NEW RAPID UPLC METHOD FOR THE ESTIMATION OF IMPURITIES IN THE CAPSULE DOSAGE FORM OF SILODOSIN

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Abstract
The present paper deals with development and validation of a new stability indicating UPLC method for the estimation of process related and degradation impurities of Silodosin in capsule dosage form. Chromatographic separation was achieved on HSS C18, 100 mm x 2.1 mm, column with 1.7µm particles column, using pH 3.2 phosphate buffer and acetonitrile in gradient elution mode. The detector was set at 225 nm and the flow rate was kept at 0.5mL/min. The optimized method was found to produce symmetric and sharp peaks with good separation between processes related impurities and degradation impurities. Formulation samples were subjected to forced degradation and stability indicating nature of the method was assessed by monitoring the sample using PDA detector. No interference was found due to any of the degradation impurities. The proposed method has been extensively validated in terms of specificity, precision, linearity, accuracy, limit of detection (LOD) and quantification (LOQ), and robustness. The precision was expressed with respect to the intra- and inter-day variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery of the known amount of impurities added to the sample preparation. The method was validated in terms of Specificity, Precision, Ruggedness, Accuracy, Robustness and Linearity as per ICH guidelines.

Key words: Silodosin, UPLC method, Method validation, Forced degradation.

1.0 Introduction
Silodosin (Fig.1) chemically 1-(3-Hydroxypropyl)-5-[(2R,-2-(2,2,2trifluoroethoxy)phenoxymethyl] amino) propyl]-2,3-dihydro-1H-indole-7-carboxamide, its molecular formula is C25H32F3N3O4 with a molecular weight of 495.53. Silodosin is a selective alpha-1 adrenergic receptor antagonist, which results in smooth muscle relaxation in the tissues of the prostate, bladder base and neck, and prostatic urethra.[1-2].

Smooth muscle relaxation in these tissues leads to improved urine flow; relief of symptoms associated with benign prostatic hyperplasia (BPH), and increased quality of life. Silodosin is indicated for treatment of signs/symptoms associated with BPH, is a medication for the symptomatic treatment of benign prostatic hyperplasia [3-4]. Silodosin is marketed under the trade names Rapaflo in the US and Silodyx in Europe and Rapilif in India (Ipca Uro sciences).

Very few methods have been published for the estimation of Silodosin using UV – visible Spectrophotometer, by HPLC and by LC-MS/ in bulk and dosage forms, in biological matrices [5-8]. To the best of our knowledge till date there was no stability indicating method was available for Silodosin and its related impurities in literature. The present method is rapid, simple, sensitive and stability indicating reverse phase UPLC method in which all impurities as well as degradation impurities was well separated. Present method has been validated as per ICH, current industrial trend and acceptable analytical practices [9-10].

2.0 Experimental
2.1 Chemicals
Samples of Silodosin and its three impurities (Fig. 2) were procured from Hetero Labs, Hyderabad, India. Gradient grade acetonitrile purchased from JT Baker, Germany. ACS grade potassium di hydrogen phosphate and ortho phosphoric acid were purchased from Sigma Aldrich, India. High pure water was used from Millipore Milli Q water purification system.

Equipment
The LC system, used for method development, forced degradation studies and method validation was Waters
The analytical method to separate degradation products from each other (using 3.0% HCl), base hydrolysis (using 1 N NaOH), and oxidative degradation (at 105°C), acid hydrolysis was attempted to stress conditions like thermal degradation indicating of the stability indicating property and specificity of the proposed method. Intentional degradation degradants, matrix, etc. be present. Typically these might include impurities, in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity was tested by injecting the sample by spiking with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Moreover, identification of each impurity was confirmed with relative retention times as compared with those of pure standards.

2.5 Method validation

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2 (R1) [9-10]. The described method has been extensively validated in terms of specificity, precision, linearity, accuracy, limit of detection (LOD) and quantification (LOQ), and robustness. The precision was expressed with respect to the intra- and inter-day variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery of the known amount of impurities added to the sample preparation.

2.5.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity was tested by injecting the sample by spiking with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Moreover, identification of each impurity was confirmed with relative retention times as compared with those of pure standards.

2.5.2 Forced degradation studies

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions like thermal degradation (at 105°C), acid hydrolysis (using 1 N HCl), base hydrolysis (using 1 N NaOH), and oxidative degradation (using 3.0% H2O2) to evaluate the ability of the proposed method to separate degradation products from each other and active ingredients as well. To check and ensure the

2.2 Chromatographic conditions

The chromatographic column used was an Acquity UPLC HSS C18, 100 mm x 2.1 mm, column with 1.7µm particles. The mobile phase A consists of 50mM phosphate buffer with pH adjusted to 3.2 with dilute ortho phosphoric acid. The mobile phase B consists of acetonitrile. The flow rate of the mobile phase was kept at 0.5 ml/min. The UHPLC gradient was set as: T/%B: 0/28, 3/28, 5.5/80, 7.5/80, 7.7/28 and 10/28. The column temperature was maintained at 30°C and the wavelength was monitored at 225 nm. The injection volume was 5µl. Phosphate buffer and acetonitrile in the ratio of 7:3 used as diluent for samples preparation.

2.3 Preparation of solutions

2.3.1 Preparation of standard solutions

A working solution containing of 2.5µg/ml of silodosin was used for the determination of impurities.

2.4 Preparation of sample solution

A test solution containing of 500µg/ml of silodosin was prepared by taking capsule powder equivalent to 50mg of silodosin into a 100ml volumetric flask.

2.4.1 Preparation of spiked sample solution

Another sample was prepared with spiked with impurities (Impurity 1, Impurity 2, Impurity 3 and Impurity 4(RC-05)) at 0.5% of sample concentration i.e., 2.5µg/mL. For the determination of impurities added to the sample preparation.

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homogeneity (peak purity) of silodosin peak in the stressed sample solutions, photo diode array detector was employed.

2.5.3 Precision

The precision of the related substance method was checked by injecting six individual sample preparations of (500µg/ml) silodosin spiked with 0.5% of Imp-1, Imp-2, Imp-3 and Imp-4 (RC-05) with respect to analyte concentration. %R.S.D. of area for Imp-1, Imp-2, Imp-3 and Imp-4 (RC-05) was calculated. The intermediate precision of the method was also evaluated using different analyst, different day and different make instrument in the same laboratory.

2.5.4 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for Imp-1, Imp-2, Imp-3 and Imp-4 (RC-05) were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp-1, Imp-2, Imp-3 and Imp-4 (RC-05) and calculating the % R.S.D. of the area.

2.5.5 Linearity

The linearity of the method was tested in order to demonstrate proportional relationship of response versus analyte concentration over the working range. It is usual practice to perform linearity experiments over a wide range of analyte. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed using a single reference standard/working standard, rather than the equation of a calibration line.

The linearity of detector response to different concentrations of impurities was studied by preparing a series of solutions using silodosin and its related impurities at five different concentration levels ranging from 0.05% to 0.75% w/w of test concentration (500µg/mL). The data were subjected to statistical analysis using a linear-regression model.

2.5.6 Accuracy

The accuracy of the related impurities method for the quantification of all four impurities in formulation samples. The study was carried out in triplicate at 0.05%, 0.1%, 0.2% 0.5% and 0.75% of the analyte concentration (500µg/ml). The % recoveries for Imp-1, Imp-2, Imp-3 and Imp-4 were calculated from the slope and Y-intercept of the calibration curve.

2.5.7 Robustness

To determine the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between Imp-1 and Imp-2 was evaluated. To study the effect of flow rate on the resolution, the same was altered by 0.05 units, i.e. from 0.45 to 0.55 ml/min. The effect of pH on resolution of impurities was studied by varying ±0.2 pH units (at 3.0 and 3.4 buffer pH). The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C. All the other mobile phase components were held constant.

3.0. Results and discussion

3.1 Optimization of chromatographic conditions

The potential impurities present in bulk samples produced by Hetero Labs, Hyderabad, India. The main target of the chromatographic method is to get the separation of all impurities and degradants generated from analyte peak. Impurities were co-eluted by using different stationary phases like C18 and C8 and different mobile phases containing buffers like potassium phosphate and ammonium acetate with different pH and using organic modifiers like acetonitrile and methanol in the mobile phase. Apart from the co-elution of impurities, we have also observed poor peak shapes for impurity 2 and degradants. pH of the buffer and organic composition of diluent found to be critical in achieving symmetrical peak shapes as well recoveries of respective impurities (1, 2, 3 & 4). The separation between major degradant & impurity 1 and another degradant and impurity 2 was achieved by optimized gradient programme. Satisfactory chromatographic separation was achieved using a mobile phase mobile phase A consists of 50mM phosphate buffer with pH adjusted to 3.2 with dilute ortho phosphoric acid. The mobile phase B consists of acetonitrile. The UHPLC gradient was set as: 0/28, 3/28, 5.5/80, 7.5/80, 7.7/28 and 10/28. In the optimized conditions the Silodosin, Imp-1, Imp-2, Imp-3 and Imp-4 were well separated with a resolution of greater than 2 and the typical retention times of Imp-1, Imp-2, Imp-3, Imp-4 and silodosin were about 1.9, 3.6, 1.1 and 6.3 min (Fig. 3). The system suitability results were given in Table 1 and the developed LC method was found to be specific for Silodosin and its three impurities, namely Imp-1, Imp-2, Imp-3 and Imp-4.
Silodosin bulk samples, Formulation samples were subjected to forced degradation. To identify interference from excipients place samples were also subjected to similar stress conditions and chromatograms were compared with that bulk drug’s and formulation samples. Under these conditions the degradation of drug substance was observed during oxidative stress, acid, base hydrolysis, thermal degradation and photolysis (Fig. 4). Silodosin was degraded predominantly into unknown impurity eluting at about 0.54 RRT, and impurity at about RRT 0.84 and impurity – 4. All the degradation products were well separated. Homogeneity and purity of all the peaks monitored using PDA detector. The mass balance of stressed samples was close to 99.5% (Table 2). This confirms the stability indicating power of the developed method.

### 3.3 Results of method validation experiments

#### 3.3.1 Precision

The % R.S.D. of area of Imp-1, Imp-2, Imp-3 and Imp-4 in related substance method precision study was within 5% confirming good precision of the method.

The limit of detection (LOD) and limit of quantification (LOQ) of Imp-1, Imp-2, imp-3 and Imp-4(RC-05) were 0.004%, 0.0029%, 0.034% and 0.0024% (of analyte concentration, i.e. 500µg/ml) for 5µl injection volume. The limit of quantification (LOQ) of Imp-1, Imp-2, Imp-3 and Imp-4(RC-05) were 0.013%, 0.011%, 0.011 and 0.010% (of analyte concentration, i.e.1mg/ml) for 5µl injection volume. The method precision for Imp-1, Imp-2, Imp-3 and Imp-4(RC-05) at LOQ level was below 7% R.S.D.

#### 3.3.2 Linearity

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. 0.05% to 0.75% for Imp-1, Imp-2, Imp-3 and Imp-4(RC-05). The correlation coefficient obtained was greater than 0.996. The results show that an excellent correlation existed between the peak area and concentration of Imp-1, Imp-2, Imp-3 and Imp-4.

#### 3.3.3 Accuracy

The percentage recovery of Imp-1, Imp-2, Imp-3 and Imp-4 in bulk drug and formulation samples was ranged from 97.1 to 102.6 (Table 3 & 4) HPLC chromatograms of blank, pure sample and all four impurities spiked in Silodosin formulation sample were shown in Fig. 3 (Blank, sample, spiked).

#### 3.3.4 Robustness

In all the deliberate varied chromatographic conditions (flow rate, pH and column temperature) the resolution between Imp-1 and Imp-2 was greater than 4.0, illustrating the robustness of the method.

#### 3.3.5 Stability of Analytical solutions

The stability of the standard and sample solutions was tested at regular intervals. The stability of solutions was determined by comparing results with freshly prepared standard solutions. The differences in values were within 0.05% for identified and unidentified impurities and 0.2% for total impurities up to 48hrs.

### Table 1 System suitability Report

<table>
<thead>
<tr>
<th>Compound</th>
<th>USP Resolution</th>
<th>USP Tailing factor(Rs)</th>
<th>Number of Theoretical plates(N)</th>
<th>Relative Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silodosin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imp-4(RC-05)</td>
<td>8.9</td>
<td>1.2</td>
<td>1</td>
<td>7896</td>
</tr>
<tr>
<td>Imp-1</td>
<td>26.5</td>
<td>1.03</td>
<td>2</td>
<td>8001</td>
</tr>
<tr>
<td>Imp-2</td>
<td>6.4</td>
<td>1.03</td>
<td>2</td>
<td>109285</td>
</tr>
<tr>
<td>Imp-3</td>
<td>22.2</td>
<td>1.05</td>
<td>2</td>
<td>196404</td>
</tr>
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<td></td>
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</tr>
</tbody>
</table>

### Table 2 Summary of Forced degradation study

<table>
<thead>
<tr>
<th>Stress conditions</th>
<th>% of Degradation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>As such sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Hydrolysis (1N HCl)</td>
<td>3.97%</td>
<td>Degraded into Imp-4(RC-05) and Other unknown impurity.</td>
</tr>
<tr>
<td>Base Hydrolysis (1N NaOH)</td>
<td>4.80%</td>
<td>Degraded into Imp-4(RC-05) and Other unknown impurity.</td>
</tr>
</tbody>
</table>
Oxidation (3% H$_2$O$_2$) 2.90%

Degraded into Imp-4(RC-05) and Other unknown impurity.

Table 3: Accuracy Data

<table>
<thead>
<tr>
<th>Accuracy level</th>
<th>Impurity-1</th>
<th>Impurity-2</th>
<th>Impurity-3</th>
<th>Impurity-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy at 0.05%</td>
<td>97.3</td>
<td>98.9</td>
<td>100.3</td>
<td>99.0</td>
</tr>
<tr>
<td>Accuracy at 0.20%</td>
<td>98.1</td>
<td>97.1</td>
<td>97.9</td>
<td>101.3</td>
</tr>
<tr>
<td>Accuracy at 0.50%</td>
<td>100.2</td>
<td>97.6</td>
<td>98.3</td>
<td>98.1</td>
</tr>
<tr>
<td>Accuracy at 0.75%</td>
<td>102.6</td>
<td>99.1</td>
<td>100.6</td>
<td>102.0</td>
</tr>
<tr>
<td>Mean Recovery</td>
<td>99.6</td>
<td>98.2</td>
<td>99.3</td>
<td>100.1</td>
</tr>
</tbody>
</table>

n=3 number of determinations

Table 4: Precision and Ruggedness data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Impurity-1</th>
<th>Impurity-2</th>
<th>Impurity-3</th>
<th>Impurity-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>%RSD (Precision)</td>
<td>2.21</td>
<td>3.13</td>
<td>1.83</td>
<td>0.93</td>
</tr>
<tr>
<td>%RSD (Ruggedness)</td>
<td>3.27</td>
<td>1.16</td>
<td>2.49</td>
<td>1.90</td>
</tr>
</tbody>
</table>

n=6 number of determinations

3.3.6 Conclusions
The validated stability-indicating UPLC method has proved to be rapid, simple, accurate, precise and reliable. The proposed method provides a good resolution between all the impurities and potential degradants. The behavior of eszopiclone under various stress conditions were studied and presented for the first time. The information presented herein could be very useful for quality monitoring of bulk samples and formulation samples as well during stability studies.

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References
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