DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULTANEOUS DETERMINATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN A FORMULATION BY RP-UPLC

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ABSTRACT

In the present study, a rapid, an accurate and precise Ultra Performance Liquid Chromatography (UPLC) method was developed and validated for simultaneous estimation of Saxagliptin and Dapagliflozin in its tablet dosage form (10mg Dapagliflozin and 5mg Saxagliptin) by selecting chromatographic parameters. The UPLC method was developed using 2.1 × 100 mm, reverse phase C18 column (Acquity UPLC ethylene bridge hybrid (BEH) C18 1.7 μm) with mobile phases containing 0.1% ortho phosphoric acid and acetonitrile (40:60) as mobile phase. Flow rate was 0.3 ml/min with PDA detection at (λmax) 254 nm and the injection volume was set at 1 μl with run time 3 min. The method was validated by using various validation parameters like accuracy, precision, linearity and robustness. These results show the method could find practical application as a quality control tool for analysis of the drug in its tablet dosage forms in pharmaceutical industries.

KEYWORDS: Saxagliptin, Dapagliflozin, UPLC, Validation, method.

INTRODUCTION

Diabetes mellitus is one of the most common medical conditions globally. The number of people with diabetes is increasing due to population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity.

Some conventional therapies for type 2 diabetes mellitus (T2DM) fail to address the progressive nature of the disease, Saxagliptin was approved by the US Food and Drug
Administration in July 2009 and by the European Medicines Evaluation Agency in October 2009 for use as monotherapy or in combination regimens for the treatment of type 2 diabetes mellitus.\textsuperscript{[1]}

Saxagliptin monohydrate is a white to light yellow or light brown, non-hygroscopic, crystalline powder. Saxagliptin is a competitive dipeptidyl peptidase-4 (DPP4) inhibitor that slows the inactivation of the incretin hormones, thereby increasing their bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose-dependent manner in patients with type 2 diabetes mellitus. Chemical name is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0] hexane-3-carbonitrile.\textsuperscript{[2]} The chemical structure of Saxagliptin was shown in Figure.1.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{saxagliptin.png}
\caption{Chemical structure of Saxagliptin.}
\end{figure}

Dapagliflozin is a white to half white crystalline powder which is soluble in water, ethanol, methanol and dimethyl formamide. It is a highly selective, orally active and reversible inhibitor of the human Sodium-Glucose Co-Transporter 2 (SGLT2), the major transporter responsible for the renal glucose reabsorption. It improves glyceamic control in patients with Type 2 Diabetes Mellitus by inhibiting the Sodium-Glucose Co-Transporter 2, intern by reducing glucose reabsorption. Dapagliflozin’s mechanism of action is complementary to and different from the mechanisms of currently available antidiabetic drugs as it involves the direct and insulin independent elimination of glucose by the kidney. Dapagliflozin selectively block for SGLT2 over SGLT.\textsuperscript{[3]} It is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. The chemical structure of Dapagliflozin was shown in Figure.2.
The literature survey reveals that several analytical methods are reported for quantitative estimation of Saxagliptin alone and in combination with other anti-diabetic agents.\textsuperscript{[4-13]} Several analytical methods are also reported for quantitative estimation of Dapagliflozin.\textsuperscript{[14-20]} On 28 February 2017, US Food and Drug Administration (FDA) has approved once-daily Qtern (10mg Dapagliflozin and 5mg Saxagliptin) for the treatment of type-2 diabetes. The new medicine is indicated as an adjunct to diet and exercise to improve glycaemic (blood sugar level) control in adults with type-2 diabetes who have inadequate control with Dapagliflozin (10mg) or who are already treated with Dapagliflozin and Saxagliptin. So far to our present knowledge, no validated UPLC method is available for the simultaneous estimation of Saxagliptin and Dapagliflozin in dosage form. This prompted the present work. The aim of the present work is to develop a simple yet quick, accurate and precise RP-UPLC method for estimation of Saxagliptin and Dapagliflozin in their marketed formulation.

**MATERIALS AND METHODS**

**Instruments and Apparatus**

The chromatography was done on a WATERS UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbencies of Saxagliptin and Dapagliflozin solutions.

**Reagents and materials**

Saxagliptin was collected as a gift sample from Aurobindo Pharmaceuticals Limited, Hyderabad, India. Dapagliflozin was provided as a gift sample by SUN Pharmaceuticals limited [Mumbai, India]. Dosage form QTERN tablets purchased from the market. HPLC-
grade acetonitrile, methanol, ortho-phosphoric acid (OPA) purchased from Merck Ltd, Mumbai, India were used in the study.

**Preparation of solutions**

**Preparation of Standard stock solutions**

Accurately weighed 5 mg of Saxagliptin and 10 mg of Dapagliflozin transferred to 10ml of volumetric flasks separately. 8ml of diluent was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2 (500 µg/ml of Saxagliptin and 1000 µg/ml of Dapagliflozin).

**Preparation of Standard working solution (100% solution)**

1ml from each stock solution was piped out and taken into a 10ml volumetric flask and made up with diluents (50µg/ml of Saxagliptin and 1000µg/ml of Dapagliflozin).

**Preparation of marketing Sample stock solution**

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 8ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters (500µg/ml of Saxagliptin and 1000µg/ml of Dapagliflozin).

**Preparation of Sample working solution (100% solution)**

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent (50 µg/ml of Saxagliptin and 1000µg/ml of Dapagliflozin).

**Buffer preparation (0.1% OPA)**

1ml of ortho phosphoric acid solution taken in a 1000ml of volumetric flask, add about 100ml of Milli-Q water and final volume makes up to 1000 ml with mill-Q water. Buffer pH adjusted to 2.8 with triethylamine.

**Diluent**

Based upon the solubility of the drugs, diluent was selected, acetonitrile and water taken in the ratio of 50:50.
Method Development

Chromatography

Chromatographic analysis was performed on Acquity uplc BEH C18 x 1.7 μ. The mobile phase consists of 0.1%ortho phosphoric acid buffer: acetonitrile (40: 60 v/v) was used throughout the analysis. The flow rate was 0.3 ml/min, the injection volume was 1.0 μl, column temperature was 30ºC, run time 3min and detection was performed at 254 nm using a PDA detector.

Determination of maximum wavelength for Saxagliptin and Dapagliflozin using a PDA detector

The maximum wavelength for the Saxagliptin and Dapagliflozin were observed at 254 nm using a UV detector in UPLC. At 254 nm both drugs were showing absorbance. Then for the estimation of this combination we have selected 254 nm for Saxagliptin and Dapagliflozin respectively.

Calibration curve of Saxagliptin

Aliquots of working standard solution (50μg/ml) of Saxagliptin (0.25, 0.5, 0.75, 1, 1.25 and 1.5 ml) were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with diluent to get concentrations 12.5, 25, 37.5, 50, 62.5 and 75 μg/ml. Solutions were injected into the system with stated chromatographic conditions. The graph of area of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.

Calibration curve of Dapagliflozin

Aliquots of working standard solution (100μg/ml) of Dapagliflozin (0.25, 0.5, 0.75, 1, 1.25 and 1.5 ml) was transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with diluent to get concentrations 25, 50, 75, 100, 125 and 150 μg/ml. Solutions were injected into the system with stated chromatographic conditions. The graph of area of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.
Method validation

System suitability parameters
The system suitability parameters were determined by preparing standard solutions of Saxagliptin (50 μg/ml) and Dapagliflozin (100 μg/ml) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity
Linearity was demonstrated from 25% to 150% of standard concentration using minimum six calibration levels (25%, 50%, 75%, 100%, 125% and 150%) for both the title drugs. The method of linear regression was used for data evaluation.

Accuracy
The accuracy of an analytical method expresses the nearness between the reference value and found value. The accuracy of the method was measured in triplicate at three concentration levels, i.e. 50%, 100% and 150% of standard solutions of Saxagliptin and Dapagliflozin.

LOD and LOQ
Increasingly dilute solution of each drug was injected into the chromatograph and signal to noise (S/N) ratio was calculated at each concentration. The limit of detection (LOD) & limit of quantitation (LOQ) was calculated on the basis of signal to noise ratio of 3:1 and 10:1 respectively.

Robustness
Robustness of the method was determined to ensure its capacity to remain unaffected by small deliberate variation in the method parameters such as a mobile phase ratio, temperature of the column and flow rate of the mobile phase.

Forced degradation studies
A thorough verification of method selectivity was carried out by forcing degradation studies, also known as stress testing. They are performed to determine possible degradation products, and confirm the ability of the developed method to detect and separate impurities, which can possibly arise during the lifetime of an API or drug product. Stress tests are conducted in conditions exceeding those used in accelerated stability testing. Stress studies were performed under conditions of dry heat (thermal studies), hydrolysis (in the presence of acidic, alkaline and water), oxidation, and photolysis. A minimum of four samples was generated for every
stressed condition, the blank solution stored under normal conditions. 100% sample solution subjected to stress treatment. Hydrolytic decomposition of Saxagliptin and Dapagliflozin was conducted at 30°C in 2N HCl, water, and 2N NaOH. For oxidative stress studies, sample was dissolved in 20% H₂O₂ and kept for one day at room temperature. A photolytic study, drug solution was exposed to UV light for one day.

RESULTS AND DISCUSSION

System suitability

The column efficiency, resolution and peak symmetry were calculated for the standard solutions. The peaks obtained for Saxagliptin and Dapagliflozin were sharp and have clear baseline separation (Figure 3). It was observed from the results that the system suitability parameters meet the requirement of method validation. System suitability and method validation results are summarized in Table 1.

![UPLC Chromatogram of standard.](image)

Table 1: Results from system suitability studies and validation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saxagliptin</th>
<th>Dapagliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates</td>
<td>2173</td>
<td>5120</td>
</tr>
<tr>
<td>K prime (retention factor)</td>
<td>6.28</td>
<td>11.93</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.52</td>
<td>1.25</td>
</tr>
<tr>
<td>Linearity (r²)</td>
<td>0.9987</td>
<td>0.9996</td>
</tr>
<tr>
<td>% RSD for Accuracy</td>
<td>0.54**</td>
<td>0.98**</td>
</tr>
<tr>
<td>% RSD for repeatability and intra day precision</td>
<td>0.69* and 0.4*</td>
<td>0.4* and 0.5*</td>
</tr>
<tr>
<td>LOD</td>
<td>0.13 µg/ml</td>
<td>0.53 µg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>8 µg/ml</td>
<td>1.59 µg/ml</td>
</tr>
</tbody>
</table>

*Results are mean of six injections.

**Results are mean of three injections.
Linearity
The calibration curves plotted for Saxagliptin and Dapagliflozin were linear over the concentration range of 12.5-75 µg/ml for Saxagliptin, 25-150 µg/ml for Dapagliflozin (Figure 4 and 5). Peak areas were plotted against concentrations and linear regression analysis performed for the resultant curve. The correlation coefficient values of Saxagliptin and Dapagliflozin are 0.998 and 0.9996. The results are summarized in Table.2.

**Fig. 4: Linearity curve for Saxagliptin.**

**Fig. 5: Linearity curve for Dapagliflozin.**
Table 2: Linearity table for Saxagliptin and Dapagliflozin.

<table>
<thead>
<tr>
<th>% level (Approx)</th>
<th>Saxagliptin</th>
<th>Dapagliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc (μg/ml)</td>
<td>Peak area</td>
<td>Conc (μg/ml)</td>
</tr>
<tr>
<td>25</td>
<td>12.5</td>
<td>142670</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
<td>247961</td>
</tr>
<tr>
<td>75</td>
<td>37.5</td>
<td>354691</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>487413</td>
</tr>
<tr>
<td>125</td>
<td>62.5</td>
<td>611423</td>
</tr>
<tr>
<td>150</td>
<td>75</td>
<td>728553</td>
</tr>
<tr>
<td>Slope</td>
<td>9607</td>
<td>7838</td>
</tr>
<tr>
<td>Intercept</td>
<td>7249</td>
<td>10435</td>
</tr>
</tbody>
</table>

**Precision**

The precision of an analytical method gives information on the random error. It expresses of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The percentage RSD values for the precision study was 0.8%, 0.3% (inter-day precision) and 0.5%, 0.5% (intra-day precision) for Saxagliptin and Dapagliflozin respectively. This is confirming good precision of the method.

**Accuracy**

Accuracy of the proposed method was determined by analyzing Saxagliptin and Dapagliflozin samples spiked at three different concentration levels in triplicate. To find out the accuracy a known amount of standard drug was added to the fixed amount of pre-analyzed sample solution at three different concentration levels in triplicate. Percent recovery of the drugs was calculated by comparing the area before and after the addition of the standard drug. Percent recovery of Saxagliptin ranged from 99.62% to 100.94% and for Dapagliflozin 98.71% to 101.28% showing better accuracy of the method.

**LOD and LOQ**

The lower limit of detection for Saxagliptin and Dapagliflozin was found to be 0.13 μg/ml & 0.53 μg/ml respectively (Figure.6). The lowest limit of quantitation for Saxagliptin and Dapagliflozin was found to be 0.38 μg/ml & 1.59 μg/ml respectively (Figure.7).
Robustness.

No significant effect was observed on system suitability parameters such as theoretical plates, purity angle, and purity threshold, when small but deliberate changes were made for chromatography conditions such as change in flow rate (± 5%) and organic content (± 2%). The results are summarized in Table.3.

Table 3: Robustness Data For Saxagliptin And Dapagliflozin

<table>
<thead>
<tr>
<th>Condition</th>
<th>Saxagliptin</th>
<th>Dapagliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>Peak Area</td>
</tr>
<tr>
<td>Flow rate (-) 0.2ml/min</td>
<td>0.63</td>
<td>458503</td>
</tr>
<tr>
<td>Flow rate (+) 0.4ml/min</td>
<td>0.52</td>
<td>319300</td>
</tr>
<tr>
<td>Mobile phase (-) 40B:60A</td>
<td>0.57</td>
<td>610805</td>
</tr>
<tr>
<td>Mobile phase (+) 50B:60A</td>
<td>0.58</td>
<td>619610</td>
</tr>
<tr>
<td>Temp (-)25°C</td>
<td>0.58</td>
<td>475897</td>
</tr>
<tr>
<td>Temp (+)35°C</td>
<td>0.07</td>
<td>385882</td>
</tr>
</tbody>
</table>

Fig. 6: UPLC chromatogram for Limit of detection of Saxagliptin and Dapagliflozin.

Fig. 7: UPLC chromatogram for Limit of quantitation of Saxagliptin and Dapagliflozin.
Forced degradation studies

The specificity of the method was also evaluated by the forced degradation study. The peak purity angle is smaller than that of peak threshold angle means there was no interface with the analyte peak from degradation products. Major degradation occurred for Saxagliptin and Dapagliflozin under acid hydrolysis condition up to 4.54 % and 3.54% respectively.

Table 4: Degradation data of Saxagliptin and Dapagliflozin.

<table>
<thead>
<tr>
<th>Type of degradation</th>
<th>Saxagliptin</th>
<th>Dapagliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (%)</td>
<td>% Recovered</td>
</tr>
<tr>
<td>Acid</td>
<td>465326</td>
<td>95.46</td>
</tr>
<tr>
<td>Base</td>
<td>469298</td>
<td>96.27</td>
</tr>
<tr>
<td>Peroxide</td>
<td>480905</td>
<td>98.65</td>
</tr>
<tr>
<td>Thermal</td>
<td>482115</td>
<td>98.90</td>
</tr>
<tr>
<td>UV</td>
<td>485827</td>
<td>99.66</td>
</tr>
<tr>
<td>Water</td>
<td>487062</td>
<td>99.66</td>
</tr>
</tbody>
</table>

CONCLUSION

A rapid and robust method for the analysis of Saxagliptin and Dapagliflozin was developed and its applicability as a method for analyzing stability was checked. The method was found to be specific, accurate, precise and reproducible. Force degradation studies confirmed its ability to determine stability because no interference from degradation products was observed. Moreover, no influence from excipients was found, allowing it to be used in the final drug product analysis. The method was also validated in accordance with ICH requirements.

REFERENCES


