SPECTROPHOTOMETRIC DETERMINATION OF GLIPIZIDE IN BULK AND TABLET DOSAGE FORM BY ABSORPTION MAXIMA, FIRST ORDER DERIVATIVE SPECTROSCOPY AND AREA UNDER THE CURVE

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ABSTRACT
Glipizide (GZ) is chemically 1-cyclohexyl-3-[p[2-(5-methylpyrazinecarboxamido)ethyl]phenyl] sulfonyl]urea, used in the treatment of type II diabetes mellitus. The drug is commercially available as tablets for oral administration. In the present work three simple, economical, precise and accurate UV spectrophotometric methods have been developed for the estimation of glipizide in bulk and pharmaceutical formulation. Method A is absorption maxima method in which \( \lambda_{\text{max}} \) was found to be 274 nm. Method B is first order derivative spectroscopy where drug showed \( \lambda_{\text{max}}=286 \) nm and \( \lambda_{\text{min}}=263 \) nm. Amplitude difference (dA/d\( \lambda \)) was calculated and was plotted against concentration and regression equation was calculated. Method C is area under the curve (AUC) in which area in the wavelength range of 255 nm - 295 nm was selected for analysis of glipizide. Linearity was observed in the concentration range 5-25 \( \mu \)g/ml (\( r^2=0.999 \)) for all the three methods. The % assay for the marketed formulation for absorption maxima, first order derivative and area under the curve method was found to be 99.03%, 100.16% and 99.06% respectively. The methods were validated with respect to linearity, precision and accuracy studies. Recovery studies for absorption maxima, first order derivative and area under the curve was found to be 100.83 %, 99.41% and 100.51% respectively. The methods were found to be simple, precise and accurate and can be employed for routine quality control analysis of glipizide in bulk as well as from its dosage form.

Keywords: Glipizide (GZ), Spectrophotometric method, First order derivative, Area Under the Curve (AUC).

INTRODUCTION
Glipizide (GZ) is chemically 1cyclohexyl3[[p[2(5methylpyrazinecarboxamido)ethyl]phenyl] sulfonyl]urea, used in the treatment of type II diabetic drug. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. It acts by blocking potassium channels in the betacells of the islets of Langerhans. Literature survey reveals estimation of glipizide HPTLC \( 1^{st} \) method, HPLC \( 2^{nd} \) method, RP-HPLC \( 3^{rd} \) in plasma, and RP-HPLC \( 4^{th} \) in dosage form has been reported. In combination with other drugs LC \( 1^{st} \) method, ion pair RP-LC \( 2^{nd} \), HPLC \( 3^{rd} \), RP-LC \( 4^{th} \), and HPTLC \( 5^{th} \) method has been reported for estimation of glipizide.

No spectrophotometric method is available for estimation of glipizide in single dosage form. In the present work, an attempt has been made to estimate the drug by spectrophotometric methods.

MATERIALS AND METHODS
For the present study JASCO double beam UV/Visible spectrophotometer (Model V-530) was used with slit width fixed at 1.5 nm equipped with Spectra Manager software (Version 1.5). A pair of 1-cm matched quartz cells were used to measure the absorbance of solution. The samples were weighed on electronic analytical balance (Gotech Model CB-50).

Reagents
Glipizide (GZ) was obtained as a gift sample from Supra Chemicals, Mumbai. The marketed formulation used for tablet analysis was Glide by Franco-Indian and Glynase by USV Ltd. The label claim states that each uncoated tablet contains 5 mg of Glipizide.

Solvents-Methanol spectroscopic grade

Preparation of Standard Solution
50 mg of the pure drug was accurately weighed and dissolved in methanol and the volume was made up to 50 ml with methanol to give standard stock solution of 1000 \( \mu \)g/ml. Aliquots of standard stock solution were suitably diluted with distilled water to get working standard solutions of concentration of 5, 10, 15, 20, and 25 \( \mu \)g/ml. These were scanned in the wavelength range 200-400 nm.

Selection of wavelength
Standard stock solution of 1000 \( \mu \)g/ml was prepared in methanol and further aliquots were made using distilled water. The standard solution of concentration 10 \( \mu \)g/ml was scanned between 200-400 nm and \( \lambda_{\text{max}} \) was found to be at 274 nm.

Fig 1: Structural formula of Glipizide

Fig 2: Spectra of glipizide (concentration 10 \( \mu \)g/ml)

METHOD A: Absorbance Maxima Method
Aliquots of standard stock solution of concentration 1000 \( \mu \)g/ml were taken and suitably diluted with distilled water to get working standard solutions in the increasing concentration range. These were scanned in the range of 200-400 nm. The absorbance maximum was found to be at 274 nm. The calibration curve was...
plotted with concentration v/s absorbance and regression equation was calculated.

Fig 3: absorbance maxima method for glipizide (concentration 5-25 μg/ml)

Method B: First order derivative spectroscopy

The first order derivative spectra showed λmaxima 286 nm and λminima 263 nm (Fig.4). The absorbance difference at n=1 (dA/dλ) was calculated by the inbuilt software of the instrument. The derivative amplitudes were calculated by considering the maxima and minima of the curve. Amplitude difference was measured for the respective concentration of standard and was plotted against concentration and regression equation was calculated. The concentration range of 5-25 μg/ml for GZ was chosen for the derivative analysis. The equation obtained to determine concentrations of GZ is as follows.

$$CGZ = \frac{dA/d\lambda - \text{intercept (C)}}{\text{slope (m)}} \ldots [I]$$

Analysis of Tablet Formulation

For the estimation of glipizide in the commercial formulations, twenty tablets each containing 5 mg of GZ were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent to 50 mg of GZ was transferred to 50 ml volumetric flask and dissolved in sufficient quantity of methanol. It was sonicated for 30 mins and volume was made up to obtain a stock solution of 1000 μg/ml of GZ. This solution was then filtered through Whatmann filter paper # 42. Further dilutions of the stock solution were made in distilled water to get required concentration. In method A, the concentration of GZ was determined by measuring absorbances of sample solutions at 274 nm (λmax of GZ). In method B i.e first order derivative spectroscopy the concentration of GZ was determined by measuring amplitudes of sample solutions at 286 nm and 263 nm. In method C, the concentration of GZ was determined by measuring absorbances of sample solutions in wavelength range of 255 nm - 295 nm. Results of tablet analysis are shown in Table No. 1. The assay procedure was repeated six times (n=6).

Table 1: Result of Marketed Formulation Analysis

<table>
<thead>
<tr>
<th>Methods</th>
<th>Label claim (mg)</th>
<th>Mean</th>
<th>Std deviation</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5 mg</td>
<td>99.0482</td>
<td>0.73853</td>
<td>0.7456</td>
</tr>
<tr>
<td>B</td>
<td>5 mg</td>
<td>100.1591</td>
<td>0.2804</td>
<td>0.2800</td>
</tr>
<tr>
<td>C</td>
<td>5 mg</td>
<td>99.05753</td>
<td>0.8292</td>
<td>0.8371</td>
</tr>
</tbody>
</table>


Validation

The methods were validated according to ICH guidelines to study linearity, accuracy and precision.

Linearity

The linearity was evaluated by analyzing different concentrations of the standard solutions of GZ. From the standard stock solution of 1000 μg/ml appropriate dilutions were made in distilled water. These were scanned in the wavelength range 200-400 nm. Beer’s law was obeyed in the concentration range 5-25 μg/ml for all the three methods. The correlation coefficient was found to be 0.999.

Accuracy (Recovery studies)

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for GZ by all the three methods, was found in the range of 98.26%- 102.3% (TableNo.2).
Table 2: Result Of Recovery Studies

<table>
<thead>
<tr>
<th>Pre analysed tablet solution µg/ml</th>
<th>Std. Drug added µg/ml</th>
<th>Recovery level</th>
<th>% Recovery</th>
<th>% RSD Method A</th>
<th>% RSD Method B</th>
<th>% RSD Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>80</td>
<td>102.32</td>
<td>99.96</td>
<td>99.71</td>
<td>0.76</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100.30</td>
<td>98.97</td>
<td>101.22</td>
<td>0.86</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>120</td>
<td>99.96</td>
<td>99.26</td>
<td>100.61</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Precision**

The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intraday precision) and on three different days (Inter-day precision).

**RESULTS AND DISCUSSION**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ_{max} (nm)</td>
<td>274 nm</td>
<td>λ_{max} 286nm</td>
<td>Area range 255-295nm</td>
</tr>
<tr>
<td>Beer’s range (µg/ml)</td>
<td>5-25</td>
<td>5-25</td>
<td>5-25</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9997</td>
<td>0.9962</td>
<td>0.9982</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.0504x + (0.0036)</td>
<td>y = 0.0012x + (0.0001)</td>
<td>y = 0.5203x + (0.0128)</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0504</td>
<td>0.0001</td>
<td>0.0128</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.4756</td>
<td>0.4222</td>
<td>0.3778</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>0.7456</td>
<td>0.4222</td>
<td>0.3778</td>
</tr>
</tbody>
</table>

For method A, the absorbance maxima was found to be at 274 nm, for method B λ_{max} at 286 nm and λ_{min} at 263 nm were selected for first order derivative spectra and for method C area under the curve in range of 255-295 nm were selected for the analysis. The % assay by the three methods was found to be in the range 99.03-100.16% for Glipizide. No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima, first order derivative spectroscopy and area under the curve was found to be in the range of 99.96% - 102.32%, 98.97% - 99.96% and 99.71% - 101.22% respectively. Hence, the proposed methods were validated in terms of linearity, precision and accuracy. The present work provides an accurate and sensitive method for the analysis of GZ in bulk and tablet formulation.

**CONCLUSION**

The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of glipizide in bulk and formulation. The proposed methods were found to be simple, economical, rapid, precise and accurate for the determination of GZ in tablet dosage form. Thus, it can be easily and conveniently adopted for routine quality control analysis.

**ACKNOWLEDGEMENT**

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**REFERENCES**