

# ESTIMATION GLIBENICLAMIDE DRUGS IN BULK AND PHARMACEUTICAL USING RPHPLC METHOD

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**ABSTRACT:** The prime aim of the current work is to develop and validate a novel, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique for the estimation of Glibenclamide in dosage form. Chromatographic separation was achieved on a Chromosil column, (150mm×4.6mm x5μ) using an isocratic method with mobile phase composed of Potassium dihydrogen phosphate buffer (pH 4.5): Acetonitrile in the ratio 60:40 v/v. The flow rate was 1 ml/min, temperature of the column was maintained at ambient and detection was made at 233 nm. The run time was 12 min. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. The developed method was linear for Glibenclamide from 10 - 50 μg/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 98.2% to 100.9% with R.S.D. values < 1.5 %. The method is precise, accurate, linear, robust and fast. The short retention time allows the analysis of a large number of samples in a short period of time and, therefore, should be cost-effective for routine Quality Control in the pharmaceutical industry.

**KEYWORDS:** HPLC, Method development, Validation, Reverse Phase and Glibenclamide.

## INTRODUCTION

Glibenclamide is the most extensively used sulphonylurea in many parts of the world for the management of non-insulin-dependent diabetes mellitus (NIDDM) 1. It is practically insoluble in water; slightly soluble in alcohol and in methyl alcohol; sparingly soluble in dichloromethane. It is a second-generation sulphonylurea 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. Glibenclamide chemically 1-{4-[2-(5-Chloro-2-methoxybenzamido) ethyl] benzenesulphonyl 1-3-cyclohexylurea. Its molecular structure is given in figure 1.

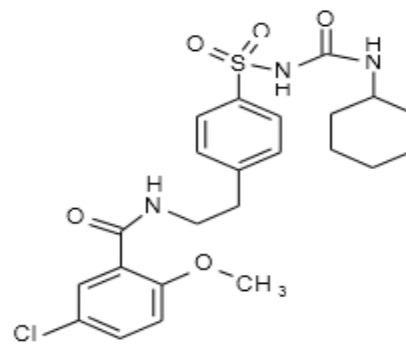


Figure 1. Structure of Glibenclamide

Literature suggests few HPLC methods coupled with UV detection<sup>3-9</sup>, fluorescence detection<sup>10</sup> or mass spectrometry<sup>11-13</sup> has been developed for the determination of glibenclamide in biological fluids. However, some of these methods were not sufficiently specific and sensitive, some were not validated and some were time-consuming and expensive. It is, therefore, felt necessary to develop a new rapid method for the determination of Glibenclamide by HPLC method. Hence a reproducible RP HPLC method was developed for the quantitative determination of Glibenclamide by using Chromosil (150mm×4.6mm x5 $\mu$ ) HPLC column. The proposed method was validated as per the guidelines suggested by ICH 14-15.

#### **MATERIALS AND REAGENTS:**

Glibenclamide working Standard was procured from Spectrum laboratories, Hyderabad, India. Commercially available glibenclamide purchased from local pharmacy. Acetonitrile HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

#### **Chromatographic conditions**

Chromatography separation was performed on Cyber lab HPLC system with UV detector. The output signal was monitored and processed using LC solutions software. The Chromosil column, (150mm×4.6mm x5 $\mu$ ) using an isocratic method with mobile phase composed of Potassium di- hydrogen phosphate buffer (pH 4.5): Acetonitrile in the ratio 60:40 v/v. The flow rate was 1 ml/min, temperature of the column was maintained at ambient and detection was made at 233 nm. The run time was 12 min.

#### **Preparation of solutions**

##### **Preparation of Phosphate buffer:**

Weighed accurately 1.625 grams of KH<sub>2</sub>PO<sub>4</sub> and 300 mg of K<sub>2</sub>HPO<sub>4</sub> into a 500ml volumetric flask, dissolved with small portion of HPLC Water and make up to the mark with water. This solution was adjusted to pH 4.5 with ortho phosphoric acid, degassed in ultrasonic water bath for 5 minutes and passed through 0.45 $\mu$  filter under vacuum filtration.

##### **Preparation of mobile phase:**

The mobile phase was prepared by mixing 60 ml of mixed phosphate buffer pH 4.5 and 40ml of Acetonitrile (HPLC grade) in 100ml of volumetric flask.

#### **Preparation of the glibenclamide Standard & Sample Solution:**

##### **Standard Solution Preparation**

mg of Glibenclamide was taken in 100ml volumetric flask. It was dissolved in mobile phase and made up to the mark with the same to get a concentration of 1000 $\mu$ g/ml. It was degassed in ultra sonicator and then filtered through membrane filter of 0.45 $\mu$  pore size.

##### **Sample Solution Preparation**

10 tablets were crushed and powder equivalent to 61.8mg was taken into 100ml volumetric flask. It was made to dissolve with mobile phase and made up to the mark with mobile phase to get the concentration of 1000  $\mu$ g/ml solution. The solution was degassed and filtered through membrane filter of pore size 0.45  $\mu$ .

##### **Method validation**

##### **Method precision:**

100 $\mu$ g/ml concentration was injected thrice into the chromatographic system. Peak areas were noted down. Average, Standard deviation, %RSD were calculated.

##### **System precision:**

100  $\mu$ g/ml solution was taken and injected once into the present chromatographic system Again the solution was injected once into the other chromatographic system .The peak areas were noted down and %RSD were calculated.

##### **Accuracy**

The accuracy of the method was evaluated by determination of recovery of Glibenclamide at three levels of concentrations. The sample solutions were spiked with standard solutions corresponding to 50, 100, and 150% of nominal analytical concentrations. (% Recovery should be between 97.0 to 103.0%).

##### **Linearity**

Test solutions were prepared from stock solution at 5 concentration levels. The peak area vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

##### **Limit of Detection (LOD) Limit of Quantification (LOQ)**

LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

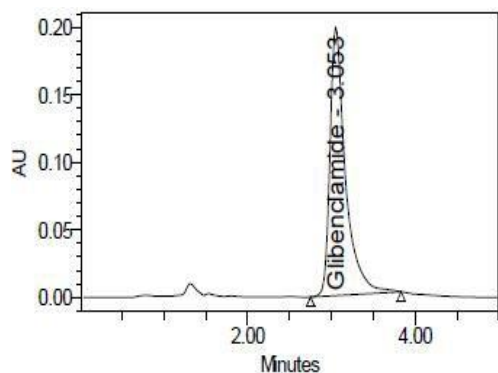
##### **Robustness**

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

## RESULTS AND DISCUSSION:

### Method development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Total of seven trials have been conducted. Some of the trials yielded a peak with tailing factor of 2. Some of the trials have showed longer retention time, some of them were not efficient in terms of theoretical plates, some them were possessed poor resolution but trial 7 with chromatographic conditions of stationary phase Chromosil column, (150mm×4.6mm x5 $\mu$ ), Mixed phosphate Buffer: Acetonitrile (60:40) as mobile phase with runtime of 12 minutes and the flow rate of 1 ml/min at detection wavelength of 233nm yielded a perfect chromatogram. The typical chromatogram obtained for Glibenclamide from final HPLC conditions are depicted in Figure 2.



**Figure 2: Typical chromatogram of Gliabenclamide by proposed method**

### Method validation

Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system

suitability, linearity, accuracy, precision, LOD, LOQ, robustness and sensitivity as follows.

### System suitability

The system suitability results for the proposed HPLC method are Tailing factor obtained from the standard injection is 1.20. Theoretical Plates obtained from the standard injection is 2354. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits.

### Precision

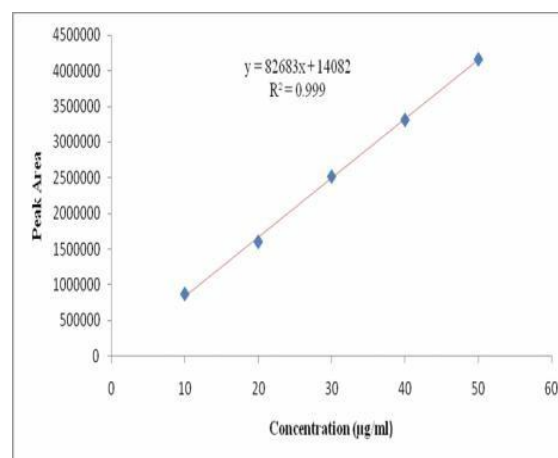
The % R.S.D. of glibenclamide assay during the method precision was found to be 0.27%, indicating good precision of the method. The results are summarized in table 1.

**Table 1: Results of precision**

Injection	Area
Injection-1	2587374
Injection-2	2585151
Injection-3	2596144
Injection-4	2587725
Injection-5	2576851
Average	2586649
Standard Deviation	6893.7
%RSD	0.27

### Linearity

The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 10 - 50  $\mu$ g/ml for three times, and the correlation coefficient obtained was 1.000, thus indicating excellent correlation between peak areas and concentrations of the analyte. The linearity data depicted in figure 3.



**Figure 3: linearity curve of Glibenclamide**

Limits of detection (LOD) and quantification (LOQ) LOD and LOQ for Glibenclamide were 0.032 and 0.09 $\mu$ g/ml, respectively. Since the LOQ and LOD values of Glibenclamide

are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

### Accuracy

Percentage recovery of Glibenclamide samples ranged from 98.2% to 100.9% and the mean recovery is 99.8%, showing the good accuracy of the method. The result is shown in Table 2.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1475340	5.68	5.78	98.2%	99.8%
100%	2544955	10.0	9.97	100.3%	
150%	3299867	13.05	12.93	100.9%	

### Robustness

In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 10 -50 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate  $\pm 10\%$  and change in the Mobile phase  $\pm 10\%$ . The results are summarized in table 3.

**Table 3: Results of Robustness**

Change in Organic Composition in the Mobile Phase / flow rate	System Suitability Results	
	USP Plate Count	USP Tailing
10% less	2653	1.30
Actual	2545	1.25
10% more	2465	1.2
0.6	2708	1.30
0.8	2545	1.25
1.0	2354	1.20

### CONCLUSION:

Literature review revealed that few methods for the assay of Glibenclamide have been reported. All the methods were time consuming and costlier. Therefore it was thought worthwhile to develop simple, precise, accurate HPLC method for the estimation of Glibenclamide. A simple, rapid and reproducible HPLC method was developed and validated for the estimation of Glibenclamide. Chromosil, 150×4.6mm; 5µ column, in isocratic mode with mobile phase containing Phosphate buffer, Acetonitrile (60:40) was used. The flow rate was 1ml/min and the analyte was monitored at 233 nm. The retention time for Glibenclamide was 6.2 minutes. The system was validated for system suitability, accuracy, precision and linearity, the system suitability parameter were within the limit, hence it was concluded that the system was suitable to perform the assay.

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