SPECTROFLUORIMETRIC METHOD FOR THE DETERMINATION OF SILODOSIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate, sensitive and reproducible spectrofluorimetric method has been developed for the analysis of Silodosin in pure and pharmaceutical dosage form. Silodosin shows strong native fluorescence in methanol having excitation wavelength at 272 nm and emission wavelength at 450 nm. The calibration graph was linear in the range from 0.01 to 1µg/ml. The proposed method was statistically validated and successfully applied for analysis of capsule dosage form. The limit of detection and limit of quantification were found to be 0.003µg/ml and 0.0091µg/ml respectively. The percentage recovery was found to be in the range of 98.53% ± 0.53 to 99.27% ± 0.49.

Keywords
Spectrofluorimetry, Silodosin.

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INTRODUCTION

Silodosin, chemically known as (-)-1-(3-hydroxypropyl)-5-[(2R)-2-((2-(2,2,2-trifluoroethoxy)-phenoxy) ethyl) amino] propyl -2,3-dihydro-1H-indole-7-carboxamide (figure 1), is used for the symptomatic treatment of benign prostatic hyperplasia. It acts as α1-adrenoceptor antagonist with high uroselectivity (selectivity for the prostate)[1].

![Figure 1 Structure of Silodosin.](image)

Several methods have been described in the literature for the determination of Silodosin such as: spectrophotometry method using acetonitrile [2] and 0.1N HCl as solvent[3]; UV and HPLC method for dissolution testing [4]; RPHPLC methods[5-7]; stability indicating HPLC method [8]; stability indicating UPLC method [9]; HPTLC method [10]; determination of Silodosin in human plasma by LC-MS/MS method [11]; LC method for determination of the enantiomeric purity of Silodosin [12] and RPHPLC method for determination of Silodosin in combination with Dutaseride [13].

The purpose of this study was to develop a fluorimetric method which is less tedious, more rapid and sensitive than the previously reported ones, for estimating Silodosin. This selective fluorimetric assay is simple and sensitive enough to be useful for determining this drug in commercialized pharmaceutical samples and can also be used for its determination in physiological fluids.

EXPERIMENTAL

Instrumentation

All fluorescence measurements were performed on the spectrofluorimeter, RF-5301 PC (Shimadzu, Japan), with non-fluorescent glass cell of 1 cm path length. Data acquisition was performed using RFPC software version 2.04. The instrument parameters are given in Table 1.

<table>
<thead>
<tr>
<th>Method parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Wavelength</td>
<td>272 nm</td>
</tr>
<tr>
<td>Emission Wavelength</td>
<td>450 nm</td>
</tr>
<tr>
<td>Scan Speed</td>
<td>Fast</td>
</tr>
<tr>
<td>Slit width</td>
<td>5</td>
</tr>
<tr>
<td>Solvent</td>
<td>Methanol</td>
</tr>
</tbody>
</table>

Materials and chemicals

Silodosin was purchased from HangzuDayang, Co., China. AR grade methanol (Spectrochem) was used as a solvent. The formulation of Rapilif capsule (IPCA laboratories) was used for analytical application. Each capsule contains 4 mg of Silodosin.

Standard and sample solutions

A stock solution of Silodosin(1000μg/ml) was prepared in methanol. From this 100 μg/ml solution was prepared in methanol. Aliquots of 100μg/ml solution were suitably diluted with methanol to give the final concentration in the range of 0.01-1μg/ml. The solution was scanned in the range of 200 to 700 nm against methanol as blank, to obtain the excitation and emission wavelength. The excitation and emission wavelength were found to be 272 nm and 450nm, respectively.

For analysis of Silodosin in solid dosage form, a commercial brand (Rapilif, IPCA Laboratories, India), was procured from local market. Twenty capsule contents of the marketed formulation were accurately weighed. The capsule powder equivalent to 4 mg of Silodosin was weighed, transferred to 10 ml volumetric flask, dissolved in methanol and the final volume was made up to the mark. The solution was sonicated for 5 minutes and filtered through whatmann filter paper No. 40. From this solution, 0.25 ml of aliquot was taken and conveniently diluted up to 10 ml with methanol and further again an aliquot of 1 ml was taken from later and diluted up to 10 ml with methanol to get the required concentration.

RESULTS AND DISCUSSION

The fluorescence of Silodosin was analysed in various solvents like single distillwater, 0.1M hydrochloric acid, 0.1M sodium hydroxide, methanol, ethylacetate and acetonitrile. Silodosin showed stronger native fluorescence property in methanol and 0.1M NaOH; but the sensitivity of the method was observed high in methanol as compared to 0.1M NaOH and hence methanol was selected.
as an optimum solvent for spectrofluorimetric analysis. The fluorescence property of the drug was studied at two excitation wavelengths i.e. 272 nm and 340 nm. At both these wavelengths the emission wavelength was found to be 450 nm. At 272 nm excitation wavelength the drug exhibited linearity in the range of 0.01–1 µg/ml whereas at 340 nm the linearity range was 0.1–3 µg/ml. However at 272 nm the sensitivity of the drug was comparatively better than at 340 nm and hence 272 nm was selected as the excitation wavelength. Figure 1 shows the overlain spectra of Silodosin over linearity range at 450 nm emission wavelength.

**Method validation**

The proposed method was validated as per ICH guidelines[14] for linearity, precision, accuracy, sensitivity and robustness.

Linearity study was evaluated by analyzing a series of standard solutions of seven different concentrations in the range of 0.01–1 µg/ml (y = 721.4x + 7.726; correlation coefficient $R^2 = 0.9989$). The overlain spectra over the linearity range are shown in figure 2.

![Overlaid spectra of Silodosin in methanol](image)

**Figure 2 Overlaid spectra of Silodosin in methanol.**

Recovery studies were done at three different levels. The pre-analyzed samples were spiked with 80%, 100% and 120% of the standard Silodosin and the mixtures were reanalyzed by the proposed method. The estimation was made in triplicate. Percentage recovery was calculated from the amount of drug found in the solution. The percentage recovery studies revealed that the recovery levels lie between 98.53% and 99.27%. Recovery results demonstrated that the proposed method was unaffected in the presence of formulation excipients and thus highly accurate as shown in Table 2.

Precision was estimated by determination of the repeatability of the method. Repeatability was assessed by analyzing three different concentrations (covering the specified range of the method) in triplicate, in a day for intra-day precision and on three different days for inter-day precision. Both inter-day as well as intra-day precision showed that the relative standard deviation ($\%$ RSD) was less than 2.0 (Table 2).

For LOD and LOQ determination, the calibration curve was repeated for three times and the standard deviation (SD) of the intercepts was calculated. According to ICH recommendations (1996), the approach based on the SD of the response and the slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were measured as:

\[ \text{LOD} = 3.3 \times \text{SD/slope of calibration curve} \]
\[ \text{LOQ} = 10 \times \text{SD/slope of calibration curve} \]

The theoretical values were assessed practically. The LOD and LOQ limits were found to be 0.003 µg/ml and 0.0091 µg/ml respectively.

For assessment of stability, the standard and sample solutions of Silodosin prepared in methanol, were stored at room temperature for 48 hrs and at 5°C in refrigerator for up to 5 days. The solutions were found to be stable and no change in the fluorescence was observed.

The method was found to be specific as the results were unaffected by the presence of the excipients, which can be observed from the results of the assay of commercial formulation.

The proposed spectrofluorimetric method was applied to the determination of Silodosin in commercial Rapilif capsule formulation. Satisfactory results were obtained which were in good agreement with the label claim and showed 99.19±0.41% recovery (Table 2).
Table 2 Summary of validation parameters and assay results.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range(µg/ml)</td>
<td>0.01-1</td>
</tr>
<tr>
<td>Detection limit (µg/ml)</td>
<td>0.003</td>
</tr>
<tr>
<td>Quantitation limit (µg/ml)</td>
<td>0.0091</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 721.4x + 7.726$</td>
</tr>
<tr>
<td>Correlation coefficient($R^2$)</td>
<td>0.9989</td>
</tr>
<tr>
<td>Accuracy (% recovery ±SD)*</td>
<td>80% 98.53±0.53</td>
</tr>
<tr>
<td></td>
<td>100% 98.67±0.63</td>
</tr>
<tr>
<td></td>
<td>120% 99.27±0.49</td>
</tr>
<tr>
<td>Precision (%RSD)*</td>
<td>Intraday 0.63</td>
</tr>
<tr>
<td></td>
<td>Interday 1.39</td>
</tr>
<tr>
<td>Assay results</td>
<td>Label amount (mg/capsule) 4</td>
</tr>
<tr>
<td></td>
<td>% Recovery ±SD$^2$ 99.19±0.41</td>
</tr>
</tbody>
</table>

*Mean value for n=3 determinations; SD=standard deviation.
*Mean value for n=3 determinations at 3 levels.

CONCLUSION

The results indicated that this method can be used for routine quality control of silodosin in bulk and its solid dosage form. As an added benefit of spectrofluorimeter owing to the detectability and sensitivity of the drug in the nano range (i.e. 10-1000ng/ml), the method can also be used for bioanalytical purpose.

REFERENCES
