Silodosin and Dutasteride both are approved drugs by USFDA (Food & Drug Administration). On literature survey, it was found that no methods have been reported for simultaneous estimation of Silodosin and Dutasteride. Therefore, it was thought of interest to develop a simple, accurate, precise, sensitive and economic analytical method and to validate as per ICH guidelines. So RP-HPLC method was developed and validated for simultaneous estimation of Silodosin and Dutasteride in multiunit system. Separation was achieved on Agilent technology HPLC; Zorbax SB C8 Column (250 mm × 4.6 mm, 5 μm) at 40°C temperature by using a mobile phase containing Buffer (Dipotassium hydrogen phosphate, pH 3) & Organic mixture (methanol : acetonitrile in 50:50 ratio) in the ratio of 20:80. Analysis was done at the flow rate of 1.0 mL/min and UV detection was carried out by wavelength gradient at 270 nm (0 to 5 min.) and 210 nm (5 min to 10 min). The retention time of Silodosin and Dutasteride was found to be 2.7 min & 6.7 min respectively. The specificity of the method was determined by assessing interference from placebo. Samples were subjected to forced degradation study by using 5N HCl, 5N NaOH, 30% hydrogen Peroxide, Humidity, thermal and photo degradation. Degradation was found at 30% peroxide condition for Silodosin and mild degradation was found for Dutasteride. The method was validated in terms of linearity, precision, accuracy, specificity, robustness, ruggedness, solution stability. The linearity were found to be in the range of 39.56 -118.68 μg/mL & 2.43-7.29 μg/mL for Silodosin and Dutasteride with correlation coefficient of 0.997 for Silodosin and 0.995 for Dutasteride. %RSD of method precision was found to be less than 2%, this indicates that the method is precise. Keywords: Forced degradation; Method development; RP-HPLC; Validation.
INTRODUCTION

Silodosin [1], a novel indoline 7-carboxamide derivative used in the treatment of Benign Prostatic Hyperplasia and Urinary Tract Infections. Silodosin is designated chemically as 1-(3-hydroxypropyl)-5-[(2R)-{(2-[2-(2, 2, 2-trifluoroethoxy) phenoxy] ethyl) amino} propyl] indoline-7-carboxamide. Structure of silodosin is shown in figure 1. Silodosin is α1A adrenergic receptor antagonist[2-3] that selectively affects the prostate and urinary bladder as a therapeutic agent for the treatment of the signs and symptoms of the benign prostatic hyperplasia. It causes smooth muscle relaxation by antagonizing the α1A adrenergic receptor in the lower urinary tract.

Dutasteride, chemically known as (5α, 17β)-N-{2,5bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide, is a synthetic 4-azasteroid compound with antiandrogenic activity. Structure of Dutasteride is shown in figure 2. Dutasteride is used to treat benign prostatic hyperplasia in men having an enlarged prostate gland and in the treatment of male pattern baldness. It belongs to a class of drugs called 5α-reductase inhibitors, which competitively and specifically inhibits type 1 (active in the sebaceous glands of most regions of skin and liver) and type 2 (primarily active in the reproductive tissues like prostate, seminal vesicles, epididymides, hair follicles and liver) isoforms of 5α-reductase, an intracellular enzyme that converts testosterone to 5α-dihydrotestosterone. The decrease in dihydrotestosterone levels may mitigate or prevent enlargement of the prostate gland [4-6].
The recent literature survey showed that a rapid, sensitive LC/MS [7-8] methods were developed for the determination of silodosin in human plasma. There are several method available for estimation of silodosin by UV Spectrophotometric methods [9-10], by RP-HPLC[11-13] and HPTLC method[14] were reported. A limited number of analytical method have been reported for the quantitative determination of Dutasteride in pharmaceutical preparations and human plasma when present alone or in combination with other drugs (Alfuzosin and Tamsulosin)[15-17]. These techniques are LC–MS[18-19], HPTLC[20], Enzyme-linked immunosorbent assay[21], HPLC and stability-indicating RP-HPLC[22-23], UV spectrophotometric method[24] · UPLC method[25].

None of the reported methods, however, addressed the analysis multiunit system of Silodosin and Dutasteride. The objective of this study was therefore to develop a simple, sensitive, and precise HPLC method for the simultaneous analysis of Silodosin and Dutasteride.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Silodosin: Working standard grade was supplied by Zydus Cadila Healthcare Limited (Ahmedabad, India), its claimed purity was 98.99%. Dutasteride: Working standard grade was supplied by Zydus Cadila Healthcare Limited (Ahmedabad, India), its claimed purity was 97.20% Combination drug products of Silodosin and Dutasteride (Label claim of 8 mg & 0.5 mg respectively) and Placebo were manufactured and supplied by Zydus Cadila Healthcare Limited (Ahmedabad, India). Acetonitrile, methanol used were of HPLC grade and were purchased from Spectrochem Private Limited, India.

**Instrumentation and chromatographic conditions**

Optimization of Method

Various method development trials were done and according to that trials, the method was optimized. The HPLC was of Agilent technology 1200 series with UV Visible detector. Column used was ZorbaxSB C8, 5 μm (250 mm × 4.6 mm). The system was run at a flow rate of 1.0 mL/min, 5 μL of sample was injected in the chromatographic system and a UV Visible detector was used for simultaneous determination of Silodosin and Dutasteride. Mobile phase comprising of 10.5mmol/l dipotassium hydrogen phosphate buffer: Organic mixture: (20:80) adjust pH 3.0 with 5% o-phosphoric acid. Organic mixture comprising of methanol:acetonitrile (50:50). Column temperature was maintained at 40°C and UV detection at 270 nm for 0 to 5 min and 210 nm for 5 to 10 min.

**Preparation of standard solutions**

50 mg of working standard of Dutasteride was dissolved in methanol in 100 mL volumetric flask, sonicate it for 2 min and volume was made up to the mark with methanol(Stock A). 40 mg of Silodosin was dissolved in 20 mL methanol in 50 mL volumetric flask, then add 5 mL of stock A solution and sonicate it for 2 min and volume was made up.
by methanol(B). 5 mL aliquot from B solution was transferred in 50 mL volumetric flask and volume was made up to the mark with mobile phase. The final concentration of Silodosin and Dutasteride was 80 ppm and 5 ppm for Silodosin and Dutasteride respectively.

![Figure 3 Chromatograph of standard solution of silodosin and dutasteride at optimized chromatographic condition.](image)

Preparation of sample solution

5 capsules were weighed and all contents of 5 capsules were taken in 500 mL volumetric flask. Add 100 mL methanol and sonicate it for 10 min, after that Volume was made up with mobile phase. The solution filtered through 0.45μ PVDC filter. The final concentration of Silodosin and Dutasteride was 80 ppm and 5 ppm respectively.

Validation of analytical method [26-27]

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The linearity for Silodosin and Dutasteride was found to be 39.56 -118.68 μg/mL & 2.43-7.29 μg/mL respectively

Procedure for linearity

The linearity was determined at 5 levels over the range of 50% to 150% of standard concentration. Standard stock solution of Silodosin and Dutasteride was prepared (B solution). From that, aliquots of 2.5, 4, 5, 6, 7.5 mL was taken in to 50 mL volumetric flask. Volume was made up to the mark with Diluent. A graph of mean area versus concentration was plotted and the correlation co-efficient, y intercept, slope of regression were calculated.
Table 1: Calibration data for Silodosin & Dutasteride:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Silodosin</th>
<th>Dutasteride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>0.9974</td>
<td>0.9951</td>
</tr>
<tr>
<td>Standard deviation of response</td>
<td>280328.05924</td>
<td>52355.58529</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>96540.00</td>
<td>235159.00</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>257801.00</td>
<td>146229.00</td>
</tr>
</tbody>
</table>

Table 2: Linearity data for Silodosin and Dutasteride:

<table>
<thead>
<tr>
<th>Linearity level</th>
<th>Silodosin</th>
<th>Dutasteride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. in µg/ml</td>
<td>Peak area</td>
<td>Conc. in µg/ml</td>
</tr>
<tr>
<td>50%</td>
<td>39.56</td>
<td>2.43</td>
</tr>
<tr>
<td>80%</td>
<td>63.29</td>
<td>3.88</td>
</tr>
<tr>
<td>100%</td>
<td>79.12</td>
<td>4.86</td>
</tr>
<tr>
<td>120%</td>
<td>94.94</td>
<td>5.83</td>
</tr>
<tr>
<td>150%</td>
<td>118.68</td>
<td>7.29</td>
</tr>
</tbody>
</table>

**Figure 4** Calibration curve for Silodosin

\[ y = 96540x + 257801 \]
\[ R^2 = 0.9974 \]

**Figure 5** Calibration curve for Dutasteride

\[ y = 235159x + 146229 \]
\[ R^2 = 0.9951 \]
Method Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It was performed by preparing 6 samples as per the test method representing a single batch.

Intermediate Precision (Ruggedness)

Intermediate Precision was determined by analyzing in combined solution their respective calibration range by changing the analyst, equipment and column etc.

Accuracy

Accuracy may often be expressed as percentage recovery. It was determined by calculating the recovery of Silodosin and Dutasteride by application of the analytical method to mixtures of the drug product contents to which known amount of analyte have been added within the range of the method. It was determined at 3 levels of 50% to 150%, each in triplicate.

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD & LOQ was calculated by Calibration curve.

$$\text{LOD} = 3.3 \times \sigma/s$$
$$\text{LOQ} = 10 \times \sigma/s$$

Here $\sigma = \text{Standard deviation of Response}$
$s = \text{Slope of the calibration curve}$

Specificity

It is the ability of the analytical method to measure specifically the analyte of interest without interferences from blank and placebo.

Check for interference from blank and placebo

Diluent was used as blank. Standard and sample were prepared as per test procedure. Diluent, standard preparation, placebo preparation, placebo spiked with standard solution, sample solution were prepared and injected.

Check for interference from forced degradation study

Procedure

In order to establish whether the analytical method for the assay was stability indicating, capsules, pure API of both Silodosin and Dutasteride, Placebo were stressed
under various conditions of acid/base hydrolysis, oxidation, thermal, UV light and humidity, as mentioned in ICH Q1A (R2). Thermal, UV light and humidity degradation of drug substance and drug product was performed in the solid state. For hydrolytic and oxidative degradation, solutions were prepared. All solutions used in forced degradation studies were prepared by dissolving API or drug product in small volume of methanol due to free solubility in methanol and then aqueous hydrochloric acid, aqueous sodium hydroxide or aqueous hydrogen peroxide was added. After the degradation, these solutions were diluted with diluents to yield final concentration.

Acid degradation: Treated with 5 mL 5 N HCl and heated on boiling water bath for 3 hours at 70°C then cool at room temperature after that add 5 mL 5 N NaOH for neutralize the solution.

Base degradation: treated with 5 mL 5 N NaOH and heated on boiling water bath for 3 hours at 70°C then cool at room temperature after that add 5 mL 5 N HCl for neutralize the solution.

Peroxide degradation: treated with 5 mL 30% hydrogen peroxide heated on boiling water bath for 3 hours at 70°C

Thermal degradation: Exposed at 100°C for 24 hours in oven.

UV light degradation: exposed under UV light in a UV chamber for 24 hours.

Humidity degradation: exposed in humidity chamber at 40 °C and 75% RH for 7 days.

Table 3: Results from forced degradation study

<table>
<thead>
<tr>
<th>Stress Condition</th>
<th>% assay</th>
<th>% degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silodosin</td>
<td>Dutasteride</td>
</tr>
<tr>
<td>5.0NHCl / 70°C/3h</td>
<td>98.00</td>
<td>99.00</td>
</tr>
<tr>
<td>5.0NaOH/70°C/3h</td>
<td>97.00</td>
<td>98.00</td>
</tr>
<tr>
<td>30%H$_2$O$_2$/70°C/3 h</td>
<td>56.00</td>
<td>98.00</td>
</tr>
<tr>
<td>Thermal/100°C/24 h</td>
<td>99.00</td>
<td>99.05</td>
</tr>
<tr>
<td>UV/24 h</td>
<td>98.00</td>
<td>98.05</td>
</tr>
<tr>
<td>Humidity/7days</td>
<td>99.05</td>
<td>98.08</td>
</tr>
</tbody>
</table>

Robustness

The following parameters were changed one by one and their effect was observed on system suitability.

- Flow rate of mobile phase: (±10%) to 0.8 mL/min and 1.2 mL/min
• Column Oven temperature: (±5°C) to 35°C and 45°C
• Mobile phase ratio: (±2%): 18:78 and 22:82
• pH: (±0.2 absolute) to 2.8 and 3.2

Solution stability

Standard and sample preparation was prepared as per test procedure. The standard and sample preparation were analyzed by HPLC system at regular intervals for 24 hours. The area of the analyte peak for both standard and sample solutions were monitored. The % difference of analyte peak area from initial for both standard and sample solutions were calculated and recorded.

System suitability parameters

System suitability was performed and calculated at the start of study of each validation parameter.

Table 4: The values of system suitability parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Silodosine</th>
<th>Dutasteride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>No. of theoretical plates</td>
<td>4693</td>
<td>8562</td>
</tr>
<tr>
<td>2.</td>
<td>Retention time (min)</td>
<td>2.7</td>
<td>6.7</td>
</tr>
<tr>
<td>3.</td>
<td>Asymmetry</td>
<td>1.5%</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Table 5: Summary of validation parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Silodosin</th>
<th>Dutasteride</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>39.56 -118.68 μg/ml</td>
<td>2.43-7.29 μg/ml</td>
<td>Linear</td>
</tr>
<tr>
<td>Correlation co-efficient</td>
<td>0.9974</td>
<td>0.9951</td>
<td>Complies</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.7 -101.9 %</td>
<td>99.3-101.4</td>
<td>Complies</td>
</tr>
<tr>
<td>Precision</td>
<td>0.2%</td>
<td>0.4%</td>
<td>Complies</td>
</tr>
<tr>
<td>LOD</td>
<td>9.58 μg/ml</td>
<td>0.73 μg/ml</td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>95.8 μg/ml</td>
<td>7.30 μg/ml</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>Peak purity angle is less than purity threshold.</td>
<td>Peak purity angle is less than purity threshold.</td>
<td>Specific</td>
</tr>
<tr>
<td>Forced degradation</td>
<td>Degraded at 30% peroxide condition</td>
<td>Mild degradation</td>
<td>Peak purity passes.</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>%RSD was less than 2%</td>
<td>%RSD was less than 2%</td>
<td>Rugged</td>
</tr>
<tr>
<td>Robustness</td>
<td>%RSD was less than 2%</td>
<td>%RSD was less than 2%</td>
<td>Robust</td>
</tr>
<tr>
<td>Solution stability</td>
<td>Stable for 36 h</td>
<td>Stable for 36 h</td>
<td>Stable</td>
</tr>
</tbody>
</table>

The system suitability parameters and system precision are evaluated and found within the limits. A plot is drawn between concentration of the component and the instrument response; It is found to be linear in the concentration range 39.56 -118.68 μg/mL & 2.43-7.29 μg/mL for Silodosin and Dutasteride respectively with good correlation coefficient greater than (r2  0.995). Precision and accuracy of the developed method are
expressed in %RSD and % of recovery of the active pharmaceutical respectively. Low %RSD value and high percent of recovery indicate that the method is highly precise and accurate. All system suitability parameters were found within the standard limit.

Table 5  Summary of validation parameters

CONCLUSION

All these factors leads to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully for the estimation of Silodosin&Dutasterid in multiunit system without inference and with good selectivity. The proposed validated method was successfully applied to determine Silodosin and Dutasteride in bulk powder and in solid dosage form.

ACKNOWLEDGMENTS

The authors are thankful to ZyduscadilaHealthcare Pvt. Ltd. ,Ahmedabad for the gift sample of Pure Silodosin and Dutasteride.

REFERENCES