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1. AIM AND OBJECTIVE:
The objective of this study is to develop and validate a rapid UV spectrophotometric method used for determination of Glibenclamide in tablet dosage form.

2. PLAN OF WORK:

- Method development
  - Drug physico-chemical properties
  - Drug literature survey
  - Fixing various chromatographic parameters
- Optimization of Developed Method
- Validation of method.
3. DRUG PROFILE: GLIBENCLAMIDE

Name                           : Glibenclamide  
Category                       :  Antidiabetic agent  
Molecular formula              :  C_{23}H_{28}ClN_3O_5S  
Molecular weight               :  494.0  
Solubility                     : It is practically insoluble in water; slightly soluble in alcohol and in methyl alcohol; sparingly soluble in dichloromethane.  
pKa                             :  14.17  
Chemical name                  : 1-{4-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulphonyl-3-cyclohexylurea.  

Mechanism of Action: It is a second-generation sulfonylurea antidiabetic agent, appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. With chronic administration in Type II diabetic patients, the blood glucose lowering effect persists despite a gradual decline in the insulin secretory response to the drug. Glibenclamide bind to
ATP-sensitive potassium channels on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin.

**Pharmacokinetic data:**

- **Bioavailability**: 90%
- **Protein binding**: 93%
- **Metabolism**: Liver.
- **Half-life**: 21 to 54 hours
- **Excretion**: About 50% of a dose is excreted in the urine and 50% via the bile into the faeces.

**Adverse effects**: Nausea, vomiting, heartburn, anorexia, diarrhoea, and a metallic taste. Skin rashes and pruritus may occur and photosensitivity has been reported.
4. LITERATURE REVIEW

4.1. Rajendran.SD. et al.\(^3\) have developed a high performance liquid chromatographic method is described for estimation of Glibenclamide in human serum. After precipitation with methanol, the separation of Glibenclamide and internal standard was accomplished using reversed phase chromatography. The mobile phase, a combination of acetonitrile and 25 mM phosphate buffer (pH 3.5) at 3:2 ratio was run isocratically through a C18 analytical column. The UV detection was done at 253 nm for Glibenclamide. Analytical run time was less than 12 min. Mean recovery was 92% for 0.5 µg/ml concentrations. The assay exhibited good linear relationship between peak area ratios and serum concentration. Quantification limit was at least 25 ng/ml of Glibenclamide and accuracy and precision were over the concentration range of 50-500 ng/ml. Assay was successfully applied to the measurement of Glibenclamide in serum for therapeutic drug monitoring.

4.2. Bandarkar.FS. et al.\(^4\) have proposed a rapid, precise, sensitive, economical, and validated analytical method for simultaneous separation and quantification of three anti-diabetic drugs, viz., Glibenclamide (GLB), gliclazide (GLC), and metformin hydrochloride (MHC) using ultrafast liquid chromatography (UFLC). The separation of the three drugs was achieved using a XR-ODS C\(_{18}\) column (30°C) with a mobile phase comprised of acetonitrile-water-trifluoroacetic acid-triethylamine (54:46:0.1:0.1 v/v) in isocratic elution mode at a flow rate of 0.38 mL/min and detected at 230 nm. System suitability tests essential for the assurance of quality performance of the method were performed. The method was validated for accuracy, precision, reproducibility, robustness, detection (LOD), and quantification (LOQ) limits according to FDA and ICH guidelines. MHC (\(R_t = 0.98\) min), GLC (\(R_t = 4.10\) min), and GLB (\(R_t = 6.40\) min) separated with good resolution in a single chromatographic run of 7.5 min. Linear relationship (\(r^2 > 0.999\)) was observed between the peak area and concentration for all the three compounds within the range of 5–50µg/mL. Accuracy ranged from 98 to 103% and the coefficient of variation for precision was found to be less than 3%; in all cases. LOD and LOQ values were 10ng/mL and 20ng/mL, respectively, for GLC and GLB; whereas 25ng/mL and 35ng/mL, respectively, for MHC. The method was found to be robust with minor changes in injection volume and column temperature. Validation results indicated that the method shows satisfactory linearity, precision, accuracy, and ruggedness. The extremely low flow rate, short run time, and simple mobile phase composition makes the method cost effective, rapid, non-tedious, and can also
be successfully employed for simultaneous analysis of the three anti-diabetic drugs from commercial products.

4.3. AbuRuz S. et al.\(^5\) described the development of SPE and HPLC methods for the simultaneous determination of metformin and glipizide, gliclazide, Glibenclamide or glimperide in plasma. Several extraction and HPLC methods have been described previously for the determination of each of these analytes in plasma separately. The simultaneous determination of these analytes is important for the routine monitoring of diabetic patients who take combination medications and for studying the pharmacokinetics of the combined dosage forms. In addition this developed method can serve as a standard method for the plasma determination of these analytes therefore saving time, effort and money. The recoveries of the developed methods were found to be between 76.3% and 101.9%. The limits of quantification were between 5 and 22.5 ng/ml. The intraday and interday precision (measured by coefficient of variation, CV%) was always less than 9%. The accuracy (measured by relative error %) was always less than 12%. Stability analysis showed that all analytes are stable for at least 3 months when stored at −70 °C.

4.4. Emilsson H. et al.\(^6\) have demonstrated a selective and sensitive high-performance liquid chromatographic method for determination of intact Glibenclamide in human plasma or urine. With glibornuride as internal standard, acid-buffered plasma or urine was extracted with benzene. The organic layer was evaporated and the residue was dissolved in equilibrated mobile phase (acetonitrile—phosphate buffer 0.01 M pH 3.5, 50:50). An aliquot of 20 µl was chromatographed on a Spherisorb ODS reversed-phase column, and quantitation was achieved by monitoring the ultraviolet absorbance at 225 nm. The response was linear (0–1000 ng/ml) and the detection limit was 5–10 ng/ml in plasma or urine. The within-assay variation was 10%. No interferences from metabolites or endogenous constituents could be noted. The utility of the method was demonstrated by analysing Glibenclamide in samples from diabetic subjects on therapeutic doses of the drug.

4.5. Niopas I et al.\(^7\) have developed and validated a rapid, sensitive, precise, accurate and specific HPLC assay for the determination of Glibenclamide in human plasma. After addition of flufenamic acid as internal standard, the analytes were isolated from human plasma by liquid-liquid extraction. The method was linear in the 10-400 ng/ml concentration range (r > 0.999). Recovery for Glibenclamide was greater than 91.5% and for internal standard was 93.5%. Within-day and between-day precision, expressed as the relative standard deviation (%RSD), ranged from 1.4 to 5.9% and 5.8 to 6.6%, respectively. Assay accuracy was better
than 93.4%. The assay was used to estimate the pharmacokinetics of Glibenclamide after oral administration of a 5 mg tablet of Glibenclamide to 18 healthy volunteers.

4.6. Abdel-Hamid.ME. et al.\textsuperscript{8} have proposed a rapid high-performance liquid chromatography (HPLC) determination of Glibenclamide in human serum. Serum samples to which flufenamic acid had been added as internal standard were treated with acetonitrile as a protein precipitant. After centrifugation, separation and reconstitution, the redissolved residue was eluted from 5 \( \mu \) Spherisorb C\textsubscript{8} reversed phase column at ambient temperature using a mobile phase consisting of acetonitrile-water (45: 55 v/v) at pH 3.7-3.8 and pumped at a flow rate 2 ml/min. The effluent was monitored at 230 nm. The analysis time was no longer than 12min. A linear relationship between the peak height ratio (Glibenclamide/flufenamic acid) and concentration was obtained in the range 20–400 ng/ml. A typical calibration curve has a regression equation \( y = 0.0035x + 0.015 \) \( (r^2 = 0.9999) \). The detection limit of Glibenclamide in serum was 20ng/ml. The mean recovery of drug from serum samples spiked with known amounts of Glibenclamide was 96.77%. Within-day and between-day coefficients of variation were 1.6-4.0% and 1.4-3.5%, respectively. Stability testing indicated that Glibenclamide was stable for at least 10 days in serum — 20°C. The method developed was applied to determine some pharmacokinetic parameters after the oral administration of 5 mg Glibenclamide tablets to a human volunteer.

4.7. Sami El Deeb. et al.\textsuperscript{9} presented a fast method for the simultaneous separation and determination of glimepiride, Glibenclamide, and two related substances by RP LC. The separation was performed on a Chromolith Performance (RP-18e, 100 mm×4.6 mm) column. As mobile phase, a mixture of phosphate buffer pH 3, 7.4 mM, and ACN (55 : 45 v/v) was used. Column oven temperature was set to 30°C. The total chromatographic run time was 80 s. This was achieved using a flow program from 5 to 9.9 mL/min. Precisions of the interday and the intraday assay for both retention times and peak areas for the four analyzed compounds were less than 1.2%. The method showed good linearity and recovery. The short analysis time makes the method very valuable for quality control and stability testing of drugs and their pharmaceutical preparations.
5. EXPERIMENTAL

5.1. Materials

Glibenclamide standard of was provided by Aarti Drugs Ltd., Boisar (India). Glibenclamide tablets containing 5 mg Glibenclamide and the inactive ingredient used in drug matrix were obtained from market. Analytical grade methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India).

5.2. Diluent Preparation

Methanol and Water (50:50, v/v) used as a diluents.

5.3. Standard Preparation

Accurately weigh and transfer 10mg of Glibenclamide Working standard into a 10 mL volumetric flask add about 7 mL of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

5.4. Test Preparation

Weigh 5 Glibenclamide Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Glibenclamide into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicated to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipetted 5 ml of the above stock solution into a 50ml volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipetted 3 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.
5.5. INSTRUMENTATION:

The various instruments required for the study is listed in Table 1.

Table 1. List of Equipment used in the method

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Make/ Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Analytical balance</td>
<td>Aicoset</td>
</tr>
<tr>
<td>2</td>
<td>UV-Visible Double Beam Spectrophotometer</td>
<td>Systronics 2202</td>
</tr>
<tr>
<td>3</td>
<td>Detector</td>
<td>UV detector</td>
</tr>
<tr>
<td>4</td>
<td>Sonicator</td>
<td>SONICA 2200MH</td>
</tr>
<tr>
<td>5</td>
<td>pH meter</td>
<td>Metler Toledo</td>
</tr>
</tbody>
</table>
6. METHOD DEVELOPMENT

6.1. Development and Optimization of the Spectrophotometric Method

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of Glibenclamide, the analytical conditions were selected after testing the different parameters such as diluents, wavelength, and other spectroscopic conditions.

Our preliminary trials using different composition of diluents consisting of water with buffer and methanol. By using diluent consisted of methanol: water (50:50, v/v) best result was obtained and degassed in an ultrasonic bath. Belowfigures represent the spectrums of blank, standard and test preparation respectively.

6.1.1 Selection of Wavelength

- Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank.
- The spectrums are shown in Figure 2, 3&4.

![Figure 2. UV spectrum of a Glibenclamide in Diluent (Blank) solution](image-url)
Glibenclamide shows $\lambda_{\text{max}}$ at 308 nm. The proposed analytical method is simple, accurate and reproducible.
7. METHOD VALIDATION:
Based on International Conference on Harmonization (ICH) guidelines, the proposed method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ, robustness and sensitivity as follows.

7.1. Specificity:
Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. The specificity of the method was determined by checking the interference of placebo with analyte.

7.2. Precision:
The precision of the method was evaluated by carrying out six independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

7.3. Intermediate Precision/Ruggedness:

7.3.1 Intra-day precision: The precision of the assay method was evaluated by carrying out six independent assays Glibenclamide (50, 100, 150% i.e. 5.0, 10.0, 15.0 µg/ml.) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

7.3.2 Intermediate precision (inter-day): Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the six samples of Glibenclamide against qualified reference standard. The percentage of RSD of six assay values was calculated. The %RSD for the absorbance of six replicate samples was found to be within the specified limits (% RSD should not be more than 2%).

7.4. Accuracy:

Recovery of the assay method for Glibenclamide was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was
Chapter VIII  

Glibenclamide

sampled thrice (n=3) into spectrophotometer and the average absorbance value was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

7.5. Linearity:

Test solutions were prepared from stock solution at 5 concentration levels (10, 20, 30, 40, 50, 60 and 70 µg/ml). The absorbance vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

7.6. Robustness:

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method.
Chapter VIII

8. RESULTS AND DISCUSSION:

8.1. Specificity

The specificity of the method was determined by checking the interference of placebo with analyte. There was no interference is observed.

8.2. Linearity

Seven points of calibration curve were obtained in a concentration range from 10-70µg/ml for Glibenclamide. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was \( y = 0.0229x + 0.2414 \) with correlation coefficient 0.9995. The results were illustrated in Figure 5.

![Figure 5. Linearity curve for Glibenclamide](chart)

8.3. Precision

The result of repeatability and intermediate precision study are shown in Table 2. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were < 0.98 % and < 0.79 %, respectively, which confirm that method was precise.
### 8.4. Accuracy

The spectrophotometer absorbance responses for accuracy determination are depicted in Table 3. The result shown that best recoveries (98.82 - 100.61%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

<table>
<thead>
<tr>
<th>Level (%)</th>
<th>Amount added$^\dagger$ (mg/mL)</th>
<th>Amount found$^\dagger$ (mg/mL)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.1960</td>
<td>0.01953</td>
<td>99.65</td>
<td>1.82</td>
</tr>
<tr>
<td>100</td>
<td>0.4040</td>
<td>0.03992</td>
<td>98.82</td>
<td>1.13</td>
</tr>
<tr>
<td>150</td>
<td>0.6067</td>
<td>0.06104</td>
<td>100.61</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Each value corresponds to the mean of three determinations

### 8.5. Solution stability study

Table 4 shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2 - 5°C and ambient temperature, as during this time the result was not decrease below the minimum percentage.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Intra-day (n=6)</th>
<th>Inter-day (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.2</td>
<td>98.6</td>
</tr>
<tr>
<td>2</td>
<td>100.6</td>
<td>99.3</td>
</tr>
<tr>
<td>3</td>
<td>99.1</td>
<td>99.0</td>
</tr>
<tr>
<td>4</td>
<td>101.5</td>
<td>100.5</td>
</tr>
<tr>
<td>5</td>
<td>101.8</td>
<td>98.2</td>
</tr>
<tr>
<td>6</td>
<td>101.3</td>
<td>99.0</td>
</tr>
</tbody>
</table>

Mean

| Standard Deviation | 0.99 | 0.78 |

% RSD

| % RSD | 0.98 | 0.79 |
### Table 4. Evaluation data of solution stability study

<table>
<thead>
<tr>
<th>Intervals</th>
<th>% assay for test preparation stored at 2-8°C</th>
<th>% assay for test preparation stored at ambient temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial</td>
<td>101.3</td>
<td>100.5</td>
</tr>
<tr>
<td>12 h</td>
<td>98.7</td>
<td>98.7</td>
</tr>
<tr>
<td>24 h</td>
<td>100.2</td>
<td>101.0</td>
</tr>
<tr>
<td>36 h</td>
<td>100.1</td>
<td>101.8</td>
</tr>
<tr>
<td>48 h</td>
<td>100.8</td>
<td>100.2</td>
</tr>
</tbody>
</table>

### 8.6. Robustness

The result of robustness study of the developed assay method was established in Table 5. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

### Table 5. Evaluation data of robustness study

<table>
<thead>
<tr>
<th>Robust conditions</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol: Water (55:45, v/v)</td>
<td>101.6</td>
</tr>
<tr>
<td>Methanol: Water (45:55, v/v)</td>
<td>99.6</td>
</tr>
<tr>
<td>Analyst change</td>
<td>102.0</td>
</tr>
</tbody>
</table>
9. SUMMARY:

✓ The prime objective of the proposed method was to estimate the concentration of Glibenclamide by UV spectroscopy method.

✓ Developing a UV method was to reduce the cost, time and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Glibenclamide was adequate at 308 nm.

✓ While developing the present method, various solvents have been tried, but Methanol and Water (50:50, v/v) system shown good specificity and selectivity.

✓ The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

✓ The present method was linear for Glibenclamide from 10-70µg/ml and the linear regression obtained was 0.999. Precision, evaluated by intra- and inter-day assays had relative standard deviation (R.S.D) values within 1.5 %. Recovery data were in the range of 98.82 and 100.61% with R.S.D. values < 1.5 %.
10. CONCLUSION:

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of Glibenclamide either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.
11. REFERENCES:


10. ICH, Q2 (R1) validation of analytical procedures: text and methodology, International conference on harmonization; Nov.1996.