Research Article

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DESIGN AND VALIDATIONOF SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF GLIBENCLAMIDE IN TABLET DOSAGE FORM

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ABSTRACT:

A rapid, specific and economic UV spectrophotometric method has been developed using a solvent composed of Methanol and Water (50:50, v/v) to determine the Glibenclamide content in bulk and pharmaceutical dosage formulations. At a predetermined λmax at 308 nm, it was proved linear in the range of 10.0–70.0µg/mL, and exhibited good correlation coefficient ($R^2$0.9998) and excellent mean recovery (98.82- 100.61%). This method was successfully applied to the determination of Glibenclamide content and the results were in good agreement with the label claims. The method was validated for linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of Glibenclamide in bulk as well as in the commercial formulations.

KEYWORDS:

Glibenclamide, UV Spectroscopy, method development, ICH guidelines

1. INTRODUCTION

Glibenclamide is chemically 1-{4-[2- (5 –Chloro-2 –methoxy benzamido) ethyl] benzenesulphonyl} -3-cyclohexylurea [1]. The structure is illustrated in Figure 1.Glibenclamide 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 secretary 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 [2].

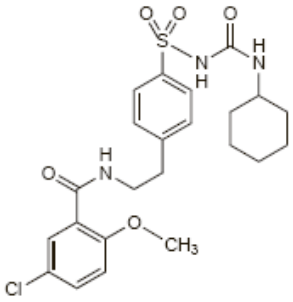

Figure No.1.Structure of Glibenclamide

Several assay techniques have been described for quantitative determination of glibenclamide in biological fluids; these include procedures based on high performance liquid chromatography (HPLC) [3-7],UV spectrophotometry [8] and colorimetry[9]. In thisstudy,efforts were made to develop a simple, easy and economic UV spectrophotometric method using a diluents composed of Methanol and Water (50:50, v/v) for the determination of Glibenclamide in the raw materials as well as in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH) [10].

2. MATERIALS AND METHODS

2.1 Materials

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

2.2 Diluent Preparation

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

2.3 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. (Stocksolution).

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

2.4 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 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.

2.5 Instrumentation

UV-VISIBLE double beam Spectrophotomer model✓ no.2202 made by SYSTRONICS and Sonicator model no.2200MH, made by SONICA.

3. METHOD DEVELOPMENT

3.1 Development and Optimization of the Spectrophotometric Method

Proper wave length selection of the methods depends upon the nature of the sampleand its solubility. To develop a rugged and suitable spectrophotometric method for thequantitative determination of Glibenclamide, the analytical conditions were selected after testingthe 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 resultwas obtained and degassed in an ultrasonic bath. Belowfigures represent the spectrums of blank, standard and test preparation respectively.

3.1.1 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.**

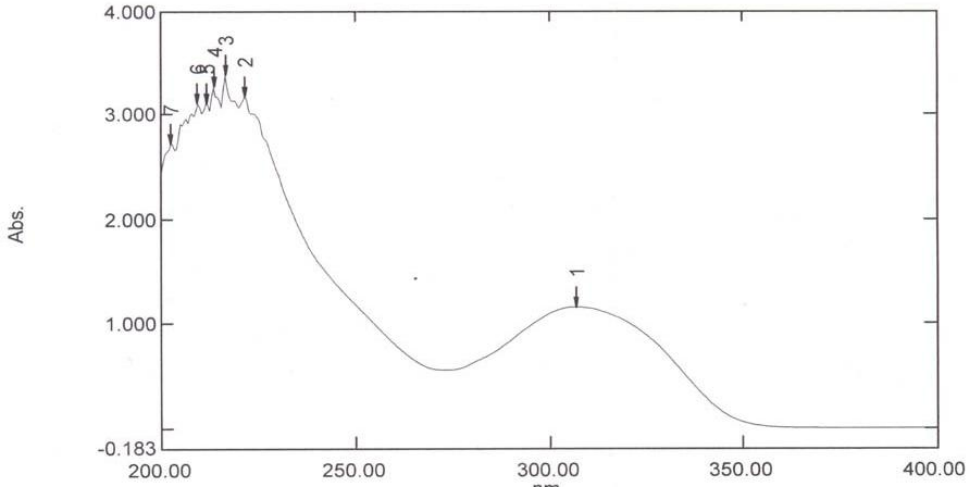

Figure 2: UV spectrum of Glibenclamide in Standard solution

Glibenclamide shows λ_{max} at 308 nm. The proposed analytical method is simple, accurate and reproducible.

4. 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.

4.1 Specificity

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

4.2 Precision:

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

4.3 Intermediate Precision/Ruggedness:

4.3.1 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, 15.0µg/ml.) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

4.3.2 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 area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

4.4 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 injected thrice (n=3) into UPLC system and the average peak area was calculated from

which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

4.5 Linearity:

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

4.6 Robustness:

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

5. RESULTS AND DISCUSSION

5.1 Specificity

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

5.2 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 4.**

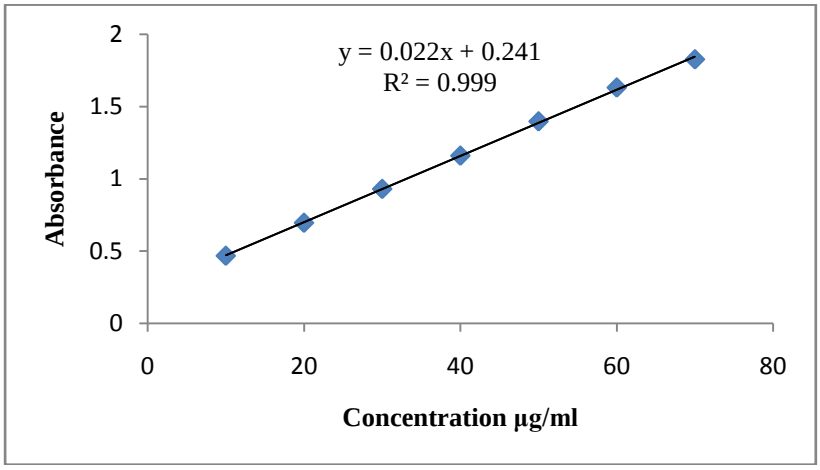

Figure 4: Linearity curve for Glibenclamide

5.3 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.

Table 2: Evaluation data of precision study

S.No.	Intra-day (n=6)	Inter-day (n=6)
1	101.2	98.6
2	100.6	99.3
3	99.1	99.0
4	101.5	100.5
5	101.8	98.2
6	101.3	99.0
Mean	101.1	99.1
Standard Deviation	0.99	0.78
% RSD	0.98	0.79

5.4 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 3: Evaluation data of accuracy study

Level (%)	Amount added* (mg/mL)	Amount found* (mg/mL)	% Recovery	% RSD
50	0.1960	0.01953	99.65	1.82
100	0.4040	0.03992	98.82	1.13
150	0.6067	0.06104	100.61	0.20

***** Each value corresponds to the mean of three determinations

5.5 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 4: Evaluation data of solution stability study

Intervals	% assay for test preparation stored at 2-8^{o} C	% assay for test preparation stored at ambient temperature
Initial	101.3	100.5
12 h	98.7	98.7
24 h	100.2	101.0
36 h	100.1	101.8
48 h	100.8	100.2

5.6 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

Robust conditions	% Assay
Methanol: Water (55:45, v/v)	101.6
Methanol: Water (45:55, v/v)	99.6
Analyst change	102.0

6. 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.

7. REFERENCES

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