"Development and Validation of a Method for Simultaneous Determination of Silodosin and Dutasteride in a Formulation by RP-HPLC."

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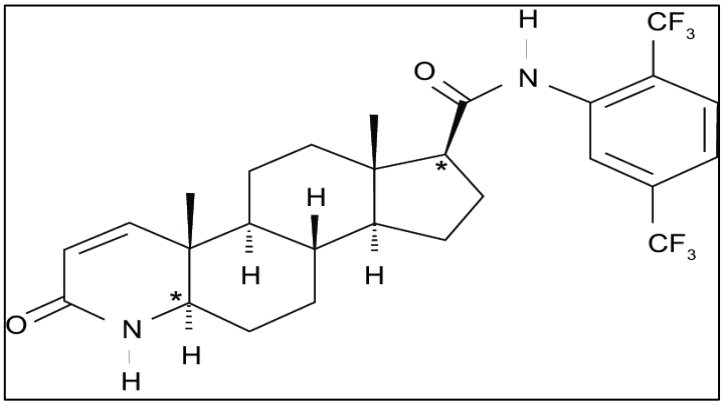
Abstract- **Silodosin and Dutasteride both are approved drugs by USFDA (Food & Drug Administration). On literature survey, it was found that no methods have been reported for simultaneous estimation of Silodosin and Dutasteride. Therefore, it was thought of interest to develop a simple, accurate, precise, sensitive and economic analytical method and to validate as per ICH guidelines. So, RP-HPLC method was developed and validated for simultaneous estimation of Silodosin and Dutasteride in multiunit system. Separation was achieved on Agilent technology HPLC; Agilent Zorbax Bonus RP (250 x 4.6 mm, 5 µ) at 30°C temperature by using a mobile phase Buffer: Methanol in the ratio of 50:50. Analysis was done at the flow rate of 1.0 mL/min and UV detection was carried out by wavelength gradient at 235 nm (0 to 5 min.). The retention time of Silodosin and Dutasteride was found to be 4.01 min & 7.29 min respectively. The specificity of the method was determined by assessing interference from placebo. The method was validated in terms of linearity, precision, accuracy, specificity, robustness, ruggedness, solution stability. The linearity was found to be in the range of 128- 192 µg/mL & 8-12 µg/mL for Silodosin and Dutasteride with correlation coefficient of 0.9998for Silodosin and 0.9994 for Dutasteride. %RSD of method precision was found to be less than 2%, this indicates that the method is precise.**

***Keywords*: Silodosin, Dutasteride, RP-HPLC, validation, method development.**

INTRODUCTION:

Silodosin (Brand Name Rapallo) Silodosin is a medication for the symptomatic treatment of benign prostatic hyperplasia. It acts as α1-adrenoreceptor antagonist with high Uros electivity (Selectivity for the prostate). The IUPAC name of this drug was1-(3-hydroxypropyl)-5-[(2R) -({2- [2- [2-(2,2,2) phenoxy] ethyl} amino) propyl] indoline-7- carboxamide. Silodosin has high affinity for the α1A adrenergic receptor; it causes practically no orthostatic hypotension (in contrast to other α1 blockers). On the other side, the high selectivity seems to be the

Fig No.1. Structure of Silodosin



cause of silodosin's typical side effect of loss of seminal emission. Silodosin is a prescription medication used for the treatment of the signs and symptoms of benign prostatic hyperplasia (BPH) 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

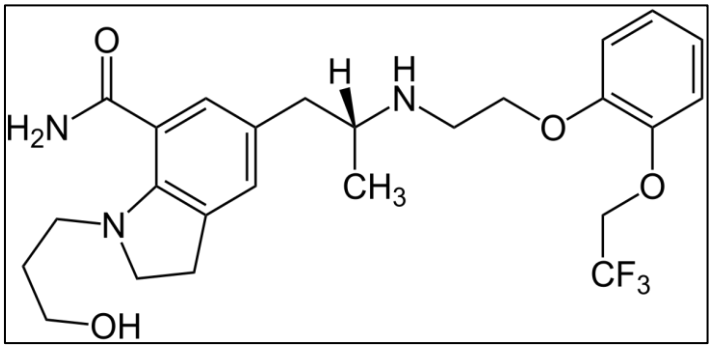


Fig No.1. Structure of Dutasteride

sex hormone testosterone into the more potent dihydrotestosterone (DHT). IUPAC name of this drug is (5α, 17β)-N-{2,5-Bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1- ene-17- carboxamide. 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. Fig

Materials and Methods: -

HPLC METHOD DEVELOPMENT FOR SILODOSIN AND DUTASTERIDE

I. Preparation of Standard Stock Solution

i. Silodosin Standard Stock Solution-I (SSSS-I):

i. Initially Prepare a Standard Stock Solution (SSS-I) of by adding 16 mg of Silodosin in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Silodosin = 1600 µg/ml).

ii. Dutasteride Standard Stock Solution-I (DSSS-I):

Then prepare a Standard Stock Solution (SSS-II) of Dutasteride by adding 10 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Dutasteride = 100 µg/ml).

iii. Then add 1 ml of SSSS-I & 1 ml DSSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Silodosin =160 µg/ml & Dutasteride = 10 µg/ml).

II. Drug Product Sample Preparation for Assay:

a) 10 tablets were weighed and average weight was calculated. And tablets were crushed & mixed in mortar and pestle.

b) Powder weight equivalent to 16 mg Silodosin and 1 mg of Dutasteride was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicate for 5 minutes and make the volume to 10 ml with diluent. (Conc. of Silodosin = 1600 µg/ml and Dutasteride = 100 µg/ml).

c) Further, pipette out 1.0 ml of above solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Silodosin = 160 µg/ml and Dutasteride = 10 µg/ml).

II. Selection of Analytical Wavelength

The sample was scanned from 200-400 nm with DAD detector. The Wavelength selected for analysis chosen was 235 nm on basis of appropriate intensity of Dutasteride.

III. Selection of Mobile Phase and its Strength

The solution of Silodosin(100µg/ml) and Dutasteride B (2 µg/ml) was prepared in HPLC water and filtered through Millipore syringe filter, then injected into HPLC system. The chromatogram was analyzed using Buffer: Methanol (Gradient)

Time (min.)	Buffer (%)	Methanol (%)
0	40	60
5	40	60
7	20	80
15	20	80
20	40	60
30	40	60

IV. Selection of column (stationary phase)

To get well resolved, symmetric peak with highest number of theoretical plates along with other system suitability parameters up to the mark, the sample solutions were analyzed using (Agilent Bonus-RP) C18 column as a stationary phase.

V. Chromatographic Conditions

- **Analytical Column:** Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5µ)
- **Mobile Phase:** Buffer: Methanol (Gradient)
- **Flow Rate:** 1 ml/min
- **Injection Volume:** 10µl
- **Detection Wavelength:** 235nm.
- **Diluent:** Buffer: Methanol (50: 50, % v/v)
- **VI. Preparation of Mobile Phase**

Preparation of Buffer:

6.8 gems of Potassium Dihydrogen Phosphate were dissolved in 1000 ml HPLC Water and the pH was adjusted to 3.3 with ortho-phosphoric acid. Filtered twice through 0.45 µ nylon Membrane filter and degassed for 15 min.

HPLC METHOD VALIDATION FOR SILODOSIN AND DUTASTEIDE

I. Specificity & Assay:

i. Individual samples of Silodosin of 160 µg/ml and Dutasteride of 10 µg/ml were prepared and peaks were for identified from Retention Time.
ii. Blank was injected to ensure there is no blank peak interfering with the main analyte peaks.
iii. Assay was calculated by using following formula;

$$\%\ \boldsymbol{Assay} = \frac{\boldsymbol{Sample\ Area}}{\boldsymbol{Standard\ Area}}\ \boldsymbol{x\ 100}$$

II. Repeatability & System Suitability:

i. A single sample was prepared as described and 6 injections were made from same sample and checked for system suitability.
ii. System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates,
3. Asymmetry (Tailing factor),
4. Resolution.

III. Linearity & Range:

i. 5 samples of varying concentrations ranging from 80-120% were made.
ii. The concentrations are given below

% Level	Silodosin Conc. (µg/ml)	Dutasteride Conc. (µg/ml)
80	128	8
90	144	9
100	160	10
110	176	11
120	192	12

iii. The sample preparations are given as below;
iv. X ml of Silodosin and Y ml of Dutasteride were added to 10 ml diluent to make up the concentrations given above:

X ml of SSSS-I	Y ml of DSSS-I	Diluted to
0.8	0.8	10 ml
0.9	0.9	10 ml
1.0	1.0	10 ml
1.1	1.1	10 ml
1.2	1.2	10 ml

IV. Accuracy

i. Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given above in table for both Silodosin and Dutasteride.
ii. Samples were injected in triplicate to calculate % RSD.
iii. % recovery was also calculated.

V. Method Precision:

6 Sample of same concentration were prepared and injected and area of all 6 samples was obtained and % RSD of six samples was calculated.

Intermediate Precision:

6 Sample of same concentration were prepared by different analyst and injected and area of all 6 samples was obtained and % RSD of six samples was calculated.

Intra & Inter-day Precision:

Single mixture working standard and drug product samples were prepared and injected twice in a day at different time intervals to evaluate intra-day precision.

Same mixture working standard and drug product samples were analyzed on second day to evaluate the inter-day precision. % Assay was calculated at each interval and stability of solutions were estimated.

VI. Limit of Detection

LOD calculated by the following formulae.

LOD = 3.3(SD/S)

Were, SD- Standard deviation; S- Slope of Curve.

VII. Limit of Quantitation LOQ calculated by the following formulae.

LOQ = 10(SD/S)

Were, SD- Standard deviation; S- Slope of Curve.

VIII. Robustness

i.The Robustness was performed by changing the column temperature by ± 2˚C.

ii.Each Sample was injected % Assay was calculated at each condition was calculated.

Condition	Increased	Normal	Decreased
Column Oven Temperature	32˚C	30˚C	28˚C

RESULTS AND DISCUSSION

PRELIMANARY ANALYSIS OF SILODOSIN AND DUTASTERIDE:

Table.1 Observations and Results of Preliminary Analysis of Silodosin

Sr.no	Tests	Observations	Results
1.	Description	White to off-white crystalline powder	Complied
2.	Solubility	Soluble in DMSO and Ethanol	Complied
3.	Melting point	104^0C	complied

Table.2 Observations and Results of Preliminary Analysis of Dutasteride

Sr.no	Tests	Observations	Results
1.	Description	White to off-white crystalline powder	Complied
2.	Solubility	Soluble in DMSO and Ethanol	Complied
3.	Melting point	240^0C	complied

RP-HPLC METHOD DEVELOPMENT FOR SILODOSIN AND DUTASTERIDE:

1. Selection of wavelength

Silodosin and Dutasteride showed the maximum absorbance at 235nm. Hence, HPLC analysis was carried out at 235nm.

2. Selection of Mobile phase

Different mobile phases like buffer and methanol in varying proportions of mobile phases were tried for better resolution of the chromatogram.

Mobile Phase	Ratio	Wavelength	Silodosin			
			RT	TP	Asymmetry	Resolution
Buffer: Eoh	50-50	250	7.30	8657	1.07	0.00
Buffer: Eoh	40-60	250	5.58	11456	1.12	0.00
Buffer: Eoh	40-60	235	5.58	11357	1.12	0.00

Buffer: Eoh	30-70	250	Not detected			
Buffer: Eoh	30-70	235	Not detected			
Buffer: Eoh	40-60	250	6.87	12773	1.16	0.00
Buffer: Eoh	40-60	235	6.87	12800	1.16	0.00
Buffer: Eoh	Gradient	235	4.01	13306	1.16	0.00

Table.3 Optimization of Chromatographic Conditions

Mobile Phase	**Ratio**	**Wavelength**	**Silodosin**			
			RT	**TP**	**Asymmetry**	**Resolution**
Buffer: Eoh	50-50	250	7.30	8657	1.07	0.00
Buffer: Eoh	40-60	250	5.58	11456	1.12	0.00
Buffer: Eoh	40-60	235	5.58	11357	1.12	0.00
Buffer: Eoh	30-70	250	Not detected			
Buffer: Eoh	30-70	235	Not detected			
Buffer: Eoh	40-60	250	6.87	12773	1.16	0.00
Buffer: Eoh	40-60	235	6.87	12800	1.16	0.00
Buffer: Eoh	Gradient	235	7.29	13306	1.16	0.00

Table.4 Optimization of Chromatographic Conditions

After several combinations of mobile phase solvents such as buffer with methanol. The mobile phase buffer with methanol was selected in gradient manner respectively using C18 column (Agilent Zorbax Bonus RP) which has given good resolution, capacity factor, acceptable system suitability parameters. The drug was eluted within 5mins which will reduces the overall analysis time and cost and the detection wavelength used was 235nm.

1. Identification of Peaks

Fig.1 Chromatogram of Working standard in optimized chromatographic conditions of mixture

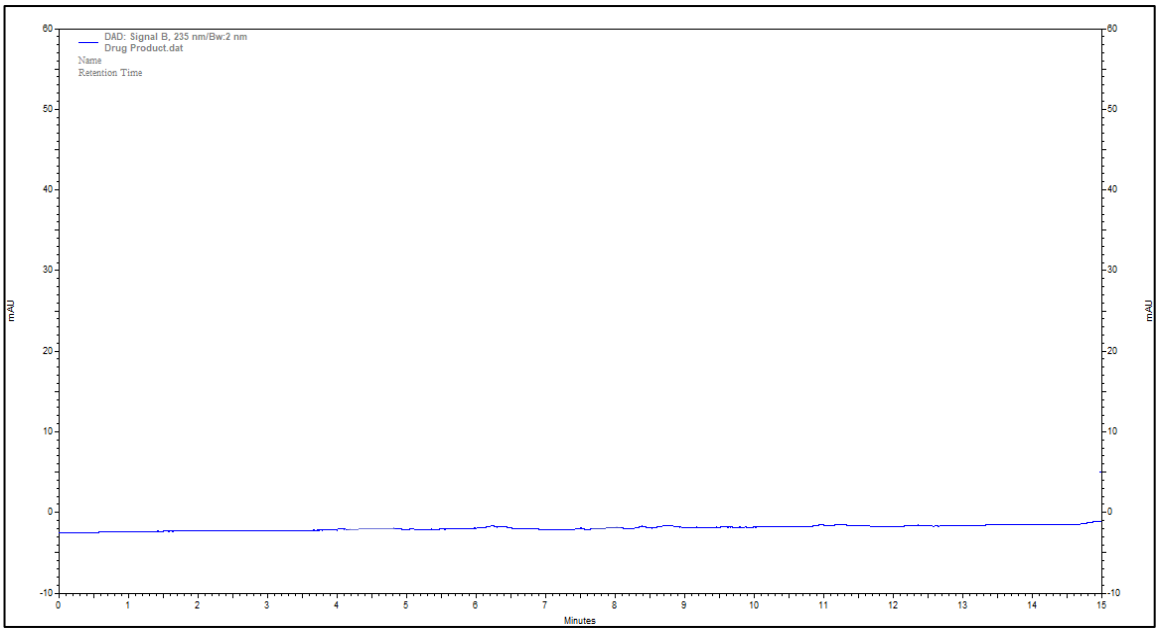


Fig.2 Chromatogram of Blank

I. Specificity and Assay:

With above optimized conditions the Silodosin and Dutasteride was eluted at 4.01 min and 7.29 min respectively. Peak was sharp, and with good resolution. Table no 5.

Sample	Silodosin			Dutasteride		
	RT	Area	% Assay	RT	Area	% Assay
Silodosin	4.01	380468	-	-	-	-
Dutasteride	-	-	-	7.29	975640	
MIX WS	4.01	380479	-	7.29	970651	-
Drug Product	4.48	380452	99.99	7.87	970564	99.99

Table No. 5. Assay results for Silodosin and Dutasteride

II. Repeatability:

The percentage RSD (<2) values obtained showed that the method developed was precise at repeatability and intermediate precision.

REPEATABILTY		
	Silodosin	Dutasteride
Reps	Area	Area
Rep 1	380449	970564
Rep 2	380468	970607
Rep 3	380482	970266
Rep 4	380467	970365
Rep 5	380504	970296
Rep 6	380489	970569
AVG	380477	970445
STDEV	19.29507709	152.5919395
RSD	0.01	0.02

Table No .6 Repeatability results for Silodosin and Dutasteride

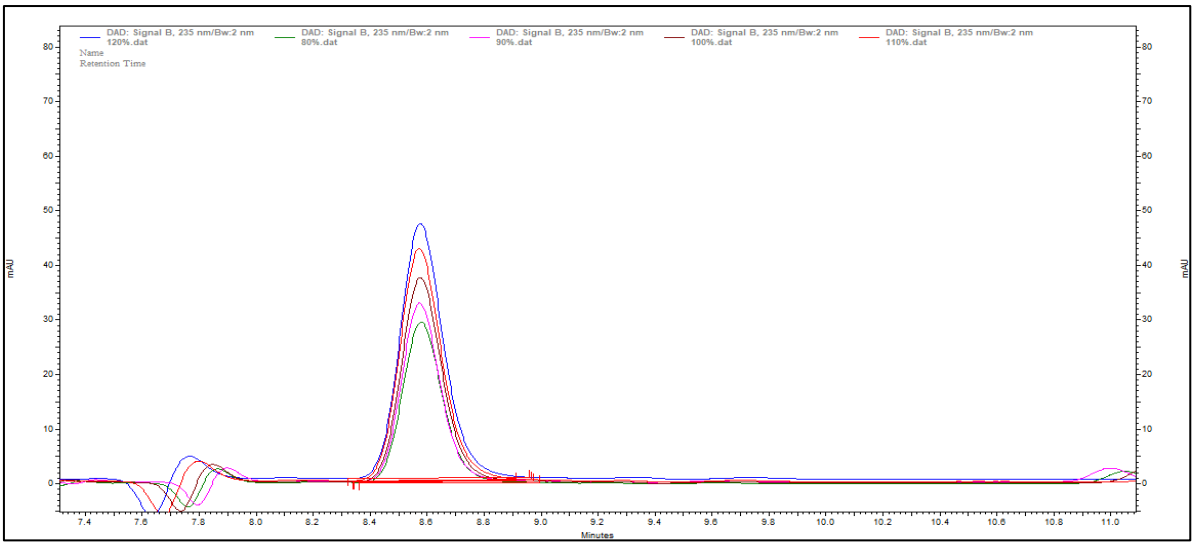


Fig.3.Overlay Chromatograms of serial dilutions of Silodosin in optimized chromatographic conditions

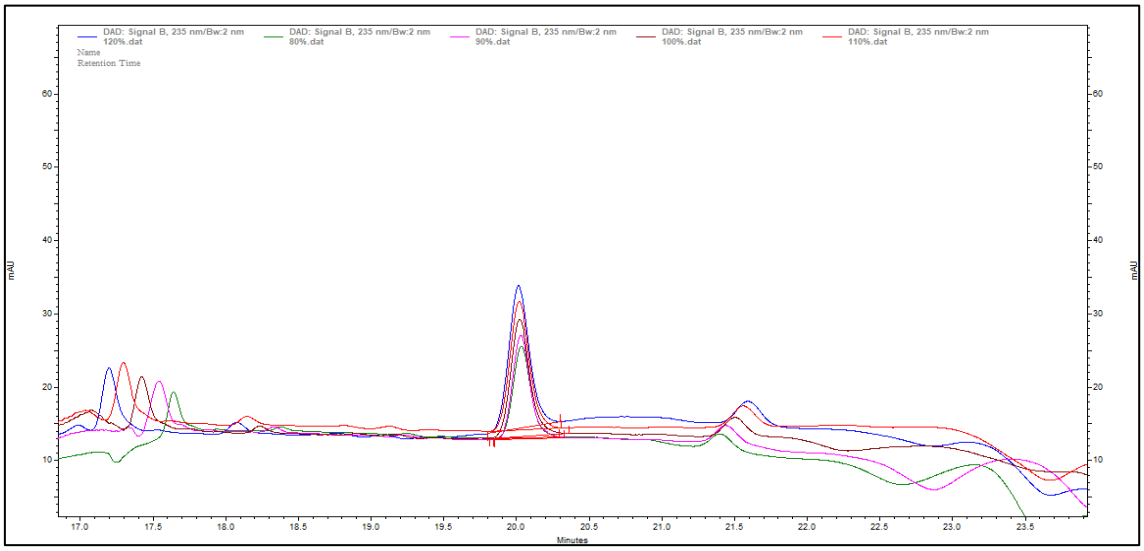


Fig. 4. Overlay Chromatograms of serial dilutions of Dutasteride in optimized chromatographic conditions

The peak response is directly proportional to the concentration of drug and was found to be linear in the range of 128-192µg/ml and 8-12µg/ml for Silodosin and Dutasteride respectively. The correlation coefficient was found to be 0.999 for both Silodosin and Dutasteride respectively, which is well within the acceptance criteria.

Linearity Rang:

Table. 7. Response of Silodosin at various linearity levels

Silodosin		
% Level	**Conc (ug/ml)**	**AREA**
80	128	300933
90	144	342222
100	160	380449
110	176	418484
120	192	458769

Table No. 8 Response of Dutasteride at various linearity levels

Dutasteride		
% Level	**Conc (ug/ml)**	**AREA**

80	8	770219
90	9	876479
100	10	970564
110	11	1062488
120	12	1162322

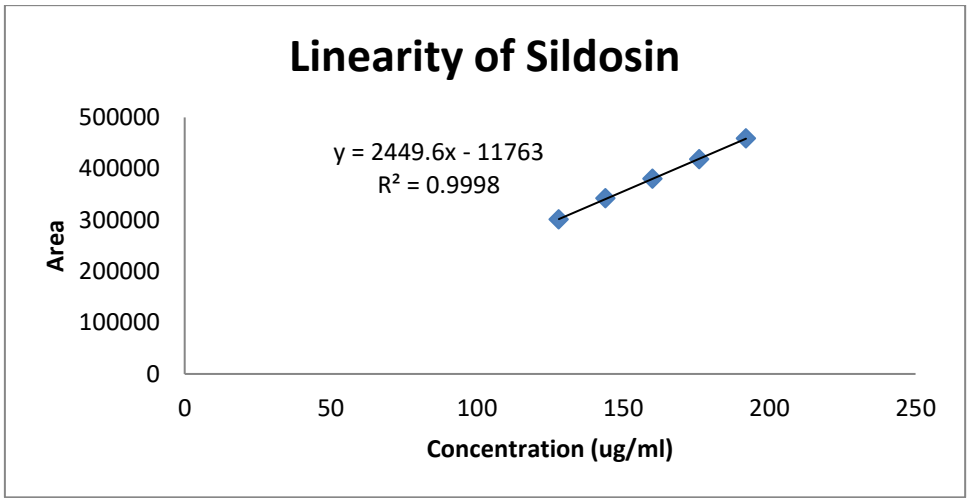


Fig.5. Calibration curve of Silodosin of RP-HPLC method

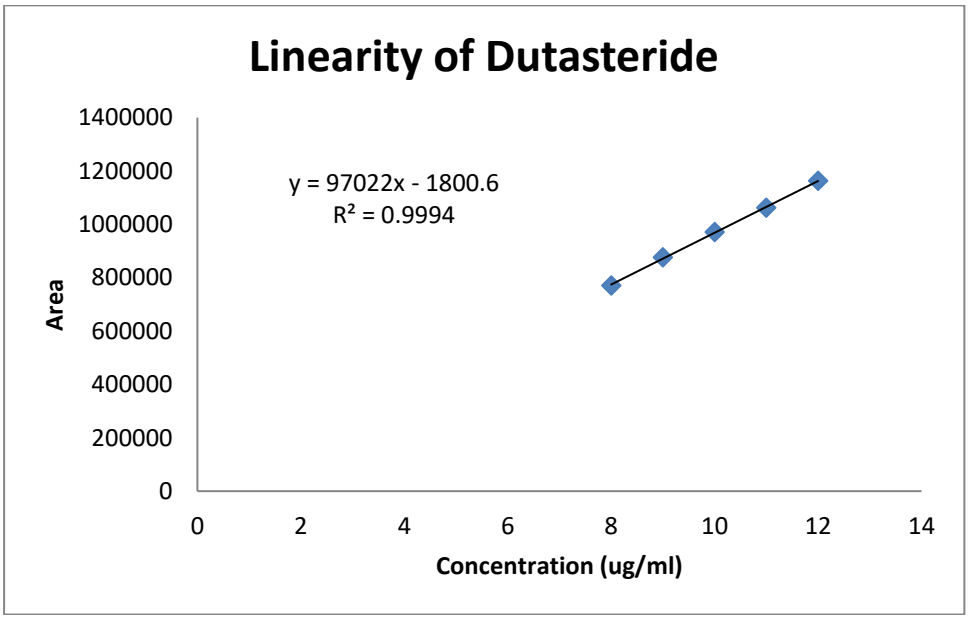


Fig6. Calibration curve of Dutasteride of RP-HPLC method

The range was from 128-192µg/ml and 8-12µg/ml for Silodosin and Dutasteride respectively.

Table.9. Range for RP-HPLC Method

Parameters	**Silodosin**	**Dutasteride**
Linearity Range (µg/ml)	128-192	8-12

IV. Accuracy:

The percentage recoveries of the results for Silodosin and Dutasteride indicates that the recoveries are well within the acceptance range, therefore, method was found to be accurate, which is having RSD less than 2.

Table 10. Accuracy data for Silodosin

Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	RSD
Rep 1	7.976	300933	7.89	98.87	99.78	0.8326	0.83
Rep 2	7.976	305909	8.02	100.50			
Rep 3	7.976	304256	7.97	99.96			
Rep 1	9.97	380449	9.97	99.99	100.00	0.00435	0.00
Rep 2	9.97	380468	9.97	100.00			
Rep 3	9.97	380482	9.97	100.00			
Rep 1	11.964	458769	12.02	100.48	100.46	0.29873	0.30
Rep 2	11.964	459976	12.05	100.75			
Rep 3	11.964	457254	11.98	100.15			

Table.11. Accuracy data for Dutasteride

Sample ID	Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	RSD
80%	Rep 1	79.76	770219	79.13	99.21	100.19	0.85235	0.85
	Rep 2	79.76	781453	80.28	100.66			
	Rep 3	79.76	781895	80.33	100.71			
100%	Rep 1	99.70	970564	99.71	100.01	100.00	0.01914	0.02
	Rep 2	99.70	970607	99.72	100.02			
	Rep 3	99.70	970266	99.68	99.98			
120%	Rep 1	119.64	1162322	119.41	99.81	99.92	0.36423	0.36
	Rep 2	119.64	1168356	120.03	100.33			
	Rep 3	119.64	1160175	119.19	99.63			

Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given above in table for both Silodosin and Dutasteride. In accuracy study, percentage recovery was calculated. The range of percentage recovery for Silodosin and Dutasteride is 98.87 % to 100.75 % and 99.21 % to 100.66 % respectively. The range of % RSD 0.30% to 0.83 % and 0.02% to 0.85 % respectively.

V. Inter-day & Intraday Precision:

The percentage RSD (<2) values obtained showed that the method developed was precise at Interday and intraday precision.

Table No. 12. Interday and Intraday precision results for Silodosin and Dutasteride

Intraday Precision					
Condition	**Sample ID**	**Silodosin**		**Dutasteride**	
		Area	**Assay**	**Area**	**Assay**
Morning	**WS**	380479	-	970651	-
	DP	380452	99.99	970564	99.99
Evening	**WS**	380599	-	1006826	-
	DP	380521	99.98	1006759	99.99
Inter-day Precision					
Condition	**Sample ID**	**Silodosin**		**Dutasteride**	
		Area	**Assay**	**Area**	**Assay**
Day 2	**WS**	381256	-	1006803	-

VI. Method Precision and Intermediate Precision:

Table No. 13. Method precision results for Silodosin and Dutasteride

METHOD PRECISION		
	Silodosin	**Dutasteride**
Sample ID	**Area**	**Area**
MP-1	380456	978563
MP-2	380468	978956
MP-3	381474	979156
MP-4	380581	981641
MP-5	380515	982689
MP-6	380564	985963
AVG	381564	981161
STDEV	393.9434816	2872.49165
RSD	0.10	0.29

Table No. 14. Intermediate precision results for Silodosin and Dutasteride

INTERMEDIATE PRECISION		
	Silodosin	**Dutasteride**
Sample ID	**Area**	**Area**
IP-1	381669	971569
IP-2	381589	972568
IP-3	381996	971965
IP-4	381471	972115
IP-5	381564	971558
IP-6	382004	971998
AVG	381716	971962
STDEV	229.263822	376.72665
RSD	0.06	0.04

VII. Detection Limit:

Table.15. Limit of Detection data of Silodosin and Dutasteride

Parameter	***Silodosin***	***Dutasteride***
LOD (µg/ml)	4.42	0.47

Detection limit was calculated on basis of standard deviation of response and slope.

VIII. Quantification Limit:

Table. 16.. Limit of Quantification data of Silodosin and Dutasteride

Parameter	***Silodosin***	***Dutasteride***
LOQ (µg/ml)	13.40	1.43

Quantification limit was calculated on basis of standard deviation of response and slope.

IX. Robustness:

Table.17. Results of Robustness

Column Oven Temp Change							
Condition	**Sample ID**	**Silodosin**			**Dutasteride**		
		RT	**Area**	**Assay**	**RT**	**Area**	**Assay**
28˚C	**WS**	4.01	375899	-	7.29	968564	-

	DP	4.48	375691	99.94	7.87	968221	99.96
30˚C	**WS**	4.01	380479	-	7.29	970651	-
	DP	4.48	380452	99.99	7.87	970564	99.99
32˚C	**WS**	4.01	390267	-	7.29	971265	-
	DP	4.48	390056	99.95	7.87	970889	99.96

The parameter, column oven temperature was changed to check robustness of the developed method. As there is negligible change in area and assay for both Silodosin and Dutasteride by the influence of column oven temperature, indicates that the method is robust.

CONCLUSION:

This research was aimed to develop and validate analytical methods such as RP-HPLC for the estimation of Silodosin& Dutasteride in API and tablet formulation. The proposed methods were found to be appropriate due to its simplicity, reliability, sensitivity, rapidness and selectivity for detection at very low concentrations.

Thus, it can be concluded that the methods developed in the present investigation were simple, sensitive, specific, linear, accurate, rapid and precise. Optimum parameters for good separation of the analytes were determined. The optimized analytical methods exhibit simplicity in terms of short analysis time, effective, clear resolution with low LOD and LOQ values and other parameters. Thus, the proposed methods were developed and validated as per the ICH guidelines.

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