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 Phenomenex 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 µg 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
Sildosin 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]. Sildosin is chemically 1-(3-Hydroxypropyl)-5-{(2R)-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 dihydrogen phosphate, HPLC grade water and Sildosin drug was used in the study. A mixture of Potassium dihydrogen 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 DEVELOPMENT
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).

Table 1. % RSD data

<table>
<thead>
<tr>
<th>S. No</th>
<th>RT</th>
<th>Peak area</th>
<th>Average peak area</th>
<th>Standard deviation</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.354</td>
<td>1110000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>2.355</td>
<td>1107193</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2.359</td>
<td>1115330</td>
<td>1108968</td>
<td>2376.6</td>
<td>0.2</td>
</tr>
<tr>
<td>4.</td>
<td>2.363</td>
<td>1110785</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>2.365</td>
<td>1112642</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>% Concentration at specification Level</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1093514</td>
<td>4.90</td>
<td>4.88</td>
<td>99.2%</td>
<td>100.1%</td>
</tr>
<tr>
<td>100%</td>
<td>2246802</td>
<td>10.0</td>
<td>10.0</td>
<td>100.3%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>3407885</td>
<td>14.8</td>
<td>15.2</td>
<td>100.8%</td>
<td></td>
</tr>
</tbody>
</table>

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 Silodosin 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
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 μg 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.

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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