DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF GLIPIZIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

RP-HPLC method was developed and validated for the estimation of Glipizide in marketed formulation (tablet). The proposed RP-HPLC method utilizes a Symmetry C18 (4.6 x 15 mm, 3.5 μm) or equivalent column, mobile phase comprising of 20 mM potassium dihydrogen phosphate:acetonitrile in the ratio of 40:60 v/v and a flow rate of 1.0 ml per minute with UV detection at 233 nm. The retention time was found to be 2.82 minutes for Glipizide. The linearity was in the concentration range of 10-50 μg/ml (r²=0.999). The % mean recovery was found to be 100.7 % w/w. The proposed method was found to be specific, linear, accurate, precise, rugged and robust. Hence the RP-HPLC method developed and validated can be used routinely for the estimation of Glipizide in the marketed formulation (tablet).

Keywords: Glipizide, RP-HPLC, Potassium dihydrogen phosphate, Acetonitrile.

INTRODUCTION

Quality control of the Active Pharmaceutical Ingredients (API’s) in the formulation is always a thrust area for the pharmaceutical industries. So the development of the reproducible, simple, and extremely inexpensive methods for the determination of APIs in the formulation is always challenging 1.

1-cyclohexyl-3-[[p[2(5-methy-1-pyrazinecarboxamido)ethyl]phenyl]sulfonyl] urea is the chemical name of Glipizide, which is used for the treatment of type II Diabetes. The drug is commercially available as tablets for oral administration 2.

Glipizide is an oral rapid- and short-acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes entero-hepatic circulation. Second generation sulfonylureas are both more potent and have shorter half-lives than the first generation sulfonylurea 3.
Glipizide is included in the United States Pharmacopoeia, European Pharmacopoeia and Indian Pharmacopoeia. The US pharmacopoeia recommends HPLC while EP & Indian Pharmacopoeia recommends titrimetry. Several HPLC methods are available for the determination of Glipizide in plasma dosage forms and urine.

MATERIALS AND METHODOLOGY

Instrument
RP-HPLC method was developed for the determination of Glipizide (Figure 1) and was validated at the laboratory. The High Performance Liquid Chromatographic was equipped with an auto sampler and DAD or UV Detector. Chromatographic analysis was performed using Symmetry Hypersil BDS C_{18} column diameter 4.6 X 150 mm and 3.5 µm particle size. Flow rate of HPLC was set to 1.0 ml/min with run time 6 min. The injection volume was 20 µl and the detection wavelength was 233 nm.

Reagent and Materials
Acetonitrile and methanol HPLC grade were purchased from MERK chemicals. Glipizide standard drugs were provided by the Pharma Train Center, Kukatpally-Hyderabad. The tablets of Glipizide were procured from local market manufactured by Pfizer.

Preparation of Standard Stock Solution
A stock solution was prepared by accurately weighing 10 mg of Glipizide drug dissolved in 10 ml of mobile phase and transferred to a 10 ml clean dry volumetric flask. The concentration of stock solution obtained was 1000 µg/ml. Further, 0.3 ml of the stock solution was pipette out and diluted with the diluents to get the concentration 30 µg/ml.

Preparation of Sample Solution
Glipizide tablets (Residronate) were crushed to give finely powdered material. Accurately weighed sample equivalent to 10 mg of Residronate was transferred into a 10 ml of volumetric flask containing 10 ml of solvent and was shaken to dissolve the drug. The volume was further diluted to obtain concentration of 1000 µg/ml. Further, 0.3 ml of the sample solution was pipette out and diluted with the diluents to get the concentration 30 µg/ml.

Method Validation
The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness as per the guidelines of the International Conference on Harmonization (ICH).

Linearity and range
The linearity of the method was determined at five concentration levels ranging from 10-50 µg/ml for Glipizide.
Accuracy
The accuracy of the method was determined by calculating recovery of Glipizide by the method of standard addition. Accurately weighed Glipizide was transferred to a pre-quantified sample solution and the weight of Glipizide was estimated by measuring the peak area ratios and by putting values to the straight line equation of calibration curve.

Precision
The precision study of Glipizide was carried out by estimating the correspondence responses. Accurately weighed Glipizide was transferred to a pre-quantified sample solution. The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within specific limits. Results summarized in. The % RSD for the area of five standard injections results should not be more than 2%.

Limit of detection and limit of quantification
Limit of detection = S/N ratio value shall be 3
Limit of quantitation = S/N ratio value shall be 10

*Signal/Noise obtained ratio

Robustness
Deliberate change in flow rate and mobile phase composition was made to evaluate the robustness. Flow rate was varied at rate 0.8 to 1.2 ml/min and mobile phase composition was varied from 70% to 50%.

RESULTS AND DISCUSSION

The UV spectra of Glipizide showed that the drug absorbs at 233 nm was selected as the detection wave length in RP-HPLC. Different mobile phases were tried but best separation; and good symmetrical peaks were obtained with the mobile phase potassium dihydrogen phosphate buffer and acetonitrile (40:60, v/v). The retention time of Glipizide was found to be 2.828 min, which indicates a good baseline. HPLC chromatogram of Glipizide was shown in (Figure 2).

Figure 2: HPLC chromatogram of Glipizide
The calibration curve for Glipizide was obtained by plotting the peak area ratio versus the concentration of Glipizide over the range of 10-50% and it was found to be linear with \( r^2 = 0.999 \), intercept 22201, slope 69828 for Glipizide. System suitability and validation parameters are given in (Table 1).

**Table 1: Regression analysis of the calibration curve**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range</td>
<td>10-50(\mu)g/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>69828</td>
</tr>
<tr>
<td>Intercept</td>
<td>22201</td>
</tr>
<tr>
<td>Correlation coefficient ((r^2))</td>
<td>0.999</td>
</tr>
</tbody>
</table>

The number of theoretical plates was found to be 3943.3, which indicates efficient performance of the column. System suitability and validation parameters were given in. The % RSD values for accuracy and precision studies obtained was less than 0.62%, which were less than 2% limit, revealed that developed method was accurate and precise. The limit of detection and limit of quantification for Glipizide was found to be 0.04 ppm and 0.01 ppm, indicates the sensitivity of the method. The system suitability and validation parameters are summarized in (Table 2).

**Table 2: System suitability and validation parameters**

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>Glipizide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>40:60 (Buffer: Acetonitrile)</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0 ml/min</td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>233nm</td>
</tr>
<tr>
<td>Retention Time</td>
<td>2.828</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>3943.3</td>
</tr>
<tr>
<td>LOD</td>
<td>0.04ppm</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.01ppm</td>
</tr>
<tr>
<td>Linearity</td>
<td>( R^2 = 0.999 )</td>
</tr>
<tr>
<td>Precision</td>
<td>% RSD = 0.62</td>
</tr>
<tr>
<td>Recovery</td>
<td>100.7</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Proposed study describes new HPLC method for the estimation of Glipizide. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows its capacity to remain unaffected by small, but deliberate variations in method parameters and
provides and indications of its reliability during normal usage. Therefore the proposed method can be used for routine analysis of estimation of Glipizide.

REFERENCES

1) Xavier CM, Basavaiah K, Vinay KB, Swamy N. “Quality Design approach for the Development and Validation of Glipizide, and Anti-Diabetic Drug, by RP-UPLC with application to formulated forms and urines”. ISRN Chromatography 2013;1


