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

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ABSTRACT
A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of glipizide in tablet dosage form and serum. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing methanol:water:0.01M KH2PO4 (70:25:5,v/v/v) was used. The flow rate was 1.5ml/min and effluents were monitored at 270nm. The retention time for glipizide was 3.211 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 15ng/ml and 45ng/ml respectively and recovery of glipizide from tablet formulation was found to be 99.6%. The proposed method was successfully applied for the quantitative determination of glipizide in tablet formulation.

Keywords: Glipizide, HPLC, Linearity, Validation, Serum.

INTRODUCTION
Glipizide is an oral medium-to-long acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. The structure on the R2 group is a much larger cyclo or aromatic group compared to the 1st generation sulfonylureas. This leads to a once a day dosing that is much less than the first generation, about 100 fold. Mechanism of action is produced by blocking potassium channels in the beta cells of the islets of langerhans. By partially blocking the potassium channels, it will increase the time the cell spends in the calcium release stage of cell signaling leading to an increase in calcium. The increase in calcium will initiate more insulin release from each beta cell.

Fig. 1: Molecular Structure of Glipizide

Literature survey revealed that numerous methods have been reported for estimation of glipizide in pharmaceutical formulations and formulations and biological fluids have been reported. Present study involves development of LC method using simple mobile phase which is sensitive and rapid for quantification of glipizide in tablet dosage forms as well as subsequent validation of developed method according to ICH guidelines.

EXPERIMENTAL

Instrument
The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and materials
Methanol of HPLC grade was purchased from E.Merck, Mumbai, India. LC grader water was obtained by double distillation and purification through milli – Q water purification system. Potassium di hydrogen phosphate of analytical grade was procured from qualigens, Mumbai, India.

Preparation of Standard Stock Solution
A stock solution of glipizide was prepared by accurately weighing 10mg of drug, transferring to 100ml of volumetric flask, dissolving in 25ml of solvent and diluting up to mark with solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 0.01mg/ml of glipizide. Resultant solution was filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper.

Preparation of sample Solution
The formulation tablets of glipizide were crushed to give finely powdered material. Powder equivalent to 10mg of glipizide was taken in 10 ml of volumetric flask containing 5ml of solvent and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 0.006mg/ml.

Calibration curve
Appropriate aliquots of standard glipizide stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 1, 2, 3, 4, 5 and 6µg/ml of glipizide. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of glipizide was constructed by plotting peak area ratio versus applied concentration of glipizide and regression equation was computed. Similarly the sample solution was chromatographed and concentration of glipizide in tablet sample was found out using regression equation.

Analysis of Glipizide in Serum
From a local hospital blood was collected and serum was separated. 0.5ml of this serum was taken in a test tube 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded.
The amount of drug present in the blood sample was calculated from linearity graph was 1.44µg/ml.

**Method validation**

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

**Accuracy**

The accuracy of the method was determined by calculating recovery of glipizide by the method of standard addition. Known amount of glipizide (3µg, 1µg and 4µg/ml) was added to a pre quantified sample solution and the amount of glipizide was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of glipizide was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

**Precision**

The intra-day precision study of glipizide was carried out by estimating the correspondence responses six times on the same day with 6µg/ml concentration and inter-day precision study of glipizide was carried out by estimating the correspondence responses six times next day with 6µg/ml concentration.

**Linearity and range**

The linearity of the method was determined at six concentration levels ranging from 1-6µg/ml for glipizide.

**Specificity**

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighted quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

**Limit of detection and limit of quantification**

Limit of detection = 0.015µg/ml  
Limit of quantification = 0.045µg/ml

**Stability**

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

**Robustness**

Robustness of the method was studied by changing the composition of organic phase by ±5% and the pH by ±0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

**RESULTS AND DISCUSSION**

The UV spectra of glipizide showed that the drug absorbs appreciably at 270nm was selected as the detection wavelength in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase methanol:water:0.01M KH₂PO₄ (70:25:5, v/v/v). The retention time of glipizide was found to be 3.211 min, which indicates a good baseline (Figure 4).
The number of theoretical plates was found to be 7523.42, which indicates efficient performance of the column. The asymmetric factor was found to be 1.48, which indicates asymmetric nature of the peak. The calibration curve for glipizide was obtained by plotting the peak area ratio versus the concentration of glipizide over the range of 1-6µg/ml, and it was found to be linear with $r^2=0.999$. The regression equation of glipizide concentration over its peak area ratio was found to be $y = -223.26 + 37027.47429x$, where $x$ is the concentration of glipizide (µg/ml) and $Y$ is the respective peak area. The data of regression analysis of the calibration curve was shown in table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantitation for glipizide was found to be 0.015µg/ml and 0.045µg/ml, indicates the sensitivity of the method. The system suitability and validation parameters were given in table 2. The high percentage of recovery of glipizide was found to be 99.6% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of glipizide in tablet formulation and serum. The result for glipizide was comparable with a corresponding labeled amount (Table 3). The absence of additional peaks indicates no interference of the excipients used in the tablets.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range (µg/ml)</td>
<td>1-6</td>
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<tr>
<td>Slope</td>
<td>37027.47429</td>
</tr>
<tr>
<td>Intercept</td>
<td>-223.26</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.999</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates (N)</td>
<td>7523.42</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>3.211</td>
</tr>
<tr>
<td>Asymmetric factor</td>
<td>1.48</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.015</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.046</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.5</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>0.868</td>
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</tbody>
</table>

**CONCLUSION**

Proposed study describes new LC method for the estimation of glipizide in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of glipizide in its tablet formulation and serum.

**REFERENCES**

