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Research Article

Analytical Method Development and Validation for the Estimation of Silodosin and Dutasteride in Capsule Dosage Form by RP-HPLC Method

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ABSTRACT

A simple, rapid, accurate, efficient and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method was developed and subsequently validated for simultaneous estimation of silodosin and dutasteride capsules. The analysis was carried out using Unison C8 column (250 mm x 4.6, 5 µm particle size) at 40°C temperature using mobile phase buffer:Methanol:Acetonitrile (20%:40%:40% v/v/v) at flow rate 1.0 ml/min. Quantification was achieved with PDA detector at a wavelength of 275nm. The silodosin peak retention time was 2.4 mins and the dutasteride retention was 10.9 mins, so the total run time was set to 14mins. The linearity range of Silodosin and Dutasteride is 20-120 µg/ml and 1.25-7.5 µg/ml respectively. Linear regression found to be 0.999. The proposed method was validated according to International Conference on Harmonisation (ICH) guidelines. The results shown in the method was accurate, precise, sensitive and economical. Thus, the current study showed that the developed reverse-phase liquid chromatography method is sensitive and selective for the estimation of Silodosin and Dutasteride in Capsule Dosage Form.

Keywords: Silodosin; Dutasteride; RP-HPLC; Method development; Validation

INTRODUCTION

Silodosin (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). The IUPAC name of this drug was 1-(3-hydroxypropyl)-5-[(2R)-({2-[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino)propyl]indoline-7-carboxamide. 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. Silodosin is a prescription medication used for the treatment of the signs and symptoms of benign prostatic hyperplasia (BPH) (Figure 1).

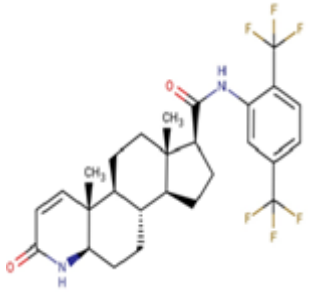

Figure 1: Structure of Dutasteride and structure of Silodosin.

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 5 α -reductase inhibitor which prevents the conversion of the

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androgen sex hormone testosterone into the more potent dihydrotestosterone (DHT). IUPAC name of this drug is (5 α , 17 β)-N-{2,5-Bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide. Dutasteride belongs to a class of drugs called 5 α -reductase inhibitors, which block the action of the 5 α -reductase enzyme that convert testosterone into DHT. It is an irreversible inhibitor of all three isoforms of 5 α -reductase, types I, II, and III.

MATERIALS AND METHODS

Instruments

HPLC with the model WATERS e 2695 separation module, PDA WATERS 2998 detector, software Empower. Digital pH meter. Weighing machine. Vacuum filter, Vacuum PR, Ultrasonic bath with the model Lab, Volumetric Flasks which are made of Borosil, Pipettes and Beakers also made of Borosil.

Chemicals and reagents

Silodosin and Dutasteride with the brand name Silo Dart, Potassium hydrogen phosphate with the brand name Merck's, Methanol for HPLC with the brand name Rankem Acetonitrile for HPLC with the brand name Rankem, Ortho phosphoric acid with the brand name RANKEM and Water with the brand name Milli-Q.

Chromatographic conditions

The chromatographic mode used in this method was RP-HPLC, the Instrument used was Waters e HPLC 2695 with PDA 2998 detector and Empower software. The column temperature was 40°C, Sample temperature was 25°C, and the Column used in this method was Unison C8 (250 mm x 4.6, 5 µm, Make: waters). The pH range was set to be 3.00 and the buffer is set to be 1.8918 g of Dipotassium Hydrogen Phosphate in 1000 ml water, pH 3.00 was adjusted with Ortho phosphoric acid. Buffer Mobile phase is 20% Buffer, 40% Methanol, and 40% Acetonitrile. Flow rate is 1 ml/min, Wavelength is 275 nm, Injection volume 10 µl and the Run time was 12 min.

PREPARATION METHODS

Preparation of standard

Preparation of Dutasteride Standard Stock Solution: Accurately weighed 50 mg of dutasteride working standard was taken in a 100 ml volumetric flask. Initially add 10 ml of methanol for dissolved dutasteride API and sonication in an ultrasonic water bath for 10 min. Make up to 100 ml volumetric flask with diluent and mixed well.

Preparation of silodosin and dutasteride standard

Accurately weighed 40 mg of silodosin working standard was taken in a 50 ml volumetric flask. Initially add 10 ml of diluent for dissolved silodosin API and sonication in an ultrasonic water bath for 5 min and add 5 ml of Dutasteride standard stock solution. Make up to 50 ml volumetric flask with diluent and mixed well. Further, Pipette out 5 ml of above solution in 50 ml volumetric flask and make up with diluent up to 50 ml mark and mixed well (Table 1).

Table 1: Gradient Program

Gradient program					
Time (min)	0	6	8	12	13
Mobile phase A (pH 3.0 buffer)	45	45	20	20	45
Mobile phase B (Organic Mixture)	55	55	80	80	55

VALIDATION PARAMETERS

System suitability

System suitability study was performed by initially injecting five successive injections and the results are reported in % RSD. The results were showed in Table 2.

System precision

The above system suitability solution were used then filtered through 0.45 µ Nylon and then six replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. The chromatograms recorded for accuracy test were shown in Figure 2.

Specificity

Preparation of Blank: Accurately measured 500 ml (50%) of Acetonitrile and 500 ml (50%) of HPLC grade water (Milli-Q water) were mixed and sonication in an ultrasonic water bath for 10 min.

Preparation of placebo

Weighed accurately 91.5 mg of placebo was transferred into 100 ml volumetric flask and then dilute and make up with diluents.

These solutions were filtered through 0.45 µ Nylon and then each concentration one replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

The chromatograms recorded for accuracy test were shown in Figure 2.

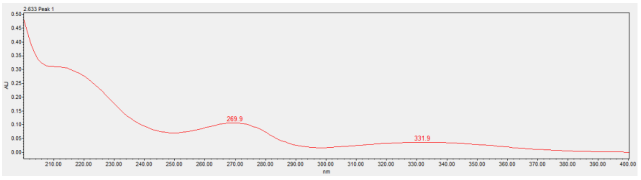

Figure 2: Spectrum for Silodosin.

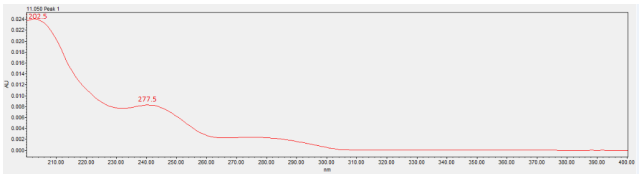

Figure 3: Spectrum for Dutasteride.

ACCURACY

Accuracy determination, three different concentrations were prepared separately i.e., 50%, 100% and 150% for the analyte and chromatograms are recorded. Results were shown in Table 3.

PRECISION

Preparation of standard solution

Accurately weighed 50 mg of dutasteride and 40 mg of silodosin in methanol and prepare the working standard solutions. Both intra-day and inter-day precision was done by performing five trials at regular intervals on same day and on five alternate days. The results are reported in the form of %RSD.

Preparation of sample solution

Prepare six sample solutions. These solutions were filtered through 0.45 µ Nylon and then each concentration two replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. The results are reported in Table 4.

LINEARITY

Preparation of stock solution

Accurately weighed 50 mg of dutasteride into 100 ml volumetric flask then add 20 ml of methanol and make up with diluents.

Accurately weighed 40 mg of silodosin into 50 ml volumetric flask then add 10 ml of diluents and add 5 ml of above solution and make up with diluents. This was marked and labelled as stock solution. The results were showed in Figure 3 and Table 5.

FILTER VALIDATION

About 50 mg of dutasteride were weighed and transferred into 100 ml volumetric flask. Initially add 10 ml of methanol for dissolved and sonication in ultrasonic water bath for 10 min. Make up to 100 ml volumetric flask with diluent and mixed well.

Accurately weighed 40 mg of silodosin was taken in a 50 ml volumetric flask. Initially add 10 ml of diluent for dissolved and sonication in ultrasonic water bath for 5 min and add 5 ml of Dutasteride standard stock solution. Make up to 50 ml volumetric flask with diluent and mixed well. The results were showed in Tables 6-10.

MOBILEPHASE STABILITY

Prepare a fresh blank solution and fresh standard solution. Used in old mobile phase (Mobile phase Prepared by before two days).The results were showed in Table 11.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of variation of temperature

The above prepared sample was analyzed at 35°C and 45°C instead of 40°C remaining conditions are same.10 µl of sample was injected twice and chromatograms were recorded.

The chromatograms recorded were shown in Figure 4.

Effect of variation of flow

The above prepared sample was analyzed at 0.8 ml/min and 1.2 ml/min instead of 1 ml/min, remaining conditions are same. 10 µl of sample was injected twice and chromatograms were recorded. The chromatograms recorded were shown in Figure 5.

RESULTS AND DISCUSSION

Method validation

The developed method was validated based on ICH guidelines to detect and quantitate both silodosin and dutasteride in dosage form with use of HPLC system equipped with PDA detector. The chromatograms are showed in Figures 6-9.

VALIDATION OF DEVELOPED METHOD

The developed method was validated according to the ICH guidelines as follows:

System suitability

System suitability study was performed by initially injecting five successive injections and the results are reported in % RSD.

Table 2: System Suitability Results for Silodosin and Dutasteride.

PARAMETER	SYSTEM SUITABILITY	
Drug	SILC	DUTC
Injection 1	129310	4756
Injection 2	127820	4767
Injection 3	128184	4763
Injection 4	129162	4740
Injection 5	129457	4701

Average	128787	4745
SD	735.145	26.876
%RSD	0.57	0.53

System precision

System precision study was performed by injecting six e injections were done and the results are reported in % RSD.

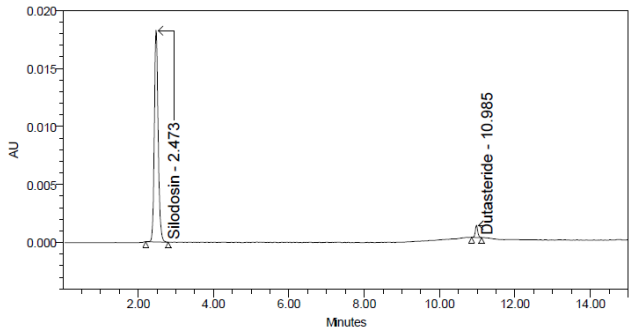

Figure 4: Chromatogram for standard injection.

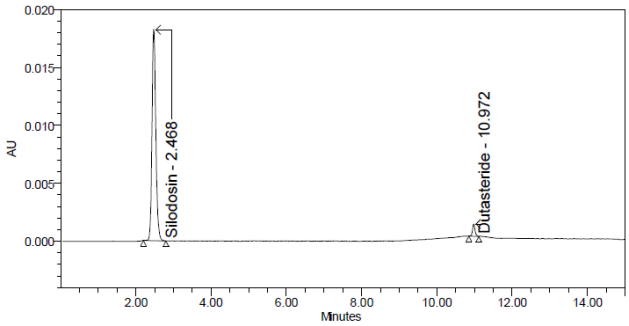

Figure 5: Chromatogram for sample injection.

Accuracy

Accuracy study was performed by injecting three different levels i.e., 50%, 100% and 150%. The results are reported in % mean recovery.

Table 3: Accuracy results for Silodosin and Dutasteride.

Drug	% level	Area	Amount added (mg)	Amount found (mg)	% recovery	Mean recovery
		64415	20	20.2	99.71	
	50%	64555	20	20.1	99.5	99.55
		64585	20	20.3	99.45	
SILO		128750	40	40.1	99.77	
	100%	128889	40	40.2	99.67	99.69
		128921	40	40.1	99.64	
		193648	60	60.1	99.5	
	150%	194256	60	60.3	99.19	99.43
		193468	60	60.2	99.6	
	50%	2365	25	25.3	100.11	
DUTA		2362	25	25	100.24	100.12
		2367	25	25.1	100.03	
		4731	50	50.1	100.09	
	100%	4735	50	50.3	100.21	100.15
		4728	50	50	100.15	
		7085	75	75.1	100.25	

150%	7092	75	75	100.15	100.16
	7096	75	75.2	100.1	

Precision

Inter and intra-day precision studies were performed by injecting a sample of 80 ppm of silodosin and 5 ppm of dutasteride six were done in same day and on six alternate days over a period of 10 days and results are reported in % relative standard deviation (%RSD).

Table 4: Inter day and Intraday Precision Results for Silodosin and Dutasteride.

PRECISION	INTERDAY		INTRADAY	
Drug	SILC	DUTC	SILC	DUTC
Injection 1	129310	4756	129462	4727
Injection 2	127820	4767	129797	4858
Injection 3	128184	4763	130198	4815
Injection 4	129162	4740	129802	4883
Injection 5	129457	4701	129852	4741
Injection 6	129085	4793	130874	4754
Average	128836.3	4753.3	129997.5	4796.3
SD	668.723	309.1	488.84	65.3
%RSD	0.52	0.65	0.38	1.4

Linearity and range

The linearity of proposed method was done by injecting various concentrations of the drug and the results are reported in the form of correlation coefficient. The range was calculated from the calibration graphs constructed by concentration versus peak areas (Figures 6 and 7).

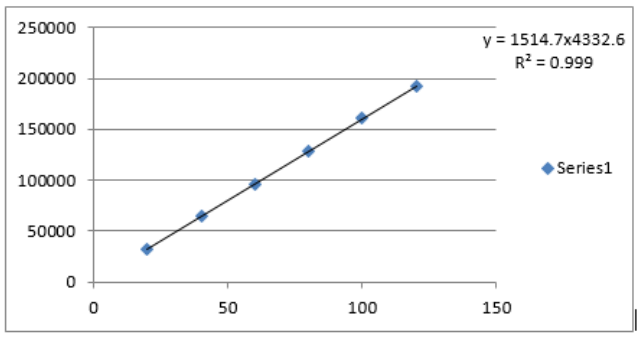

Figure 6: Calibration graph of Silodosin.

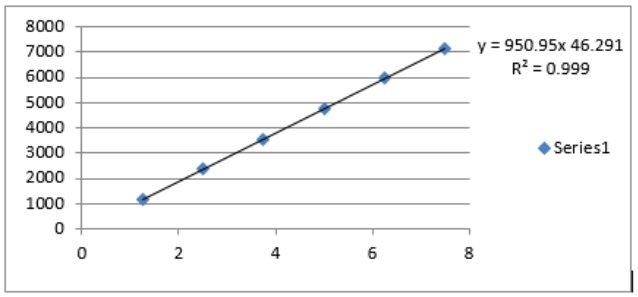

Figure 7: Calibration graph of Dutasteride.

Table 5: Linearity Results for Silodosin and Dutasteride.

Injection Level	Silodosin		Dutasteride	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
Injection 1	20	32122	1.25	1189
Injection 2	40	64244	2.5	2379
Injection 3	60	96366	3.75	3569
Injection 4	80	128489	5	4758
Injection 5	100	160611	6.25	5947
Injection 6	120	192733	7.5	7137

Filter validation

Filter validation study was performed by injecting three different levels i.e., unfiltered centrifuged, nylon filtered and PVDF filtered. Two injections of each level were done and the results are reported in % mean recovery.

Table 6: System Suitability results for filter validation.

Parameter	System suitability	
Drug	DUTC	SILC
Injection 1	127830	4765
Injection 2	129321	4740
Injection 3	128179	4757
Injection 4	129162	4730
Injection 5	129547	4762
Average	128807.8	4750.8
SD	756.097	15.123
%RSD	0.59	0.32

Table 7: Filter validation results for Silodosin.

Preparation	Sample area/std area avg	Std wt/50	5/50	Spl wt/200	Potency/100	Avg wt/LC	100	Percentage
Unfilter-1	119636	50.1/50	5/50	601.10/	99.93/100	300.55/8	100	93.03
Unfilter-2	119998	50.1/50	5/50	601.10/	99.93/100	300.55/8	100	93.31
Nylon filter-1	120147	50.1/50	5/50	601.10/	99.93/100	300.55/8	100	93.42
Nylon filter-2	120059	50.1/50	5/50	601.10/	99.93/100	300.55/8	100	93.35
PVDF filter-1	119943	50.1/50	5/50	601.10/	99.93/100	300.55/8	100	93.26
PVDF filter-2	120170	50.1/50	5/50	601.10/	99.93/100	300.55/8	100	93.44

Table 8: Filter validation results for Dutasteride.

Preparation	Sample area/std area avg	Std wt/100	25/100	Spl wt/200	Potency/100	Avg wt/LC	100	Percentage
Unfilter-1	4761	40.1/100	25/2500	601.10/200	99.93/100	300.55/0.5	100	100.19
Unfilter-2	4751	40.1/100	25/2500	601.10/200	99.93/100	300.55/0.5	100	99.98
Nylon filter-1	4751	40.1/100	25/2500	601.10/200	99.93/100	300.55/0.5	100	99.98
Nylon filter-2	4703	40.1/100	25/2500	601.10/200	99.93/100	300.55/0.5	100	98.97
PVDF filter-1	4792	40.1/100	25/2500	601.10/200	99.93/100	300.55/0.5	100	100.85
PVDF filter-2	4767	40.1/100	25/2500	601.10/200	99.93/100	300.55/0.5	100	100.32

Table 9: Solution stability results for Silodosin.

Preparation	Sample area	Run Time	Plate count	Tailing	Percentage
Fresh Sample	128574	2.47	3509	1.26	99.8
Fresh Sample	127534	2.47	3515	1.26	99
24 hrs B.T Sample	127931	2.48	3778	1.29	99.3
24 hrs B.T Sample	127752	2.48	3662	1.27	99.2
24 hrs R.F Sample	127738	2.48	3625	1.27	99.2
24 hrs R.F Sample	127993	2.48	3656	1.26	99.4

Table 10: Solution stability Results for Dutasteride.

Preparation	Sample area	Run Time	Plate count	Tailing	Percentage
Fresh Sample	4768	10.98	148206	1.2	100.4
Fresh Sample	4740	10.98	148841	1.17	99.8
24 hrs B.T Sample	4844	11	142324	1.17	101.9
24 hrs B.T Sample	4824	11	148901	1.18	101.5
24 hrs R.F Sample	4749	11	151301	1.14	100
24 hrs R.F Sample	4742	11	140946	1.11	99.8

Mobile phase stability

Mobile phase stability study was performed by injecting one fresh blank and five fresh standard injections by using previous mobile phase solution. The results are reported in Run time of peaks.

Table 11: Mobile phase stability Results for Silodosin and Dutasteride.

Parameter	Run time of peaks	
Drug	SILC	DUTC
Injection 1	2.47	10.98
Injection 2	2.47	10.98
Injection 3	2.47	10.98
Injection 4	2.47	10.98
Injection 5	2.47	10.98

Robustness

Robustness was performed by flow rate variation and temperature variation. Two injections each were done and the acceptance criteria for tailing factor and plate count were determined (Figure 8-10).

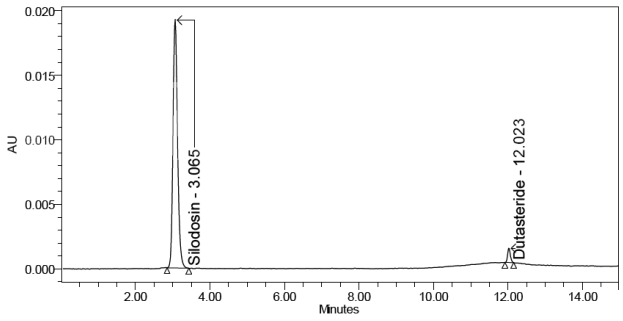

Figure 8: Chromatogram for flow rate 0.8 ml/min injection 1.

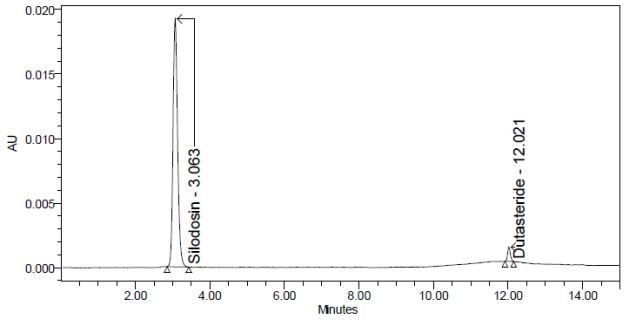

Figure 9: Chromatogram for flow rate 1 ml/min injection 2.

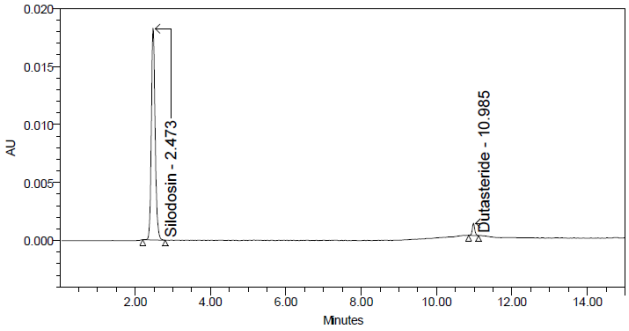

Figure 10: Chromatogram for temperature 35°C injection 1.

CONCLUSION

Method development and validation of Silodosin and Dutasteride was done by RP-HPLC method employing Unison C8 (150 mm x 4.6, 5 µm, Make: waters) using mobile phase as Phosphate Buffer: Acetonitrile: Water in 20:40:40 v/v/v at a flow rate 1 ml/min.

The linearity range of Silodosin and Dutasteride was found to be HPLC 20-120 and 1.25-7.5 µg/ml respectively. Linear regression was not more than 0.999. The values of %RSD were <2% for both the methods. The %recovery varies in the range of 99-100%.

The results show the method was accurate, precise, sensitive, and economic. The HPLC method was more rapid. Method was successfully applied to the pharmaceutical dosage form (Silo Dart capsules).

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