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Development and Validation of LC Method for Determination of the Enantiomeric Purity of Silodosin in Bulk Drug Substances

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ABSTRACT

A simple, rapid, novel and normal phase chiral HPLC method has been developed for the separation of S-Silodosin from R-Silodosin and quantitative determination of S-Silodosin enantiomer in Silodosin bulk drugs. The proposed method was based on normal phase chromatographic separation on Polysaccharide-Based Chiral Stationary Phase, Chiral pak AS-H column (250mm ×4.6mm i.d.; particle size,5µ) at a temperature of 28°C using a mobile phase consisting of n-Hexane, Ethanol and Diethyl amine (600 : 400 : 0.1 v/v/v) at a flow rate of 1mL.min^{-1} with an injection volume of 10µL. Quantitation was achieved with UV detection at 270 nm based on peak area with linear calibration curves. The elution times of S-Silodosin and R-Silodosin were 5.0 min and 6.0 min respectively. In this proposed chiral HPLC method, the resolution between S-Silodosin and R-Silodosin was found to be greater than 1.5. The developed method was validated with respect to linearity, accuracy, precision, solution stability, ruggedness, robustness, limit of detection and limit of quantification. The recovery obtained for S-isomer was in between 102.2 % and 104.4%. The detection limit obtained for S-isomer was 0.04µg.mL^{-1} and the quantification limit was 0.13µg.mL^{-1} respectively. Linearity was performed for the S-isomer from LOQ to 150%. The correlation coefficient obtained for S-isomer was more than 0.999. The solution stability of Silodosin bulk drug was determined and the compound was found to be stable up to 48 hrs. As the method has less run time (10 min), it can be useful in quality control laboratories for routine analysis.

Key words: R-Silodosin, S-Silodosin, Polysaccharide-based chiral stationary phase, High performance liquid chromatography and Method validation.

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INTRODUCTION

Chiral separation by high-performance liquid chromatography (HPLC) using a chiral stationary phase (CSP) is one of the most efficient methods for separating enantiomers, not only on an analytical scale, but also on a preparative scale, and in the past two decades, many CSPs have been developed. Polysaccharides such as cellulose, amylose, and chitin are the most abundant optically active polymers on the earth and can be readily modified to carbamates and esters through the reaction with isocyanates and acid chlorides, respectively. The CSPs based on polysaccharide derivatives are some of the most popular ones and can separate a wide range of chiral compounds[1,2]. By using hexane-alcohol eluent systems on these CSPs, 80–90% of the chiral compounds may be resolved[3,4]. The chiral resolution is essential in pharmaceutical, agriculture, and food industries. Silodosin (Figure 1) chemically 1-(3-Hydroxypropyl)-5-[(2R)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino)propyl]-2,3-dihydro-1H-indole-7-carboxamide, is a medication for the symptomatic treatment of benign prostatic hyperplasia[5]. It acts as α1-adrenoceptor antagonist with high uroselectivity[6]. Silodosin has one chiral centre and is used as a single enantiomer (R)[7]. Enantiomers of the racemic drugs often differ in pharmacokinetic behaviour or pharmacological action. The development of analytical methods for the quantitative analysis of chiral compounds are extremely challenging due to the fact that enantiomers posses virtually identical properties. Even though corresponding S-isomer is controlled in starting stages of Silodosin synthesis, it is quite important to monitor the level of other isomer in finished product. Literature reveals that there are only few analytical methods for estimation of Silodosin in bulk and pharmaceutical dosage forms. They include UV[8] and LC-MS[9,10] methods but none has reported the chiral separation of enantiomers of Silodosin.

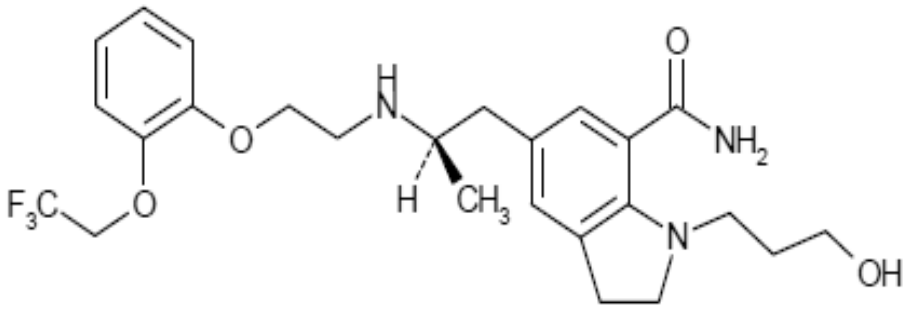

Figure 1. Chemical Structure of Silodosin

Molecular formula: $C_{25}H_{32}F_3N_3O_4$

Chemical name: 1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino)propyl]-2,3-dihydro-1*H*-indole-7-carboxamide

MATERIALS AND METHODS

Chemicals and reagents

HPLC grade n-Hexane, Ethanol and Diehyl amine were purchased from Merck. The purity of the solvents used is more than 99.0%. R-Silodosin bulk drug and S-Silodosin isomer were kindly provided by Pharma industries of Hyderabad, India.

Instrumentation

An Agilent HPLC 1260 series equipped with an auto sampler with UV-Visible detector was used in the experiment; a quartenary gradient pump system with temperature control oven used. Data acquisition and processing were conducted using the Empower software.

Preparation of Stock, Standard and Test Solutions

The stock solution of S-Silodosin was prepared by dissolving 20mg of S-isomer in 10mL of mobile phase (100%). 0.1mL of stock solution transferred into 10mL volumetric flask containing 10mL of mobile phase (1.0% solution). A series of dilutions prepared using 1.0% solution of S-Silodosin for Limit of quantification, Limit of detection, Precision, Linearity and Accuracy. The sample solution was prepared by weighing about 20 mg of the R-Silodosin bulk drug substance into a 10mL volumetric flask and dissolved and diluted to 10 mL with mobile phase.

Preparation of System suitability

20mg of R-Silodosin bulk drug substance dissolved in 10 mL of mobile phase and spiked with 0.15% of S-Silodosin. As a part of system suitability, two criteria were defined (i) resolution between R-Silodosin and S-Silodosin (ii) Tailing factor R-Silodosin. The System suitability results are tabulated Table 1.

Table 1. System suitability Results

S. No.	Parameter	1.0 mLmin^{-1}
01	The resolution between S-Silodosin and R-Silodosin	1.97
02	The tailing factor of R-Silodosin from system suitability solution	1.13

Chromatographic Conditions

The chromatographic column used was Chiral pak AS-H (250mm ×4.6mm i.d.; particle size, 5µ), make of Chiral Technologies, USA. Using Solvents like n-Hexane, ethanol and diethyl amine (600:400:0.1, v/v/v) was optimized as mobile phase as well as diluent. The flow rate of the mobile phase was 1.0 mL.min^{-1} and the column temperature set at 28°C. The injection volume was 10µL. The concentration of the sample taken was 2mg.mL^{-1}. The wavelength of method was set at 270nm.

RESULTS AND DISCUSSION

Method Development and Optimization

The HPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Silodosin Enantiomers (R-Silodosin and S-Silodosin) and also complied the general requirements of system suitability criteria. The main challenge was to get good resolution between Silodosin enantiomers with acceptable peak shapes and trace level detection. With this objective several trials were carried out initially, on Chiral pak AD-H and Chiral cel OD-H columns with respect to different flow rates and different mobile phase compositions. The results showed that the resolution was not more than 1.5, as well as peak shapes were poor with these columns. Later Chiral pak AS-H column was considered, which gave good resolution and Gaussian peak shapes with the optimized mobile phase. With respect to mobile phase, initially 2-propanol was used which gave good resolution but has longer retention times for both enantiomers. To reduce this ethanol was used as a mobile phase component and within 7mins both enantiomers were resolved with very good peak shapes. Therefore the finally optimized mobile phase consists of 60% of n-Hexane and 40% of Ethanol with 0.1% Diethyl amine as modifier with a flow rate of 1.0mL.min^{-1} and column oven temperature of 28°C. The run time was 10 minutes. In the present study 0.15% specification was set for S-isomer. The blank, system suitability chromatograms and spectra of Silodosin enantiomers were shown in the Figures 2, 3 and 4. As per ICH guidelines, for any known impurity 0.15% is the specification. The sample concentration of 2mg.mL^{-1} was considered based on the detection level. The run time was fixed for 10 minutes. In the present study 0.15% specification kept for S-isomer.

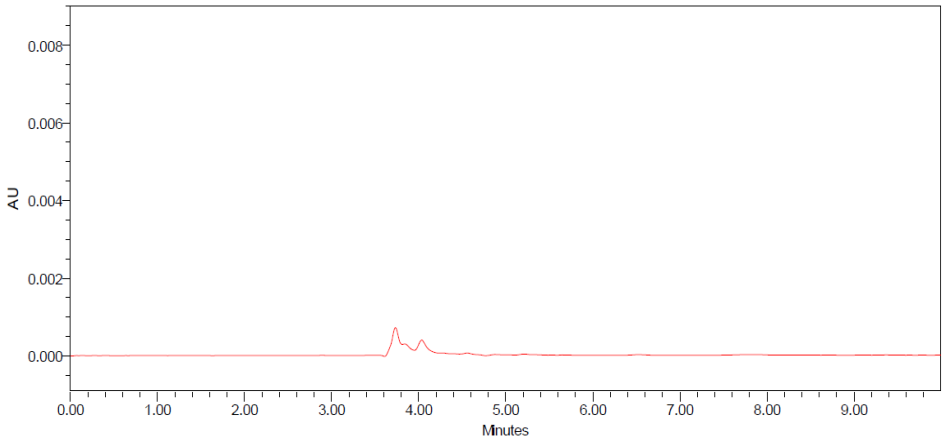

Figure 2. A typical chromatogram of Blank Mobile phase (Chiral pak AS-H column with dimensions 250mm ×4.6mm i.d.; particle size, 5µ) flow rate: 1.0mL·min^{-1}, wave length 270nm, column oven temperature 28°C).

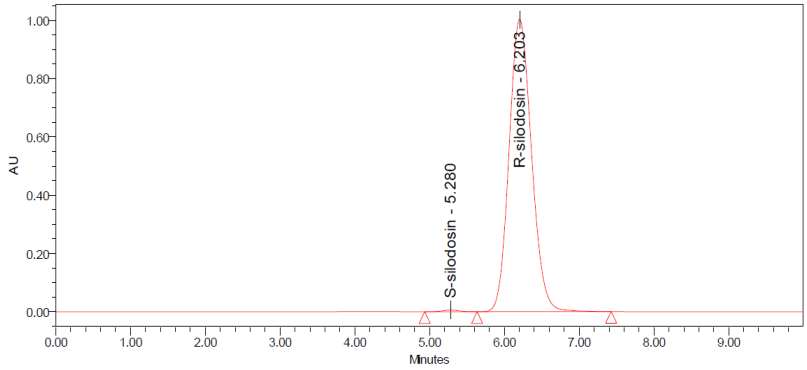

Figure 3. A typical chromatogram of System suitability in full scale (Chiral pak AS-H column with dimensions 250mm ×4.6mm i.d.; particle size, 5µ) flow rate: 1.0mL·min^{-1}, wave length 270nm, column oven temperature 28°C)

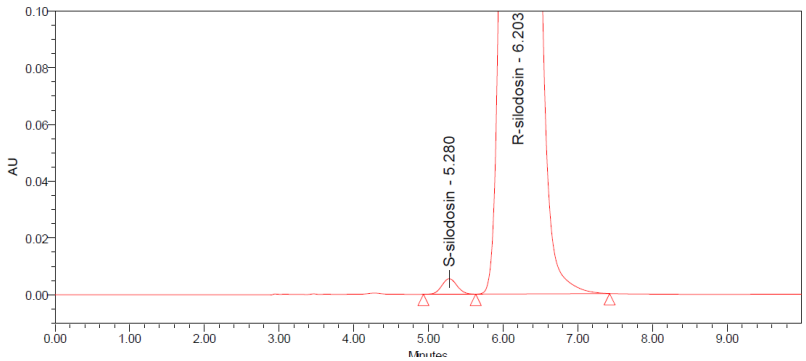

Figure 4. A typical chromatogram of System suitability in Zoom condition (Chiral pak AS-H column with dimensions 250mm ×4.6mm i.d.; particle size, 5µ) flow rate: 1.0mL·min^{-1}, wave length 270nm, column oven temperature 28°C).

Method Validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines. The validated method parameters were LOD, LOQ, Accuracy, Precision, Linearity, Range, Ruggedness and Robustness.

Sensitivity

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for S-Silodosin estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The detection limit (LOD) of the S-isomer was found about 0.04 µg/mL and the quantification limit (LOQ) was about 0.13 µg/mL respectively. The precision study was also carried out at LOQ level by injecting six individual preparations of S-Silodosin. Calculated the %RSD for the areas of S-isomer. The precision values at LOQ concentration for S-isomer was below 2%. The recovery at LOQ concentration level for S-isomer was 99.8%.

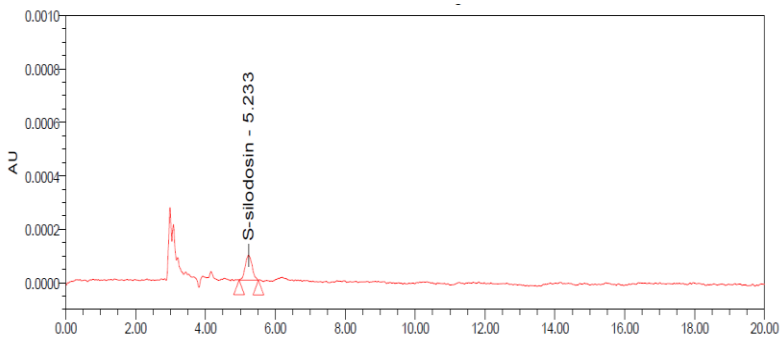

Figure 5. A typical chromatogram of LOQ solution in Zoom condition (Chiral pak AS-H column with dimensions 250mm ×4.6mm i.d.; particle size, 5µ) flow rate: 1mL.min^{-1}, wave length 270nm, column oven temperature 28°C).

Precision

The precision of the Chiral method was performed by injecting six individual preparations of R-Silodosin (2mg.mL^{-1}) spiked with 0.15% (100%) of S-Silodosin. The %RSD for area% of S-isomer was calculated. Precision study was also determined by performing the same procedures at higher level (at 150%). The %RSD values for precision study at 100% concentration and 150% concentration for S-isomer were below 2%. Intermediate precision performed for R-Silodosin spiked with 0.15% S-isomer (100% level) and the %RSD values for all the isomers found to be below 1.0%.

Linearity and Range

A linearity test solution for chiral method was prepared by diluting the S-Silodosin to the required concentrations. The solutions were prepared at six concentration levels. From LOQ to 150% of the 0.15% level of the S-Silodosin (i.e. LOQ, 50%, 75%, 100%, 125% and 150%) was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The correlation coefficient for S-Silodosin was more than 0.999. The coefficient of determination (R^2) obtained for S-Silodosin was within the acceptance criteria Figure 6.

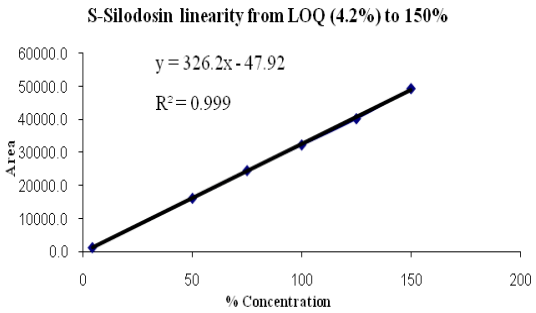

Figure 6. Linearity plot of S-Silodosin, showing the linearity range and R^2 value

Accuracy

Accuracy of the method was determined by analyzing R-Silodosin (*n*=3) spiked with (50% to 150%) 0.075%, 0.15% and 0.225% of the S-isomer. The recoveries obtained for S-isomer at all levels were in between 102.2% and 104.4% respectively. The percentage of recoveries of S-isomer was calculated and tabulated Table 2.

Table 2. Accuracy Results

Level of Accuracy	S-Silodosin	Isomer added ($mgmL^{-1}$)	Isomer Recovered ($mgmL^{-1}$)	% Isomer Recovery (n=3)
50%	1	0.0015	0.0016	102.2
	2	0.0015	0.0014	
	3	0.0015	0.0016	
100%	1	0.0030	0.0032	104.4
	2	0.0030	0.0032	
	3	0.0030	0.0030	
150%	1	0.0045	0.0046	103.7
	2	0.0045	0.0048	
	3	0.0045	0.0046	

Ruggedness

Ruggedness of the method was performed by doing precision study by injecting six individual preparations of ($2mg \cdot mL^{-1}$) R-Silodosin spiked with 0.15% of S-isomer (100% level) using different column, different analyst and different system from the same laboratory. The %RSD for area% of S-isomer was calculated. The %RSD obtained for S-isomer was below 2.0%.

Table 3. System suitability Results

S. No.	Parameter	0.8 $mLmin^{-1}$	1.2 $mLmin^{-1}$	23°C	33°C	+10% n-Hexane	-10% n-Hexane
01	The resolution between S-Silodosin and R-Silodosin	2.18	1.79	2.09	2.11	1.78	2.04
02	The tailing factor of R-Silodosin from system suitability solution	1.16	1.16	1.14	1.14	1.18	1.29

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (*Rs*) between R-Silodosin and S-Silodosin was evaluated. The flow rate of the mobile phase was 1.0 mL·min–1. To study the effect of flow rate on the developed method, 0.2 units of flow was changed (i.e. 0.8 and 1.2 mL·min–1). The effect of column temperature on the developed method was studied at 23°C and 33°C instead of 28°C. The effect of Mobile phase composition on resolution of isomers was studied by varying ± 10% of n-Hexane and Ethanol. System suitability injected in the above changed conditions to check the

resolution and RRT of both enantiomers. No peak is merged with each other and the resolution between two isomers was more than 1.5. The Robustness data was tabulated Table-3.

Solution Stability

Solution study was performed for sample, system suitability solutions upto 48 hours at room temperature and the solution was found to be highly stable at room temperature during 48 hours study and no impurities observed during this study.

CONCLUSION

A simple, sensitive and novel Chiral HPLC method was developed and validated for the quantitative determination of S-isomer related to Silodosin. Compared with the previously reported methodologies there is no specific chiral method was reported for Silodosin Active pharmaceutical ingredient. This method utilizes a Chiral pak AS-H column which is readily available in most of the quality control testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect S-isomer 0.04 $\mu g.mL^{-1}$ and can quantify upto 0.13 $\mu g.mL^{-1}$. Silodosin API is manufacturing and supplying by so many industries, if they want to check the S-isomer content this method is useful. The run time of the method was only 10 minutes, so it can reduce solvent consumption as well as analysis time.

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