Development and validation of RP-HPLC method for simultaneous estimation of Ertugliflozin and Sitagliptin in bulk drug and tablet dosage form

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Abstract: To treat type 2 diabetes, in a combined tablet dosage form the ertugliflozin and sitagliptin were administered. Considering the less complication and readily availability of HPLC, the main objective of present study was to develop a new, precise, accurate, linear, robust, and economical RP-HPLC method for the simultaneous estimation of ertugliflozin and sitagliptin in tablet dosage form. Effective chromatographic separation of ertugliflozin and sitagliptin was achieved on Kromasil C18 (5 µm 250 mm X 4.6 mm) and the mobile phase containing Methanol and 0.1% OPA in water isocratic elution mode at a flow rate of 1.0mL/min. with column temperature at 30 °C and the injection volume was 20 µL at column temperature at 30°C. At an isosbestic wavelength of 212 nm, ertugliflozin and sitagliptin were found to have retention times of 5.30 min. and 2.05 min., respectively. The method was proven to be precise (%RSD 2%), accurate (>90%), and specific for the simultaneous measurement of both drugs in tablets. As a result, the suggested method with excellent specificity, accuracy, precision, linearity and robustness as well as economical was useful for the regular quality control analysis of ertugliflozin and sitagliptin tablets.

Introduction

Ertugliflozin, chemically known as (1S,2S,3S,4R,5S)-5-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-1-(hydroxymethyl)-6, 8-dioxabicyclo (3,2,1) octane-2,3,4-triol; (2S)-5-oxopyrroldine-2-carboxylic acid, is a selective inhibitor of sodium-dependent glucose cotransporters (SGLT), more specifically type 2 diabetes (Fediuk et al., 2020). A new dipeptidyl peptidase-4 (DPP-4) inhibitor drug with the chemical name (R)-3-Amino-1-(3-(Trifluoromethyl)-5,6-Dihydro- (1,2,4) Triazolo (4,3-A) Pyrazin-7(8h)-yl)-4-(2,4,5-Trifluorophenyl) Butan-1-One is sitagliptin. Sitagliptin is an inhibitor of the protease dipeptidyl peptidase-4 (DPP-4), which breaks down the incretin GLP-1. GLP-1 levels that are elevated or sustained can enhance the pancreas's ability to secrete insulin by blocking DPP-4. Sitagliptin reduces hepatic glucose overproduction while increasing insulin production. In order to address decreased insulin levels brought on by beta-cell malfunction and the liver's unchecked synthesis of glucose, sitagliptin only functions when blood sugar levels are raised (Davis et al., 2010). To treat type 2 diabetes, ertugliflozin and sitagliptin were administered in a combined dosage form. There have been a few reported validated analytical techniques for estimating ertugliflozin and sitagliptin by RP-HPLC method (China et al., 2019; Rajeswari et al., 2022; Venkateswara et al., 2018; Raju et al., 2021; Vilas et al., 2022). It was found that no economically validated method was available from the literature for simultaneous estimation of ertugliflozin and sitagliptin in bulk and tablet dosage form. The goal of the present research is to develop and validate the economical RP-HPLC method for simultaneous estimation of ertugliflozin and sitagliptin in bulk drugs and tablet dosage forms.
Figure 1. Simultaneous estimation of Ertugliflozin and Sitagliptin in tablet dosage form

Material and Method

Material

Ertugliflozin, Sitagliptin were purchased from Vidisha Analytical. Steglujan® tablets containing Ertugliflozin 15 mg and Sitagliptin 100 mg in the ratio of 1:6.67 were purchased from the market. HPLC-grade methanol and acetonitrile were purchased from Merck Specialities Pvt. Ltd (Mumbai, India). HPLC grade Milli-Q water was purchased from Siddhi Lab. Solvents, chemicals, and reagents of HPLC grade were used throughout the validation of the analytical method.

Selection of wavelength

To get the ultraviolet-visible (UV-vis) spectra, both the drugs ertugliflozin and sitagliptin were dissolved in...
methanol separately to prepare primary standard stock solutions (500 µg/mL each). These solutions were then further diluted to prepare secondary stock solutions (at a concentration of 20 µg/mL each). Methanol was utilized as a blank. A UV spectrophotometer was used to scan a standard solution of sitagliptin and ertugliflozin (20 µg/mL each) in wavelength spectral mode between 400 and 200 nm. For both drugs, absorption maxima were found. At 212 nm, Sitagliptin and ertugliflozin displayed Q-point.

Instrumentation and Chromatographic Conditions

HPLC (1260 Infinity II, Agilent) equipped with quaternary pump (DEAX02386) and Detector (DEACX16446) was used. This system was operated by Openlab EZ Chrome for controlling the instrument parameters. Chromatographic separation of Ertugliflozin and Sitagliptin was achieved on Kromasil C18 (250 mm X 4.6 mm, 5 µm) and the mobile phase containing Methanol and 0.1% OPA in water isocratic elution mode at flow rate of 1.0mL/min. with column temperature at 30 °C and the injection volume was 20 µL.

Standard and sample solutions preparation

Standard solutions preparation

**Ertugliflozin L-pyroglutamic acid (25.9 mg)**

in 20 ml volumetric flask stock solutions (1000 µg/mL) prepared & sonicated to completely dissolve it.

**Secondary stock solutions (100 µg/mL)**

**Sitagliptin phosphate (and 25.7 mg)**

20 ml volumetric flask in stock solutions (1000 µg/mL) prepared & sonicated to completely dissolve it.

**Secondary stock solutions (100 µg/mL)**

Sample solutions preparation

Sample solution prepared by Weighing the powder material (from 20 tablets) equivalent to 100 mg of Sitagliptin and 15 mg of Ertugliflozin. Transfer it to a 100 mL volumetric flask that has been thoroughly cleaned and dried, add 70 ml of methanol, sonicate it for 15 minutes, and then level off the volume by adding more methanol. Filter the solution with an appropriate 0.45-syringe filter, and then dilute the filtrate (3.35 ml) to 50 ml with diluent to make 10 µg/mL of ertugliflozin and 67 µg/mL of sitagliptin.

Method validation

Stability of Analytical Solution

The stability of the standard and test sample solutions was studied. The stability study was carried out in normal laboratory conditions. After being kept in a normal illuminated laboratory for conditions 12 and 24 hours, the solution was analyzed.

Specificity

Following solution was prepared and injected to prove the specificity nature of the method. Blank (Mobile phase), Placebo (Placebo solution prepared using 312.34 mg of placebo material containing Lactose, Starch, Magnesium stearate, Talc, crospovidone Which is equivalent to 100 mg of Sitagliptin and 15 mg of Ertugliflozin in methanol), Sitagliptin and Ertugliflozin Standard solution mixture, Tablet test sample solution.

Linearity and range

12.95 mg Ertugliflozin L-pyroglutamic acid (Equivalent to 10 mg of Ertugliflozin) and 32.13 mg Sitagliptin phosphate (Equivalent to 25 mg of Sitagliptin) were weighed accurately and transferred separately into 50 ml volumetric flask, added 30 ml of methanol in each and sonicated to dissolve the standard completely and diluted each solution up to the mark with methanol to prepare 200 µg/mL and 500 µg/mL stock solution of Ertugliflozin and Sitagliptin respectively. Working standard solutions were prepared as a result of diluting the aforementioned solutions 1, 5, 10, 12.50, 15 for Ertugliflozin and 6.75, 33.51, 67.01, 83.77, 100.02 µg/mL for Sitagliptin. Each level was injected three times, and the mean area was computed. Ertugliflozin and Sitagliptin concentration vs peak area response plots were constructed.

Accuracy

Tests for accuracy will be conducted at three levels of accuracy: 50%, 100%, and 150% of the working concentration of the sample solution. The solution was prepared in triplicate for each accuracy level. Calculated the percent recovery, the mean percent recovery, the percent RSD for both the overall recovery and each level.
Precision
The precision of the method was calculated using %RSD. By examining the concentrations of 6 samples, the developed method's intra-day and inter-day batch precision was examined. By conducting the analysis on a different day, intermediate precision was done to ensure that results could be replicated. Sample prepared using the sample preparation protocol.

Sensitivity
As per ICH Q2R1 guidelines, the approach based on the calibration curve was used to calculate the residual standard deviation of a regression line and determine the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Robustness
By purposefully changing chromatographic conditions including flow rate, wavelength and column oven temperature, the robustness of the method was assessed. The column oven temperature was changed by ±2°C, the flow rate by ±0.1 mL/min, and the wavelength varied by ±3nm. For the purpose of determining robustness, the impact of such changes on peak resolutions, tailing factors and theoretical plates was assessed.

System suitability
A Pharmacopeial requirement known as "system suitability" is used to assess if the chromatographic system is suitable to perform the desired analysis. Data was collected from five replicate injections of the standard drugs solution during the tests, and the outcomes were recorded. 10 µg/mL of Ertugliflozin and 67 µg/mL of Sitagliptin are the working concentrations. The marketed formulation contains Ertugliflozin (15 mg) and Sitagliptin (100 mg) in the ratio of 1:6.67, hence concentration is selected in this ratio.

Results and Discussion
Method development
The development of an RP-HPLC method for simultaneous analysis of ertugliflozin and sitagliptin began using a number of mobile phase ratios composed up of methanol and 0.1% OPA in water. Finally, effective chromatographic separation of Ertugliflozin and Sitagliptin was achieved on Kromasil C18 (250 mm X 4.6 mm, 5 µm) and mobile phase Methanol and 0.1% OPA in water in the ratio 75:25 in an isocratic elution mode at a flow rate of 1 mL/min. The retention time for Ertugliflozin and Sitagliptin were found to be at 5.30 min and 2.05 min respectively.

Method Validation
Solution stability

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Time point</th>
<th>Test sample solution</th>
<th>Standard solution</th>
<th>% Absolute difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertugliflozin</td>
<td>Initial</td>
<td>7469485</td>
<td>7484047</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>12 Hours</td>
<td>7440294</td>
<td>7453170</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>24 Hours</td>
<td>7423172</td>
<td>7440318</td>
<td>0.58</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>Initial</td>
<td>17845913</td>
<td>17594023</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>12 Hours</td>
<td>17768956</td>
<td>17668406</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>24 Hours</td>
<td>17698942</td>
<td>17464513</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Specificity
Ertugliflozin and Sitagliptin did not interfere with the R.T. in the blank or placebo sample. Peak purity was found to be NLT 0.95 and within limits for Standard solution and test solution. As a result, the devised chromatographic method met the specificity requirements.
Figure 5. Typical chromatogram of Blank solution

Figure 6. Typical chromatogram of Placebo solution

Figure 7. Typical chromatogram of Peak purity of Standard solution

Figure 8. Typical chromatogram of Peak purity of Test sample solution
Linearity and range

The standard calibration curve has been discovered to be linear over the concentration range of 1.0 - 15.0 µg/mL and 6.75 - 100.02 µg/mL for ertugliflozin and sitagliptin, respectively. For ertugliflozin and sitagliptin, the correlation coefficient ($R^2$) derived from linear regression analysis was 0.999. Based on mean peak and concentration, the calibration curve's equations for ertugliflozin and sitagliptin were $y = 744986.547 \times - 19560.762$ and $y = 266629.117 \times + 4185.341$, respectively.

Precision

Precision is expressed as % RSD and % RSD NMT 2% is considered to be acceptable. Since the % assay and % RSD results were well within the acceptable range, the method is reproducible and accurate. The proposed method was found to have an overall % RSD for intra-day and inter-day precision of less than 2%.

Table 2. Peak purity of Standard and Test solution

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention Time</th>
<th>Peak Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
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<tr>
<td>Ertugliflozin</td>
<td>5.29</td>
<td>0.984</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>2.05</td>
<td>0.989</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ertugliflozin</td>
<td>5.29</td>
<td>0.988</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>2.05</td>
<td>0.993</td>
</tr>
</tbody>
</table>

Table 3. Result for Ertugliflozin of Intra-day and Inter-Day Precision of test sample assay (Where, A refer as Ertugliflozin, B refer as Sitagliptin)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample</th>
<th>Repeatability</th>
<th>Intermediate precision (Inter-Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test Sample (mg)</td>
<td>Area</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>460.3</td>
<td>7319703</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>460.5</td>
<td>7324901</td>
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<tr>
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<td>7349417</td>
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<tr>
<td>B</td>
<td>1</td>
<td>460.3</td>
<td>17339724</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>460.5</td>
<td>17413456</td>
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<td>3</td>
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<td>17240842</td>
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<td>4</td>
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<td></td>
<td>5</td>
<td>460.4</td>
<td>17445034</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>460.5</td>
<td>17584601</td>
</tr>
</tbody>
</table>
Accuracy/recovery

The accuracy of analytical method is expressed as % recovery and it determines the degree of closeness between the obtained values to the true values. The overall % recovery was observed to be in the range of 98-102% for both Ertugliflozin and Sitagliptin. % RSD for each level and overall recovery were found to be <2%. Recovery of analytical procedure was found well within the acceptance limit at all 3 levels.

Sensitivity

Ertugliflozin and Sitagliptin have been shown to have Limit of Detection (LOD) values of 0.091 µg/mL and 0.960 µg/mL, respectively. The Limit of Quantitation (LOQ) values for ertugliflozin and sitagliptin, however, were determined to be 0.275 µg/mL and 2.910 µg/mL, respectively. These results are sufficient for accurately and precisely measuring and detecting ertugliflozin and sitagliptin.

Robustness

As shown in Table 6, minor variations in wavelength, flow rate, and column oven temperature did not significantly affect retention time, theoretical plate, or asymmetry. This led to the conclusion that the analytical approach was reliable and self-sufficient to analyse the two drugs simultaneously and that the developed method was found to be well within the limits.

System suitability

It was found that the resolution was 17.76, indicating well-resolved peaks. The theoretical plate, peak resolution, and tailing factor were all found to be within the permissible range, i.e., the % RSD was 2.0%. Theoretical plates exceeded 2000 and the tailing factor (asymmetry) was less than two. It was found that the suggested validated analytical approach satisfies the system the suitability requirements.

Conclusion

The assay of commercially available formulations was conducted using the aforementioned method, and the average assay results for ertugliflozin and sitagliptin were 99.64% and 100.12%, respectively. On placebo and blank samples who were not interfered with during the retention times of these drugs, a specificity study was conducted. Thus, this method is specific. Linearity study was carried out between 50 % to 150% levels, R² value was found to be 0.99999 for both Ertugliflozin and Sitagliptin. From the results shown in the recovery table, the value was found 99.13%, 99.06%, 99.46 % for 50%, 100% and 150% which were between 98-102% which indicates the
method is accurate. The relative standard deviation values for repeatability and intermediate precision studies were less than 2%. %RSD for Repeatability for both Sitagliptin and Ertugliflozin was obtained as 0.893% & 0.466% respectively. In Intermediate precision %RSD was calculated for two drugs and obtained as 1.284 % and 0.861 % respectively for Sitagliptin and Ertugliflozin. LOD, LOQ values obtained from regression equations of Ertugliflozin and Sitagliptin were 0.091 µg/ml, 0.960 µg/ml and 0.275 µg/ml, 2.910 µg/ml respectively. The robustness result for changes in wavelength, flow rate and column oven temperature were found to be within acceptable limits. As a result, the suggested method is useful for the regular quality control analysis of ertugliflozin and sitagliptin and is specific, accurate, precise, linear and robust.

Conflict of Interest
We do not have conflict of interest.

References

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