Development and Validation of HPTLC Method for Quantification of Silodosin in Bulk and Pharmaceutical Dosage Form

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A simple, sensitive, precise and specific high-performance thin layer chromatographic method was developed and validated for the determination of silodosin in bulk and Pharmaceutical Dosage Forms. Validation was carried out in compliance with International Conference on Harmonization guidelines. The thin-layer chromatographic method employed aluminium plates precoated with silica gel 60 F254 as stationary phase. The solvent system consisted of toluene/methanol/diethylamine (8:1:1). This solvent system was found to give compact spot for silodosin with Rf value 0.37. Densitometric analysis of silodosin was carried in the fluorescence mode at 366 nm. Linear regression analysis showed good linearity with respect to peak area in the concentration range of 140-1400 ng per spot. The method was validated for precision, accuracy, specificity, and robustness.

Keyword: Silodosin, HPTLC, Silica gel, Densitometry.

INTRODUCTION: Silodosin is a highly selective third generation α1A-adrenoceptor antagonist approved by FDA in 2008 for the treatment of the signs and symptoms of benign prostatic hyperplasia (BPH) [4]. Alpha-adrenergic antagonists are effective in relieving BPH symptoms. They relax smooth muscle in the prostate and bladder neck. Chemically silodosin is 1-(3-hydroxypropyl)-5-[(2R)-{2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino] propylindoline-7-carboxamide, with an empirical formula of C25H32F3N3O4 and molecular weight of 495.534 g/mol [1,2,5]. Literature survey revealed that a liquid chromatography–tandem mass spectrometric (LC–MS/MS) method has been developed and validated for the determination of silodosin in human plasma. A UV
A spectrophotometric method for estimation of silodosin from its solid dosage form was also available.

The present work deals with development and validation of HPTLC method for the quantitative analysis of silodosin. The aim of the present work was to develop an economic, accurate, specific and reproducible HPTLC method using densitometric detection for the determination of silodosin either in bulk form or in capsules.

**Experimental**

**Instruments and Apparatus**
A Camag HPTLC instrument consisting of Linomat V automatic spotter equipped with a 100-μL syringe, Scanner-III, flat-bottom twin-trough developing chambers, and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland) were used. HPTLC plates used were of aluminium backed silica gel 60 F254 with 0.2mm thickness, 10 × 10 cm, 5 x 10 cm (E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai). Shimadzu AUX 220 analytical balance (Kyoto, Japan) and PCI analytics ultrasonic bath sonicator 421 (Mumbai, India) were used during the research work.

**Reagents and Materials**
MSN Laboratories Ltd. kindly gifted Silodosin pure powder with 99.1% purity. All the solvents used were either analytical or HPLC grade. Capsules were purchased from local pharmacy.

**Chromatographic Conditions**
Before analysis, HPTLC plates were cleaned by pre-development with methanol and activated at 110°C for 5 min for solvent removal. Solutions of silodosin were applied to plates (10 × 10 cm, 5 x 10 cm) by means of a Linomat V automatic spotter equipped with a 100 μL syringe and operated with settings of band length, 8 mm; distance between bands, 10 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin-trough chamber previously saturated for 30 min with the mobile phase, toluene/ methanol/ diethylamine (8: 1: 1, v/v/v) to 8.5 cm. For all measurements, densitometric scanning was performed using a Camag TLC scanner III in the fluorescence mode at 366 nm and operated by the winCATS software.

**Preparation of Standard Solution.**
Accurately weighed 100 mg of silodosin was transferred to a 100 mL volumetric flask, dissolved in, and diluted up to the mark with methanol to obtain a standard solution of silodosin (1000 μg/mL). From this solution, 1 mL was further diluted to 10 mL with methanol to obtain a working standard solution of silodosin (100 ng/μL).

**Preparation of Sample Solution.**
Twenty capsules (SILODAL) each containing 8 mg silodosin were taken. A quantity of powder equivalent to 10 mg of silodosin was weighed and transferred to a 10 mL standard flask and about 5 mL of methanol was added and sonicated for 30 minutes. Then the volume was made up with methanol. The solution was filtered through whatmann filter paper No. 1 and 1ml of the filtrate was diluted with methanol to get the concentration of 0.1 mg/mL of silodosin.

**Method Validation**
The HPTLC method was validated as per ICH guidelines [6].

**Linearity**
For determining the linearity, standard solution of silodosin was diluted to get a concentration of 0.02mg/ml, 0.05mg/ml, 0.1mg/ml, 0.15mg/ml, 0.2mg/ml. 7 μL of all the solutions were applied to the TLC plate to give bands containing 140-1400 ng of silodosin per spot, and the plate was developed, using the previously described optimized mobile phase, and scanned. The calibration curves were constructed by plotting peak areas versus concentrations [3].
Accuracy
Accuracy of the method was determined by standard addition method in which the known amount of standard silodosin solutions were added to pre-analyzed capsule solution. These amounts corresponded to 25, 62.5, and 125% of the amounts claimed on the label. The amounts of Silodosin were estimated. Accuracy study was performed for three times, and percentage recovery of silodosin was calculated [3].

Method Precision (Repeatability)
Precision refers to the multiple application of the same standard solution of silodosin and repeated scanning of the same spot (n = 6) of silodosin without changing the position of plate for the HPTLC method. It describes the homogeneity of chromatography across the plate, including application development and evaluation. Repeatability is reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)
The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 2 other days, one plate per day for 3 different concentrations of silodosin (350, 490, and 700 ng/spot). The results are reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were found by the standard deviation of the response and the Slope of the calibration curves of silodosin using the formulae as given below.

$$\text{Limit of Detection} = \frac{3.3 \times y - \text{intercept}}{\text{slope of the calibration curve}}$$

$$\text{Limit of Quantification} = \frac{10 \times y - \text{intercept}}{\text{slope of the calibration curve}}$$

Robustness of the Method
Robustness of the proposed method was estimated by changing different conditions like developing distance (60 mm, 70 mm, 80 mm from the lower edge of plate), chamber type (flat bottom chamber and twin trough chamber) and relative humidity. Rf values were measured after development of plates, and % RSD was calculated. A concentration level of 700 ng per band was employed.

Solution Stability
The stability of standard solutions was tested after 0, 3, 6, and 12 h of storage. The stability of the solutions was determined by comparing Rf value at 700 ng per band.

Analysis of Silodosin in Capsules
To determine the content of silodosin in commercial formulations, 20 capsules (silodal 8) were weighed, their mean weight determined and finely powdered. Powder equivalent to 10 mg of silodosin was transferred into a 10mL volumetric flask containing 5 mL methanol, sonicated for 30 min, and diluted to the mark with methanol and filtered. 1 ml of the filtrate was diluted to 10 ml with methanol to obtain 100 ng/μL of the drug. 7 μL of both working standard solution and sample solution was applied to the HPTLC plate at 700 ng per spot and followed by development. Analysis was carried out in triplicate, peak areas were measured at 366 nm, and sample concentrations calculated using the formulae as given below.

$$\frac{\text{Amount of Drug in sample}}{\text{Peak area of std}} = \frac{\text{Peak area of std}}{\text{Conc of std}} \times \text{average weight}$$

Results and Discussion
Optimization of Chromatographic Conditions
The TLC procedure was optimized with a view to develop an assay method used for the quantification of the silodosin in pharmaceutical capsules. Both the pure drug and the capsule were spotted on the TLC plate and run in different
solvent systems. The mobile phase with toluene/methanol/diethylamine in the ratio of (8:1:1, v/v/v) was selected which gave symmetric, well-defined peak with prominent Rf value of 0.37±0.02 for silodosin with scanning at 366 nm (Fig.3). The TLC plate was viewed prior to derivatization under UV 366 nm and 254 nm (Fig.1-2).

**Validation of the Method**
The calibration plot was linear over a concentration range of 140-1400 ng per spot for silodosin (Fig.4). A good linear relationship observed over this range ($r^2 = 0.99916$) indicated that the method is linear. Repeatability of sample application and sample measurement was expressed as % RSD and was found to be 1.0984% and 1.1084% for six replicate determinations. The low values of % RSD indicate that the proposed method is repeatable. The % RSD value obtained for reproducibility was between 1.1084% - 1.4084% which indicates that the proposed method is precise. RSD of Rf value during robustness studies were calculated for changes in parameters like developing distance and chamber type were less than 1% that indicates that this method is robust and reproducible. Under different humidity environments, % RSD of Rf value was found to be more than 2%, hence the chromatogram should be developed at humidity control between 50% to 70%. LOD and LOQ values were found to be 85 and 260 ng per spot, respectively, and pointed towards adequate sensitivity of the method. Accuracy was determined on previously analyzed formulations after spiking with 25, 62.5 and 125 % of the standard drug. Mean recovery obtained is 101.02%. Validation parameters are summarized in Table 1.

**Solution Stability**
There was no indication of degradation in sample solutions of silodosin as revealed by peak purity data of bands of solution stored at different times. The solution was found to be stable at ambient temperature for 12 h, and no unknown peaks were observed.

**Analysis of Formulated Capsules**
A single band was observed in samples extracted from capsules, and there was no interference from the excipients, which might have been present in the capsules. The amount of silodosin obtained in capsules is in good agreement with label claim. The content of silodosin in capsules was found to be 95.58% with standard deviation 0.12. It was therefore inferred that there is no interference of excipients during the analysis of silodosin normally present in capsules. Thus the method can be applied for the routine analysis of silodosin in pharmaceutical formulations.

**Conclusion**
The developed HPTLC procedure was simple, precise, specific and accurate. Statistical analysis indicated that the method was reproducible and selective for the analysis of silodosin in bulk drug and in capsules without interference from excipients. Hence, this method can be easily and conveniently adopted for routine analysis of silodosin in bulk and its pharmaceutical dosage forms.

**Table 1: Summary of validation parameters of proposed HPTLC method.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>140-1400 ng/spot</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2 = 0.99916$)</td>
<td>0.99916</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>85 ng/spot</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>260 ng/spot</td>
</tr>
<tr>
<td>% Accuracy</td>
<td>101.02%</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>Robust</td>
</tr>
<tr>
<td>Repeatability of sample application ($n = 6$)</td>
<td>1.0984% - 1.1084%</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>1.1084% - 1.4084%</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
</tr>
<tr>
<td>% Assay</td>
<td>95.58%</td>
</tr>
</tbody>
</table>
Figure 1: Developed HPTLC plate prior to derivatization under UV 366 nm

Figure 2: Developed HPTLC plate prior to derivatization under UV 254 nm

Figure 3: A typical densitogram of standard silodosin with Rf value 0.37±0.02
REFERENCE:


