Simultaneous Estimation and Validation of Glisoxepide in Pharmaceutical Formulations by Rp-High Performance Liquid Chromatography

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ABSTRACT
This paper describes a simple, precise and accurate HPLC method for the simultaneous estimation of Glisoxepide as the bulk drug and in tablet dosage forms. The separation was achieved using a reverse phase column (250 × 4.6 mm, 5µm, L7pack) at ambient temperature with an isocratic mixture of ammonium acetate and acetonitrile in the ratio of 30:70% V/V at a flow rate of 1mL/min and detection at 254 nm. The retention time for Glisoxepide was 1.247 min. The linearity for Glisoxepide was found to be 1 to 10 µg/ml and it obeys beer's law with correlation coefficient of 0.9991. The percent recovery obtained for Glisoxepide was 99.6 ± 0.86 The method is accurate, precise and found to be suitable for the quantitative analysis of the drugs in combinational dosage forms.

Keywords: Glisoxepide, HPLC

INTRODUCTION
Glisoxepide is a well-known as a Glisoxepid, Glisepin, Pro-Diabon, Glisoxepida. It is an anti-diabetic drug from the group of sulfonylureas [1] and it is one of the sulphonamide-derived oral anti-diabetic drug. It inhibits the uptake of bile acids into isolated rat hepatocytes. However, it inhibits taurocholate uptake only in the absence of sodium ions. Glisoxepide uptake could be further inhibited by blockers of the hepatocellular monocarboxylate transporter by the loop diuretic bumetanide, 4,4′-disothiocyanato-2,2′-stilbenedisulfonate (DIDS) and Bisulphate [3-8]. These results are consistent with the transport of glisoxepide via the transport system for the unconjugated bile acid cholate. It has the structural formula and shown in Fig. 1. The developed method was simple, precise, specific and accurate. The statistical analysis prove that method is reproducible and selective for the analysis of Glisoxepide in bulk drug and tablet formulations. The challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry. Different analytical techniques like preparative HPLC [9-14], liquid-liquid extraction [15-20], UV-Vis Spectrophotometry available for the assay and impurities of drugs, HPLC/UPLC [21-27] is Glisoxepide. The developed method was simple, precise, specific and accurate. The statistical analysis prove the method is reproducible and selective for the analysis of Glisoxepide in bulk drug and tablet formulations. This paper presents RPL-HPLC method for simultaneous determination of Glisoxepide in bulk and its formulations.

![Fig 1. Structure of Glisoxepide](image)
MATERIALS AND METHODS

Chemicals and reagents
HPLC grade acetonitrile and ammonium acetate were used for the analysis. Water obtained from Milli-Q water system. Glisoxepide used in this study is procured from NEO Medichem private limited, Hyderabad. Formulation used for this study is 10 mg of Glisoxepide.

Instrumentation
Chromatographic separation was performed on a SHIMADZU chromatographic system equipped with LC-20AT pump. Variable wavelength programmable UV-visible detector SPD – 20A and Rheodyne (7725i) with 20µL fixed loop are used and data analysis is done by using SPINCHROM software.

Chromatographic conditions
Separation and analysis was carried out on C8 (250 ×4.6 mm, 5µm, L7 pack) column. Mobile phase consisting of a mixture of acetonitrile and ammonium acetate in the ratio of 30:70 V/V was delivered at flow rate of 1 ml/min, with detection at 254 nm. The mobile phase was filtered through a 0.45 µm membrane filter and sonicated for 15 min. Analysis was performed at ambient temperature.

Preparation of standard solution
Accurately, weighed 10 mg of Glisoxepide transferred into a 100 mL volumetric flask then 5 mL methanol was added and shaken well to dissolve and sonicated for 5 min. Volume was made up to 100 mL with acetonitrile. The solution was further diluted with acetonitrile to achieve final concentration of 10 µg/mL of each drug and filtered through a 0.45 µm membrane filter before injection.

Sample preparation and assay
A pharmaceutical sample containing Glisoxepide 10 mg was weighed and transferred to 100 mL volumetric flask. The contents of the flask were dispersed in 30 mL of ammonium acetate and 70 mL of acetonitrile and shaken well to dissolve and sonicated for 30 min. and further dilution was made up to get 10 µg/mL concentration of each drug. The solution was filter through 0.45 µm membrane filter before injection. All determinations were conducted in triplicate. Both the standard and sample preparation was injected separately, and the peak area responses were recorded. The percentage of the formulations was calculated and given in Table-1.

Table 1: Assay of formulation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pharmaceutical Formulation</th>
<th>Labelled amount (mg)</th>
<th>proposed method</th>
<th>Amount found (mg)</th>
<th>t (Value)</th>
<th>F (Value)</th>
<th>Found by reference method ±S.D</th>
<th>% recovery by proposed methods ±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glisoxepide</td>
<td>5</td>
<td></td>
<td>4.98 ± 0.017</td>
<td>0.682</td>
<td>1.535</td>
<td>4.95 ± 0.081</td>
<td>99.6 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Glisoxepide</td>
<td>5</td>
<td></td>
<td>4.98 ± 0.043</td>
<td>1.414</td>
<td>2.462</td>
<td>4.94 ± 0.063</td>
<td>99.64 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>Glisoxepide</td>
<td>5</td>
<td></td>
<td>5.05 ± 0.028</td>
<td>1.426</td>
<td>1.124</td>
<td>4.95 ± 0.081</td>
<td>101.0 ± 0.56</td>
</tr>
</tbody>
</table>

RESULT AND DISCUSSION

The simultaneous estimation of Glisoxepide was carried out by RP-HPLC using ammonium acetate and acetonitrile as mobile phase in the ratio of 30:70 V/V and C8 column as the stationary phase. The results of system suitability parameters such as tailing factor, asymmetry and number of theoretical plates are indicated satisfactory results and tabulated in Table-2. The retention time for Glisoxepide was found to be 1 mL/min. The resolution value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicate the complete separation of the drug. The linearity was studied the concentration range from 1-10 µg/mL. The regression coefficient value is 0.9994 respectively, the mean recovery for Glisoxepide was 99.0 to100.0%, which is largely within the 90 to 110% range that is considered acceptable and revels that the method is accurate.

The validation of the proposed method was verified by system precision and method precision. The system precision was evaluated by measuring the peak area response of Glisoxepide for five replicate samples of the standard solutions. The method precision was determined by quantifying the sample solutions as per the proposed method. The % RSD was found to be less than 2 proposed precision, accuracy. The specificity of the method was confirmed by injecting the placebo. Robustness of the method is determined by analyzing the samples in duplicate with varying the method conditions very small changes in flow rates, showed there were no marked changes in chromatographic behavior and content of the drug as evident from the low value of RSD indicating the method is robust. The method was also confirmed by ruggedness study analyzing the product day to day, analyst and instrument to instrument. The data of ruggedness of Glisoxepide are found to be within the acceptance limit. Different validation parameters for the proposed HPLC method is summarized in Table-3 and chromatogram of Glisoxepide is

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shown in Fig- 2. The result obtained was in agreement with the labeled value of Glisoxepide formulations. The parameters are in the acceptable ranges.

![Fig 2. Chromatogram for GSP (M15)](image)

Peak table

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area%</th>
<th>Tailing factor (0%)</th>
<th>K'</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.247</td>
<td>11574</td>
<td>17.925</td>
<td>1.733</td>
<td>0.000</td>
<td>0.000</td>
</tr>
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</table>

Table-2. System suitability parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Glisoxepide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theoretical plates (N)</td>
<td>1216</td>
</tr>
<tr>
<td>2</td>
<td>Tailing factor</td>
<td>1.733</td>
</tr>
<tr>
<td>3</td>
<td>Asymmetry</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>%RSD if peak retention time</td>
<td>0.136%</td>
</tr>
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</table>

Table-3. Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glisoxepide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>1-10 µg/mL</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.9991</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>5 ng/mL</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.0 to 100.0</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>0.699%</td>
</tr>
<tr>
<td>Robustness (%RSD)</td>
<td>0.735%</td>
</tr>
<tr>
<td>Ruggedness (%RSD)</td>
<td>0.683</td>
</tr>
</tbody>
</table>

CONCLUSION

The proposed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of Glisoxepide and pharmaceutical dosage forms without any interference from the excipients.

REFERENCES

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Citation of this article