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Method Development and Validation of RP-HPLC Method for assay of Sildosin in Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid reverse-phase high performance liquid chromatographic method has been developed and validated for the drug Sildosin in pure and in capsule dosage form. The estimation was carried out on a Phenomenax Luna C18 (150mm × 4.6 mm i.d., particle size 5µm) column with a mixture of Phosphate buffer and Acetonitrile with a pH 3.0 adjusted with ortho phosphoric acid in the ratio of 40:60%v/v. UV detection was performed at 219nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.32 min. and the flow rate was 0.8 ml min $^{-1}$. The calibration curve was linear over the concentration range of 50-90 µ g mL^{-1}. The LOD and LOQ values were found to be 2.93 and 9.91. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for estimation of Sildosin in pharmaceutical dosage form.

Keywords: Sildosin, RP-HPLC, Phosphate buffer, Acetonitrile

INTRODUCTION

Silodosin is a selective antagonist of post-synaptic alpha adrenoreceptor which are located in the human prostate, bladder base, bladder neck, prostatic capsule, and prostatic urethra [1]. Blockade of these alpha-1 adrenoreceptor can cause smooth muscle in these tissues to relax, resulting in an improvement in urine flow and a reduction in BPH symptoms [2-3]. Silodosin is chemically 1-(3-Hydroxypropyl)-5-[(2R)-2-({2-[2-(2, 2, 2-trifluoro Ethoxy) phenoxyl] ethyl} amino) propyl]-2, 3-dihydro-1H- indole-7-carboxamide [4].

Literature [1, 4-5] reveals different methods for its analysis in formulations. Hence our present plan is to develop a new, simple, precise and accurate method for its analysis in formulation after a detailed study, a new RP-HPLC method was decided to be developed and validated. For the estimation of this method we used Phosphate buffer and Acetonitrile with a pH 3.0 adjusted with ortho phosphoric acid in the ratio of 40:60%v/v. The column used was Thermosilane C8, at a flow rate of 0.6 ml/min. and UV detector was employed in the study at 219nm.

MATERIALS AND METHODS

Apparatus and Chromatographic Parameters

A Waters HPLC with Alliance with Auto sampler with Empower 2.0 software with Symmetry C8 (4.6 x 150mm, 5 µm, Make: Thermosil) column and UV detector was employed in the study. An Edwa pH meter Afcoset digital balance and ambient column oven were the other instruments used for this study.

Drug Samples

The Sildosin drug used for estimation for this study was procured from capsule. The brand name SILODOL 8 was used which is marketed by RANBAXY LTD. The label claim was Sildosin 8 mg in each capsule.

Reagents and Solutions

HPLC grade Acetonitrile and Methanol, a GR grade/Merck Potassium di hydrogen phosphate, HPLC grade water and Sildosin drug was used in the study. A mixture of Potassium di hydrogen ortho phosphate buffer Acetonitrile in the ratio of 40:60%v/v was used as a mobile phase at a pH 3.0 adjusted with Ortho phosphoric Acid and it is also used as a diluent for preparing the working solution of drug. The mobile phase was degassed in ultrasonic water bath for 5 minutes and filtered through 0.45µm filter under vacuum filtration

PREPARATION OF THE SILODOSIN STANDARD & SAMPLE SOLUTION

Standard Solution Preparation

Accurately weighed and transfer 10mg of Silodosin Working standard into a 10 ml volumetric flask, added about 7 ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent(Stock solution). Further pipette out 0.7 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

Sample Solution Preparation

Weighed 5 Silodosin capsules and calculated the average weight. Accurately weighed and transfer the sample equivalent to 10 mg of Silodosin into a 10 ml volumetric flask. Add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette out 0.7 ml of this stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

METHOD DEVLOPMENT

Three trials were performed for the method development and the best peak with least fronting factor was found to be the third peak with RT= 2.32 min (Fig 1).

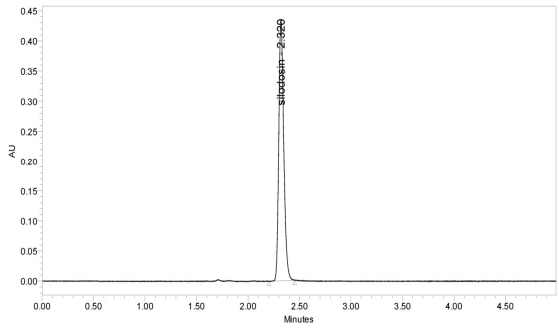


Figure 1. Chromatogram peak of sildosin

METHOD VALIDATION

Precision

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria

The % RSD for the area of five standard injections results (Table 1) should not be more than 2%.

Table 1. % RSD data

S. No	RT	Peak area	Average peak area	Standard deviation	% RSD
1.	2.354	1110000	1108968	2376.6	0.2
2.	2.355	1107193			
3.	2.359	1115330			
4.	2.363	1110785			
5.	2.365	1112642			

Accuracy

Injected the standard solutions of Accuracy -50%, 100% and 150% and calculated the Amount found, Amount added for Silodosin and the individual recovery and mean recovery values.

Acceptance Criteria

The % Recovery for each level (Table 2) should be between 98.0 to 102.0%.

Table 2. Percent recovery level of sildosin

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1093514	4.90	4.88	99.2%	
100%	2246802	10.0	10.0	100.3%	100.1%
150%	3407885	14.8	15.2	100.8%	

Recovery Studies

To determine the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits. The % recovery, Mean recovery and %Relative standard deviation value for Sildosin drug was found to be 99.2-100.8%, 100.1% and 0.2 respectively.

Linearity and Calibration Curve

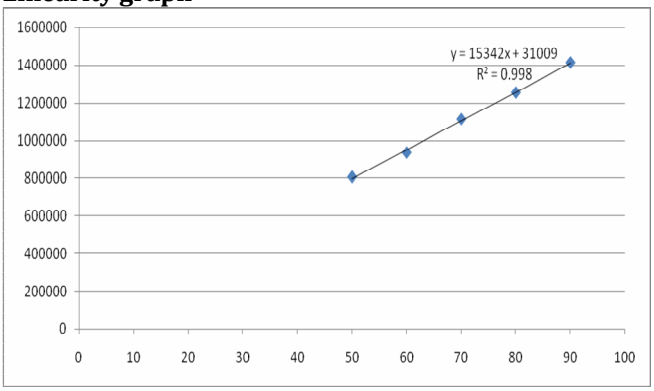
Working dilutions of Sildosin in the range of 50-90µg/ml was prepared by taking suitable aliquots of working standard solutions of drug in different 10ml volumetric flask and diluting up to the mark with mobile phase. 20µl quantity of each dilutions was injected in to the column at a flow rate of 0.8ml/min. the drug in the elute was monitored at 219nm and the corresponding chromatograms were recorded. From these the mean peak areas were calculated and a plot of concentration vs peak areas was constructed. The

regression of the plot was computed by least square regression method. The slope and intercept value for calibration curve was y=15342x+31009 (R2=0.998) founded.

Table 3. Different concentration values of sildosin

S. No	Concentration in mcg	RT	Area	Height
1.	50	2.371	806536	238291
2.	60	2.377	935934	268924
3.	70	2.385	1112681	323825
4.	80	2.375	1256127	365507
5.	90	2.388	1413552	406010

Linearity graph



X-Axis = Concentration **Y**-Axis = Peak area

Limit of Detection and Limit of Quantification

Limit of Detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level. The LOD for Sildosin was found to be 2.93 at 0.03µg/ml solution.

Limit of Quantification (LOQ) is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level. The LOQ for Sildosin was found to be 9.91 at 0.101µg/ml solution.

Robustness

Robustness is determined by making deliberate changes in the chromatographic conditions like change in flow rate, mobile phase composition and temperature and evaluated for the impact on the method. It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust.

RESULTS AND DISCUSSION

A simple, rapid and precise method has been developed and validated for the drug Sildosin. The estimation was carried out with a mixture of Phosphate buffer and Acetonitrile with a pH 3.0 adjusted with ortho phosphoric acid in the ratio of 40:60%v/v. Precision of the methods were studied by making repeated injections of the samples and system precision values were determined (Table 3, 4). The retention time was 2.32 min. The calibration curve was linear over the concentration range of 50-90 µ g mL^{-1}. The LOD and LOQ values were found to be 2.93 and 9.91. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-HPLC method developed was very much suitable for routine analysis.

Table 4. System precision values of sildosin

S. No	Parameter	Acceptance criteria	Observed value
1	Accuracy	95-105%	98.62%
2	Precision	RSD within 2%	0.76%
3	Linearity	R^2 not less than 0.99	R^2=0.998
4	LOD	S/N=3	2.93
5	LOQ	S/N=10	9.91

CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of Sildosin. The method validated was found to be simple, accurate and precise. Therefore the proposed study method can be used for quantification of Sildosin in bulk and pharmaceutical dosage form.

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