Research Article

DEVELOPMENT AND VALIDATION OF RP–HPLC METHOD FOR ESTIMATION OF SILODOSIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, rapid, precise and reliable isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of silodosin in bulk and pharmaceutical dosage forms. The separation of silodosin was carried out with mobile phase containing ammonium acetate buffer pH 4.5: acetonitrile 50:50 using Zorbax Eclipse C-8 column (150 X 4.6 mm, 5 µ). The mobile phase was pumped at a flow rate of 1ml/min and the eluent was monitored by PDA detector at 268 nm. The retention time was 2.865 min. Linearity curve was plotted and correlation coefficient is 0.999. The accuracy of the proposed method was determined and mean percentage was found to be 102.44 %. The method was successfully validated according to the ICH guidelines and it was concluded that the developed method was accurate, sensitive, precise, robust and useful for the routine quality control of silodosin in pharmaceutical dosage forms.

Keywords: Silodosin, RP-HPLC, Validation.

INTRODUCTION

Silodosin[1], a novel indoline 7-carboxamide derivative used in the treatment of Benign Prostatic Hyperplasia and Urinary Tract Infections. Silodosin is designated chemically as 1-(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2, 2, 2-trifluoroethoxy) phenoxy] ethyl} amino) propyl] indoline-7-carboxamide[1]. Its empirical formula is $C_{25}H_{32}F_3N_3O_4$ and structure of silodosin is shown in figure 1.

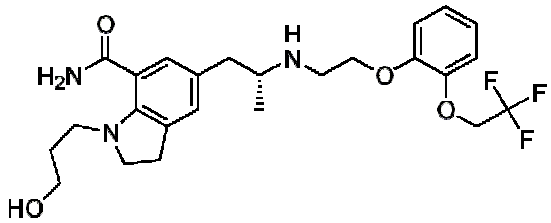


Figure 1: Chemical Structure of Silodosin

Silodosin is α_{1A} adrenergic receptor antagonist[2-3] that selectively affects the prostate and urinary bladder as a therapeutic agent for the treatment of the signs and symptoms of the benign prostatic hyperplasia. It causes smooth muscle relaxation by antagonizing the α_{1A} adrenergic receptor in the lower urinary tract. It shows more affinity towards α_{1A} adrenergic receptor than α_{1B} adrenergic receptor and minimizes the blood pressure related adverse effects.

The recent literature survey showed that a rapid, sensitive LC/MS[4] method was developed for the determination of silodosin in human plasma. Estimation of silodosin[5] by UV Spectrophotometric method was reported but there is no RP-HPLC method development was reported. Therefore the current work of my research presents a new Reverse Phase -HPLC method for the analysis of silodosin in bulk and its dosage form. After developing the method it was validated for assay determination which includes accuracy, precision, linearity range, selectivity and robustness according to the International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Reagents and Chemicals

Silodosin was obtained as gift sample from MSN laboratory in Hyderabad and marketed product was purchased from local market. Acetonitrile, Water, were obtained from Merck. Mumbai and Ammonium Acetate, Acetic Acid obtained from RANKEM Mumbai. All solvents used in this work are HPLC grade.

Standard solutions and Chromatographic conditions

Preparation of Ammonium Acetate Buffer pH 4.5

10 mM Ammonium Acetate Buffer was prepared by dissolving 0.72 g of Ammonium acetate in 1000 ml of water (HPLC grade) and pH was adjusted to 4.5 with acetic acid.

Preparation of Silodosin standard stock solution

A stock solution was prepared by dissolving accurately weighed 40 mg of Silodosin in Water: Methanol (50:50) as diluent and transferred into 50 ml of volumetric flask, from that 5ml was pipette out and transferred into 50 ml volumetric flask and made up with diluent to obtain 80 ppm solution.

Sample preparation of silodosin in tablet dosage form

To estimate the silodosin in pharmaceutical dosage form silodol capsule was selected as a commercial brand. Twenty capsules were opened and powdered was taken. Powder equivalent to 8 mg was transferred to 50ml of

volumetric flask and dissolved by adding water: methanol (50:50) as diluent. The solution was sonicated for 15 min using ultra sonicator bath and the resulting solution was filtered through a 0.45 µm membrane filter.

HPLC instrumentation and conditions

Instrument

Chromatographic separation was performed by High Performance Liquid Chromatography system (Waters 2695 separation module, USA) equipped with Waters 2996 Photodiode Array Detector, pumps (LPG-3400A, 8007455), auto sampler (WPS-300SL analytical 8008961). The analysis was carried out by using Zorbax Eclipse C-8 column (150 X 4.6 mm, 5 µ). The Empower 2 soft ware was employed in this method.

Chromatographic conditions

Mobile phase consisting of Ammonium Acetate buffer: Acetonitrile 50:50 at 1 ml/min flow rate was used. It was filtered through 0.45µm nylon filter and sonicated for 2 min in ultrasonic bath. Samples were analyzed at 268 nm at an injection volume of 10 µL and separation was carried by using Zorbax Eclipse C-8 column (150mm X 4.6mm, 5.0µ). The pump pressure was set at 1500 -1900 psi and run time was set at 10 min. The optimized chromatographic conditions are given in table 1

Table 1: Optimized chromatographic conditions

Parameter	Optimized Condition
Instrument	Waters 2695- RP-HPLC
Column	Zorbax Eclipse C-8 column
Mobile phase	Ammonium Acetate buffer pH 4.5 : ACN 50:50
Flow Rate	1 ml/ min
Detection	PDA Detector at 268 nm
Injection volume	10µl
Temperature	30°C
Retention Time	2.865 min

Method development and validation

Preliminary trials were made by changing different mobile phases at various chromatographic parameters to develop a new method for analyzing the silodosin. Finally a suitable method was developed with a mobile phase Ammonium acetate buffer: Acetonitrile 50:50 at a flow rate of 1.0 ml/min and a detection wavelength of 268 nm afforded the best separation of silodosin

After method development, validation of the current test method for silodosin was performed in accordance with ICH guidelines for assay determination of active ingredients in bulk and finished pharmaceutical products which include accuracy, precision, selectivity, robustness, limit of quantification limit of detection, linearity and range.

Accuracy (% Recovery studies)

The percentage recovery of silodosin was studied at three different concentrations. The recovery method was carried out at 50%, 100%, and 150% by standard addition method according to ICH guidelines. The detailed results were given in Table 2.

Precision

Precision method of an analytical method is usually expressed as the standard deviation. The % Relative Standard Deviation was determined by injecting five freshly prepared silodosin standard samples in the same day under the similar conditions. The results were given in Table 3.

Linearity and range

Linearity for silodosin was evaluated by calculating the correlation coefficient. Five different concentrations of silodosin were prepared and a calibration curve was constructed by plotting the peak area vs concentrations. From this, correlation coefficient was calculated and linearity range was found as 40-120µg/ml. The data was given in table 4 and figure 2.

Robustness

To evaluate the robustness, the sample was analyzed by changing flow rate and temperature. The flow rate as per the method is 1ml. This was changed to 0.8ml and 1.2ml and temperature was changed ±5°C. This deliberate change in the method has no affect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust. The results are placed in Table 5.

LOD and LOQ

LOD and LOQ were determined based on standard deviation of the response and slope. The lowest amount of the sample is determined quantitatively and its limits were expressed as concentration of analyte (parts per million). The following two formulas were used for LOD and LOQ detection.

LOD = 3.3*SD / Slope of response

SD - Standard Deviation

LOQ = 10 σ/Slope of response

σ - Standard deviation

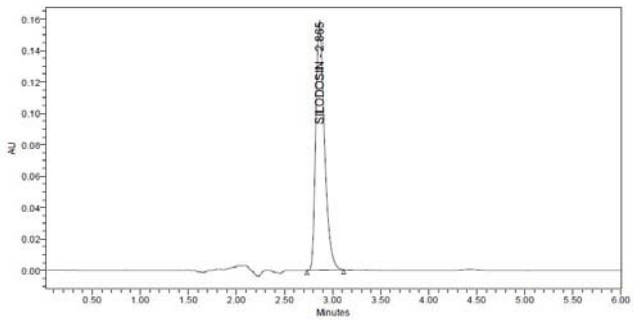
RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Different chromatographic conditions were employed for the analysis of the silodosin in bulk and pharmaceutical dosage form. The optimized mobile phase used in this method is Acetonitrile: ammonium acetate buffer (pH 4.5) 50:50 v/v at a flow rate 1 ml/min. Sample was detected by PDA detector at 268 nm. The retention time for silodosin was found to be 2.865 min (figure 3).

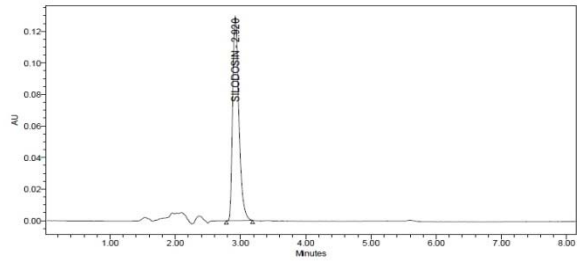
Assay of Silodosin in Capsule

Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into HPLC system and scanned at 268 nm. The assay limits were found to be 96 - 106%. A single peak was observed with retention time 2.920 (figure 4).



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	SILODOSIN	2.865	1006906	100.00	4487	1.39

Figure 3: RP-HPLC Chromatogram of Silodosin



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	SILODOSIN	2.920	856139	100.00	4367	1.40

Figure 4: Silodosin chromatogram in dosage form

Method Validation

Accuracy

The mean percentage recovery of silodosin was found to be 102 % which suggest that method development is very accurate. The acceptance limit of % recovery should be between 98.0-102.0%.

Table 2: % Recovery data of silodosin

Concentration	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
50%	99.04	100.87±1.615	1.601
	102.09		
	101.489		
100%	102.375	102.44±0.506	0.494
	102.988		
	101.983		
150%	100.569	100.44±0.294	0.293
	100.658		
	100.109		

Precision

The % relative standard deviation of silodosin was found to be 1.36. Based on the results obtained from the data it was suggested that developed method is precise.

Linearity

Calibration curve was constructed for silodosin standard solution by plotting average peak area versus and concentrations. The regression equation is y = 14214x – 10537 and regression coefficient r^2 is 0.999.

Table 3: Precision method of proposed RP-HPLC method

Injection	Area	Retention Time
Injection 1	1019440	2.83
Injection 2	1010661	2.84
Injection 3	1017344	2.84
Injection 4	1026927	2.84
Injection 5	989540	2.84
Injection 6	1026159	2.84
Average	1015011.833	2.84
SD	13849.35	0.004
% RSD	1.36444	0.141

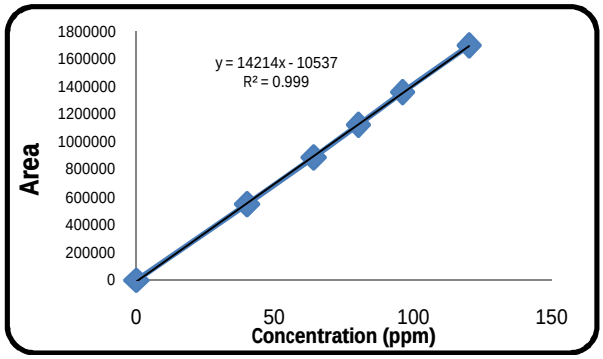


Figure 2: Linearity curve

Table 4: Linearity data

Concentration (PPM)	Area
40	548518
64	888274
80	1124864
96	1361230
120	1699463
Correlation coefficient	0.999

Robustness

Robustness was carried out by changing two parameters such as flow rate and temperature. It was found that there was no changes occur on chromatogram when flow rate and column temperature changes.

Table 5: Robustness Data

S.No	Flow Rate				Temperature (30 ±5°C)			
	0.8 ml		1.2 ml		35°C		25°C	
	Area	RT	Area	RT	Area	RT	Area	RT
1	1387730	4.05	1400268	4.069	1031362	2.88	1030261	2.86
2	1410157	4.042	1400363	4.063	1037432	2.86	1039564	2.88
3	1422751	4.01	1402023	4.06	1046021	2.9	1042021	2.83
SD	17739.1	0.0212	986.96	0.005	7365.48	0.02	6203.22	0.03
% RSD	1.26	0.524	0.07	0.11	0.71	0.56	0.598	0.89

SD- Standard Deviation, **RT**- Retention Time **% RSD**- Relative Standard Deviation

Table 6: System suitability for RP HPLC Method

Standard No	Area	Retention time	Theoretical plates	Tailing
1	1002420	2.84	4259	1.38
2	1004483	2.86	4267	1.39
3	1017561	2.87	4420	1.38
4	1014654	2.88	4335	1.39
5	1006906	2.86	4357	1.39
AVRG	1009205	2.86		
SD	6579	0.0144		
%RSD	0.651	0.50		

System Suitability

System suitability was performed for silodosin standard solution by injecting five replicates. A standard solution was prepared and was injected five times in to the HPLC. The % RSD of the retention time and area of the peak was calculated from the chromatograms obtained. The % RSD of area was found to be within the limits and it should not more than 2.0. It was found that the number of theoretical plates for silodosin is more than 2000 and tailing factor is also below 2.0. The results of system suitability and system suitable parameters were given in Table 6 & 7.

Table 7: System Suitability parameters of proposed RP-HPLC method

Parameters	Values
Retention time (min)	2.865
Theoretical plates	4487
Tailing factor	1.39
Wavelength (λmax)	268 nm
Regression equation	Y = 14214x - 10537
Correlation coefficient(r^2)	0.999
Capacity factor (k)	0.245

CONCLUSION

A novel isocratic RP-HPLC method was developed using simple mobile phase for the estimation of silodosin in bulk and pharmaceutical dosage forms. The method was validated successfully using parameters like accuracy, precision, linearity and robustness. The %RSD for all parameters was found within the limits, which indicates the validity of the method and therefore the proposed method can be used for routine analysis for silodosin in bulk and pharmaceutical formulation.

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