DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF Gliclazide

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ABSTRACT:
The aim of the present work was to develop and validate a method for the estimation of gliclazide by reverse phase HPLC method. A validation protocol was followed to develop an analytical method, using Grace Vydac C18 column (250 x 4.6 mm, 5µ) as stationary phase and acetonitrile:ammonium acetate buffer as mobile phase. The mobile phase pH, ratio of mobile phase, ionic strength of buffer, the flow rate was optimized and the method was validated for its linearity, accuracy, precision, robustness. The retention time was within 15 min, acetonitrile:ammonium acetate buffer (50:50) with an ionic strength of 20 mM and a flow rate 1ml/ml was selected for elution. The method was linear in the range from 10–70 µg/ml with (r²) 0.9995, accuracy was 99.62±1.43 (% Mean ±SD), robustness was found to be 0.92%, repeatability and intermediate precision was found to be 0.37% and 0.58% respectively, LOD and LOQ was found to be 0.1 and 0.5 µg/ml respectively. The developed method was found to be linear, accurate, robust and precise and the values obtained were within acceptable values.

Keywords: Gliclazide, RP-HPLC, Validation.

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INTRODUCTION
Gliclazide (1-(3-azabicyclo (3.3.0) oct-3-y1)-3-p-tolylsulphonylurea) is a second generation sulphonylurea derivative used for the treatment of hyperglycemia in non insulin dependent diabetes mellitus (NIDDM)¹.². Gliclazide is lipophilic (Log p (Oct/Water), 2.1), practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol with a bioavailability of approx. 60-110% (variable), T_max (h) of 4-6h, pKa (5.8). The dose of gliclazide varies between 20mg and 160 mg daily in two divided doses. Gliclazide is a comparatively newer sulfonylurea and has good efficacy, appears of particular benefit in patients previously untreated with oral antidiabetic drugs and is generally well tolerated. In the long term, it reduces hepatic gluconeogenesis, and increases insulin effects by acting at the receptor or post receptor sites. It also inhibits platelet aggregation and increases fibrinolysis. Because of these therapeutic effects it has emerged as one of the important and promising drug substances for diabetes mellitus³.⁴. The present study describes the development and validation of an analytical method for estimation of Gliclazide by RP-HPLC.

MATERIALS AND METHODS
Instrumentation
An HPLC equipped with UV detector was used for the present research work. The separation was achieved using Grace Vydac C18 column (250 x 4.6 mm, 5µ).

Chemicals and Reagents
Gliclazide was obtained as a gift sample from the Lupin Research Park, Pune. All other chemicals and reagents are of analytical grade. HPLC grade water was used to prepare all solutions.

Method Development
Stock and standard solution
Stock solution of gliclazide working standard was prepared by dissolving 10 mg of drug in 10 ml of methanol, so that final concentration is 1 mg/ml. From the stock solution 5, 10, 15, 20, 25 μg ml\(^{-1}\) dilutions were prepared by using methanol as diluents.

**Sample preparation**

Diluted concentration of 10μg/ml gliclazide was prepared from the primary stock solution using methanol as a diluent. Acetonitrile was selected as organic solvent to elute gliclazide from the stationary phase because of its favourable UV transmittance, low viscosity and low back-pressure.

**Initial separation conditions**

- **Stationary phase:** Grace Vydac C\(_{18}\) column (250 x 4.6 mm, 5μ)
- **Mobile phase:** Acetonitrile: 20mM ammonium acetate buffer, pH 4.5(50:50)
- **Run time:** Isocratic run for 20 min
- **Detection wavelength:** 230 nm
- **Flow rate:** 1 ml/min
- **Injection volume:** 20 μl
- **Temperature:** Ambient (around 25°C)
- **Auto sampler:**
- **Temperature:** 4±2°C

The standard solution of gliclazide 10 μg/ml was prepared, injected in to HPLC system and run for 30 min.

**Effect of pH**

The mobile phase pH was optimized by using different pH conditions (pH 3.0 to pH 7.0) at a flow rate of 1 ml/min and Grace Vydac C\(_{18}\) column used as the stationary phase.

**Effect of Ratio of Mobile Phase**

Acetonitrile and ammonium acetate buffer (pH 4.5) were studied in 50:50, 60:40 and 70:30 (% v/v) for the proper selection of the mobile phase.

**Effect of Ionic Strength**

The Ammonium acetate buffer (pH 4.5) was prepared in different strength such as 20, 30 and 40 mM and chromatograms were recorded.

**Effect of Flow Rate**

The flow rate of 0.9, 1.0 and 1.1 ml/min was used and chromatograms were recorded.

**Method Validation**

Method validation was carried out as per ICH Q2 R1 guidelines. Assay performance was evaluated by

**Linearity**

Solutions with concentration of 0.5, 1, 2, 5, 10, 25, and 50 μg/ml in the mobile phase and a volume of 20 μl of the solution was injected and chromatograms were recorded under the optimized chromatographic conditions. The peak area of the gliclazide was plotted against the concentration to get the regression equation and coefficient determination.

**Accuracy**

The known amount of standard drug was spiked (100, 120, 150%) in triplicate to the preanalyzed samples and the recovery of the drug was calculated at three concentrations such as 10, 12, 15 μg/ml.

**Robustness**

Evaluation of robustness leads to the generation of a series of system suitability parameters which ensure that the analytical procedure is maintained whenever used.

**Precision**

**Repeatability**

A solution containing 10 μg/ml of gliclazide was analyzed at different time intervals and the percentage relative standard deviation was calculated.

**Intermediate precision**

It is generally expressed as the percent relative standard deviation for a statistically significant number of samples. It was carried out in between days and by different analysts.

**Limit of detection (LOD) and Limit of quantitation (LOQ)**

LOQ and LOD were calculated based on the standard deviation of the slope and blank response from the calibration curve as per ICH guidelines. LOD= 3.3×SD/$S$ \(\times SD\)

**RESULTS AND DISCUSSION**

**Effect of pH**

With the increase in pH of mobile phase there has been a decrease in the retention time of gliclazide and vice versa. So, Ammonium acetate buffer, pH of 4.5 was selected as the retention time was within 15 min.

**Effect of Ratio of Mobile Phase**

The retention time of Gliclazide was found to be 7.0, 6.4 and 3.1 mins respectively with different mobile phase ratio.

**Effect of Ionic Strength**

20 mM strength of Ammonium acetate buffer (pH 4.5) was selected as no change in the retention time was observed with the change in the ionic strength of the buffer.

**Effect of Flow Rate**

1 ml/min was selected was selected as the best peaks were obtained with the all the different flow rates. Based on the above optimization parameters, the following chromatographic condition was selected for the estimation of gliclazide by HPLC method consists of

Stationary phase: Grace Vydac C\(_{18}\) column
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Mobile phase: Acetonitrile: 20mM ammonium acetate buffer, pH 4.5 (60:40)  
Run time: Isocratic run for 20 min  
Detection wavelength: 230 nm  
Flow rate: 1 ml/min  
Injection volume: 20 µl  
Temperature: Ambient (around 25°C)  
Autosampler Temperature: 4±2°C

With the above separation condition, the retention time for gliclazide was found to be 6.4 mins. The typical standard chromatogram of gliclazide was showed in the Fig. 1.

**Linearity**
The coefficient determination ($r^2$) for the present method was 0.9995 which indicated that the present method is linear and it is linear in the range from 10 – 70 µg/ml. Acceptance criteria for Linearity, ($r^2$) is >0.999. Calibration curve is shown in the Fig 2.

**Accuracy**
The recoveries at three different concentrations (10, 12, 15 µg/ml) were found to be within the range of 98 to 102% as per ICH guidelines. Mean % recovery (Mean±SD) was found to be 99.62±1.43.

**Robustness**
The overall percentage relative standard deviation in the various parameters was found to be 0.92% and the acceptance limit was <2%. The result indicated that the method was robust.

**Precision**
The repeatability and intermediate precision of the proposed method was found to be 0.37% and 0.58% respectively which were within the acceptance criteria for the repeatability and intermediate precision (<1% and <2% RSD).

**LOD and LOQ**
The present method LOD and LOQ was found to be 0.1 and 0.5 µg/ml respectively. This indicates that the developed method is sensitive for the quantification of gliclazide. Summary of the analytical method validation parameters is reported in the Table 1.

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>Validation Results</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity ($r^2$) (10-70 µg/ml)</td>
<td>0.9995</td>
<td>&gt; 0.999</td>
</tr>
<tr>
<td>Accuracy (% Mean±SD)</td>
<td>99.62±1.43</td>
<td>98–102</td>
</tr>
<tr>
<td>Robustness (% RSD)</td>
<td>0.92</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Repeatability precision (% RSD)</td>
<td>0.37</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Intermediate precision (% RSD)</td>
<td>0.58</td>
<td>&lt;2</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.1</td>
<td>S/N ratio should be 3:1</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.5</td>
<td>S/N ratio should be 10:1</td>
</tr>
</tbody>
</table>

**CONCLUSION**
An improved reverse phase HPLC method was developed for gliclazide and was validated as per guidelines. The assay was found to be linear, accurate, robust and precise and the values obtained were within acceptable values.
REFERENCE