RSD	0.3622	0.0744

Acceptance criteria:

The percentage relative standard deviation for the assay values should not be more than 2.0.

Figure No: 27 Chromatogram for intermediate precision



RESULTS:

The percentage RSD values were within the acceptance criteria.

LIMIT OF DETECTION

The parameter LOD was determined on the basis of response and slope of the regression equation. The Detection Limit (DL) may be expressed as:

LOD = 3.3 F/S

Where,

F = Residual Standard deviation of the response,

S = Slope of the calibration curve.

The LOD for this method was found to be **3.29µg/ml** and **3.31µg/ml** for Sildosin and Dutasteride respectively.

LIMIT OF QUANTIFICATION

The parameter LOQ was determined on the basis of response and slope of the regression equation. The Quantitation Limit (QL) may be expressed as:

LOQ = 10 F/S

Where,

F = Residual Standard deviation of the response,

S = Slope of the calibration curve.

The LOD for this method was found to be **9.89µg/ml** and **9.95 µg/ml** for Silodosin and Dutasteride respectively.

Table: 11 Summary of LOQ and LOD

Molecule	LOQ(µg/ml)	LOD(µg/ml)
Silodosin	9.89	3.29
Dutasteride	9.95	3.31

ROBUSTNESS

The following table shows the parameters of the method that were altered to test the robustness of the method. Small deliberate changes in method like Flow rate, mobile phase ratio, and wavelength are made but there were no recognized change in the result and are within range as per ICH Guide lines. The corresponding chromatograms are attached.

Acceptance criteria:

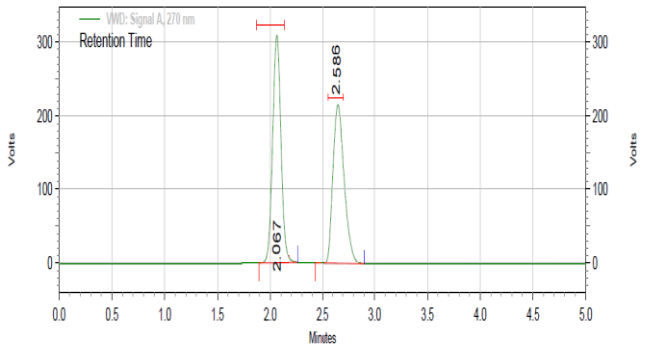
The %RSD of peak area of all peaks for the six replicate injections should be not more than 2.0

Table No: 7.9 Summary of Robustness

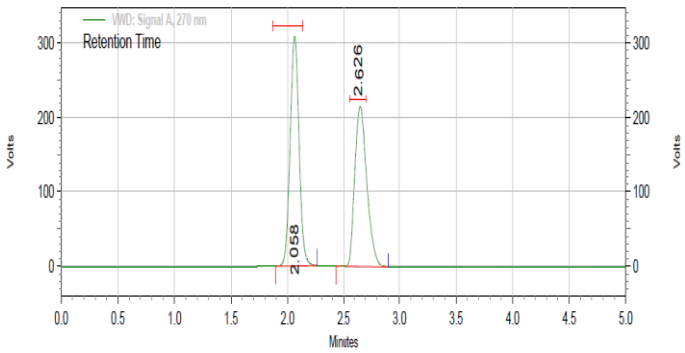
S.No	Parameters	Silodosin			Dutasteride		
		RT (min)	Theoretical plate count	Resolution	RT (min)	Theoretical plate count	Resolution
1	Standard	1.940	2452	0.00000	3.993	2969	4.74343
2	Change in mobile phase ratio(-)65:25	2.067	2891	0.00000	2.586	2971	4.47329
3	Change in mobile phase ratio(+)85:15	2.058	2839	0.00000	2.626	2265	6.56432
4	Change in flow rate(-) 0.8 ml/ min	2.083	2428	0.00000	2.650	2261	4.92815
5	Change in flow rate(+)1.2 ml/ min	2.077	2815	0.00000	2.823	2928	4.25766
6	Change in wavelength (-)265	2.057	2886	0.00000	2.638	2851	4.41817
7	Change in wavelength (+)275	2.063	2941	0.00000	2.578	2984	4.40823

Figure No: 28 Chromatograms for Robustness

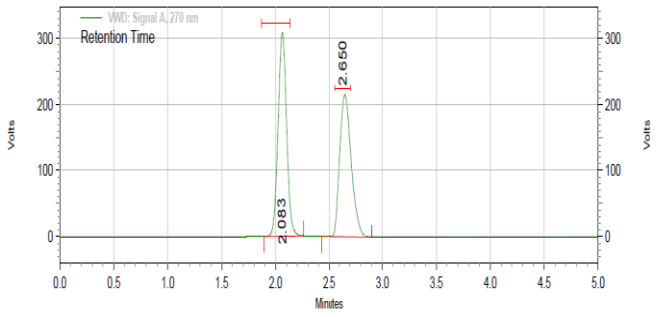
Robustness: Mobile phase 65:35 (Methanol: 0.1% Orthophaspharicacid)



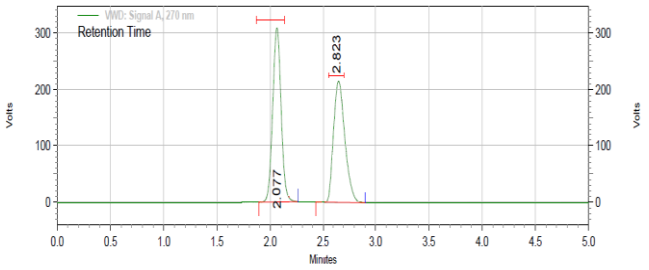
Robustness: Mobile phases 85:15 (Methanol: 0.1% Orthophaspharicacid)



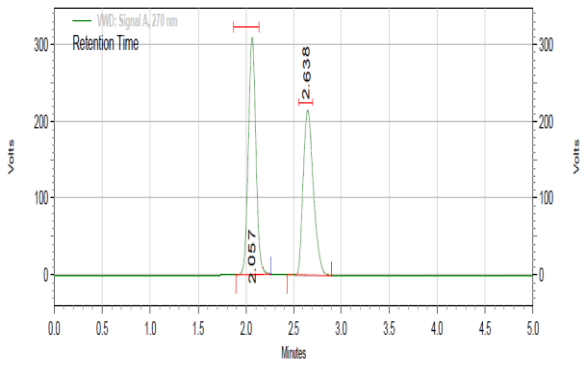
Robustness: Flow rate -0.8ml/min



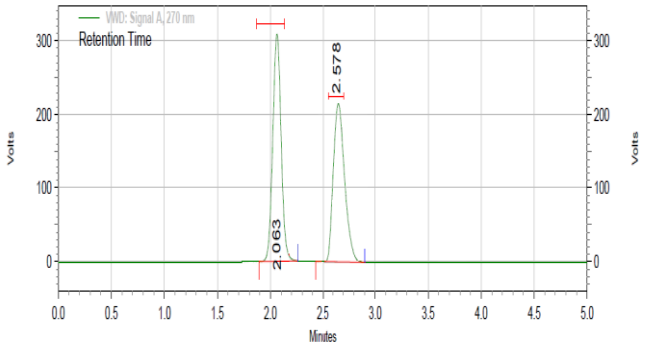
Robustness: Flow rate -1.2ml/min



Robustness: Wavelength at 265nm



Robustness: Wavelength at 275nm



RESULT:

The results of Robustness of the present method had shown that changes made in the flow and mobile phase composition did not produce significant changes in analytical results. Results were presented in the above table. As the changes are not significant we can say that the method is robust.

8.0 SUMMARY

S. No	Validation Parameter	Acceptance Criteria	Result
1	System suitability	% RSD for five replicate injections should not be more than 2.0.	% RSD for 6 replicate injections from standard solution for Silodosin and Dutasteride is 0.206and 0.167 respectively.
		The USP plate count for The Silodosin And Dutasteride peaks should not less than 2000.	The USP plate count for Silodosin And Dutasteride are 2452 and 2969 respectively.
		The USP tailing for the Silodosin And Dutasteride peaks should not more than 2.0.	Tailing factor for Silodosin And Dutasteride from first injection of standard solution 0.54 and 0.78 respectively.
2	Specificity	Peak due to placebo and other analyte/s (if any) should be separated	The peaks of Diluent and Placebo are not interfering with Silodosin And Dutasteride peaks.

		from Silodosin And Dutasteride peak.		
3.	**Precision**			
	System Precision	% RSD for 5 replicate injections should not be more than 2.0 %	**Silodosin**	**Dutasteride**
			0.6611	0.0233
		The USP plate count for the Silodosin And Dutasteride peaks should not be less than 2000.	2486	2982
	Method Precision	% RSD of the method precision results obtained from six preparations should not be more than 2 for Silodosin And Dutasteride.	**Silodosin**	**Dutasteride**
			0.1136	0.10229
		Bracketing standard should meet the system suitability criteria.	Complies	Complies
4.	**Linearity**	R^2 should be more than 0.999	0.996	0.983
			Silodosin	**Dutasteride**

5	**Accuracy**	The mean % recovery at every level should be 95.0-105.0%	**50 %**	**100 %**	**150 %**	**50 %**	**100 %**	**150 %**
			99.26	99.26	99.26	104.81	104.81	104.81
6	**Robustness**	The system suitability parameters should pass for all conditions	The system suitability parameters passed for all the conditions					

9.0 CONCLUSION

Method development & validation of Silodosin and Dutasteride was done by RP-HPLC method. The estimation was done by the analysis in RP-HPLC employing Agilent C18 (4.6 x 150mm, 5µm) using mobile phase as Methanol: 0.1% Orthophaspharic acid in 75:25 v/v at a flow rate 1ml/min. The linearity range of Silodosin and Dutasteride was found to be HPLC 25-125 µg/ml & 50-250 µg/ml respectively. Linear regression was more than 0.999. The values of %RSD was <2% for both the methods. The % recovery varies in the range of 99-101. The results show the methods are accurate, precise, sensitive, and economic. The HPLC method is more rapid. Method is successfully applied to the pharmaceutical dosage form.

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