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Development and Validation of Sensitive RP-HPLC Method for the Estimation of Glibenclamide in Pure and Tablet Dosage Forms

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ABSTRACT

Reversed phase liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the determination of Glibenclamide in pure and formulations. Standard stock solution (1.0 mg.mL^{-1}) of Glibenclamide was prepared by transferring accurately weighed 50.0 mg of Glibenclamide and dissolving in a 50 mL volumetric flask containing 10 mL of methanol. This solution was sonicated for 20 min to achieve complete dissolution and made up to the mark with mobile phase. From the standard stock solution different concentrations of working standard solutions of Glibenclamide were prepared with the same mobile phase ranging from 2.0 -10.0 µg.mL^{-1}. The linearity for HPLC method was determined at six concentration levels ranging from 2.0 -10.0 µg.mL^{-1} for Glibenclamide. The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis determination of Glibenclamide in tablet dosage forms.

Keywords: *Glibenclamide*, RP-HPLC, Validation, Accuracy, Ruggedness, Formulations and uses.

INTRODUCTION

Glibenclamide (Figure 1) [1] is chemically known as 5-chloro-N-[2-[4[(cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-2-methoxy benzamide is second generation sulphonyl ureas drug widely used in treatment of type 2 diabetic patients. It acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells causing cell membrane depolarization (increasing intracellular calcium in the beta cell) which stimulates the insulin release.

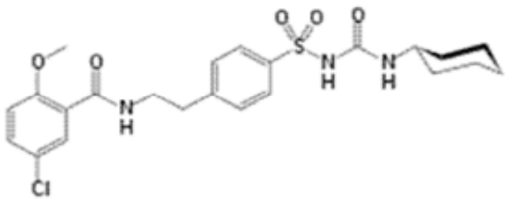

Figure 1: Chemical Structure of Glibenclamide

Several assay techniques have been reported for quantitative determination of Glibenclamide in biological fluids which high performance liquid chromatography (HPLC) [2], LC-MS [3-5] in plasma and UV-Spectropthometric techniques [6, 7] in dosage forms. To the best of our knowledge there are no UV visible Spectrophotometric and

inexpensive RP-HPLC method reported in the literature. This paper describes the application of UV-Visible spectroscopy and economical RP-HPLC method for the quantitative analysis of Glibenclamide in pharmaceutical raw material and dosage forms. Several liquid chromatographic methods [2-5] are reported for analysis of Glibenclamide in plasma and various biological fluids which suffer from undesirably long chromatographic run times and use of an internal standard. Rao et al. have published their results on different oxide materials, luminescent materials and polymers in their earlier studies [8-24]. The objective of this study was, therefore, to develop a simple, accurate, sensitive and validated RP-HPLC method for the quantification Glibenclamide in pure and in tablet dosage forms with good sensitivity. Method validation for the developed method was done according to ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents: The pharmaceutical grade pure samples of Glibenclamide (99.80 %) supplied by Hetero Labs. Hyderabad, India. Disodium hydrogen phosphate, Triethylamine , orthophosphoric acid of AR grade were procured from Qualigens Fine Chemicals, Mumbai, India and methanol HPLC grade was obtained from E Merck Ltd, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system.

Mobile Phase Preparation: The mobile phase consisted of disodium hydrogen phosphate buffer 40 mM containing 0.1 % triethylamine (adjusted to pH-4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v). The mobile phase was freshly prepared and filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, US) and degassed by an ultrasonic bath. The injections were carried out through a 10.0 μL loop. The analytes were detected and quantified by UV detection at a wavelength of 236 nm.

Stock Solutions and Standards: Standard stock solution (1.0 $mg.mL^{-1}$) of Glibenclamide was prepared by transferring accurately weighed 50.0 mg of Glibenclamide and dissolving in a 50 mL volumetric flask containing 10 mL of methanol. This solution was sonicated for 20 min to achieve complete dissolution and made up to the mark with mobile phase. From the standard stock solution different concentrations of working standard solutions of Glibenclamide were prepared with the same mobile phase ranging from 2.0 -10.0 $\mu g.mL^{-1}$. The solutions were filtered through a 0.45 μm membrane filter before injection.

Assay Procedure: Twenty tablets of Glibenclamide (MICRONASE label claim 5.0 mg) procured form local pharmacy were weighed and transferred in to a clean, dry mortar and powdered. Tablet powder equivalent to 50 mg of Glibenclamide was transferred in to a 50 mL volumetric flask and 20 mL of the mobile phase was added. The solution was sonicated for 20 min to achieve complete dissolution of Glibenclamide, made up to the mark with mobile phase and then filtered through a 0.22 µm nylon membrane filter. Further dilutions were made with mobile phase to get a concentration of 2.0 – 10.0 $\mu g.mL^{-1}$ of Glibenclamide. The solutions were filtered through a 0.45μm membrane filter before injection.

***HPLC Apparatus*:** The chromatographic separation was performed on a Waters liquid chromatographic system equipped with a Waters 1515 isocratic solvent delivery system (pump), Waters 2487 dual wavelength photo diode array detector and Rheodyne 7725i injector with 10 µL loop volume. Empower 2 software was used for data collecting and processing. Inertsil ODS C_{18} column (250 mm x 4.6 mm i.d, 5 µm) was used as stationary phase for the separation.

RESULTS AND DISCUSSION

Method Development: Several tests were performed in order to get satisfactory separation-resolution of Glibenclamide in different mobile phases with various ratios of organic phase and buffers by using C_{18} column. The ideal mobile phase used was disodium hydrogen phosphate buffer 40 mM containing 0.1 % triethylamine (adjusted to pH 4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v to obtain satisfactory and good resolution. Increasing or decreasing pH of mobile phase by ± 0.2 did not showed any significant change in retention time of each analyte. The chromatographic conditions employed for the assay of Glibenclamide are reported in the Table-1 and the typical chromatogram obtained was recorded respectively Figure-2. The retention of Glibenclamide was 4.273 minutes and was evaluated at a flow rate of 1.0 $mL.min^{-1}$.

Method Validation: The method was validated following the parameters such as specificity, linearity, precision, accuracy, limits of detection and quantization and robustness, following the ICH guidelines (ICH, 2009).

Specificity: The specificity of the method was evaluated with regards to interference due to the presence of excipients in the pharmaceutical formulation. The placebo samples consisted of all the excipients without the active substance. Then, the specificity of the method was established by determining the peak purity of Glibenclamide in samples using a UV detector, ranging between 190-400 nm. To determine specificity with respect to sample compounds the responses of standard and sample solution were compared. No interferences were detected at the retention times of Glibenclamide in sample solution.

Table -1: Chromatographic Conditions

Chromatographic Parameters	**Peak Conditions**
Mobile phase	Disodium hydrogen phosphate buffer 40 mM containing 0.1 % triethylamine (adjustedtopH4.8by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v.
Column	Inertsil ODS C_{18} column (250 mm x 4.6 mm i.d., 5 µm)
Flow rate	1.0 mL.min^{-1}
Detection	PDA : 236 nm
Injection volume	10 µL
Temperature	ambient temperature 25±2 °C
Retention time	4.273 minutes
Run time	6 minutes
Area	15620582 mAU
pH	4.8
Pressure	17-20 Pa

Selection of UV Wavelength: The detector wavelength of present study 236 nm was selected on the basis of higher sensitivity and obtained overlay spectra shown is absorptive point for Glibenclamide at 236 nm by using photodiode array detector.

Linearity: The linearity for HPLC method was determined at six concentration levels ranging from 2.0 -10.0 µg.mL^{-1} for Glibenclamide**.** The calibration curve was constructed by plotting response factor against concentration of Glibenclamide which is represented in Figure-3. The slope and intercept value for calibration curve were Y = 8E+06x - 1E+06 (R^2 = 0.9989) for Glibenclamide, where Y represents the ratio of peak area ratio of analyte to Glibenclamide and X represents analyte concentration. The results were satisfactory shown that significant correlation exists between response factor and concentration of drug. The results are shown in Table-2.

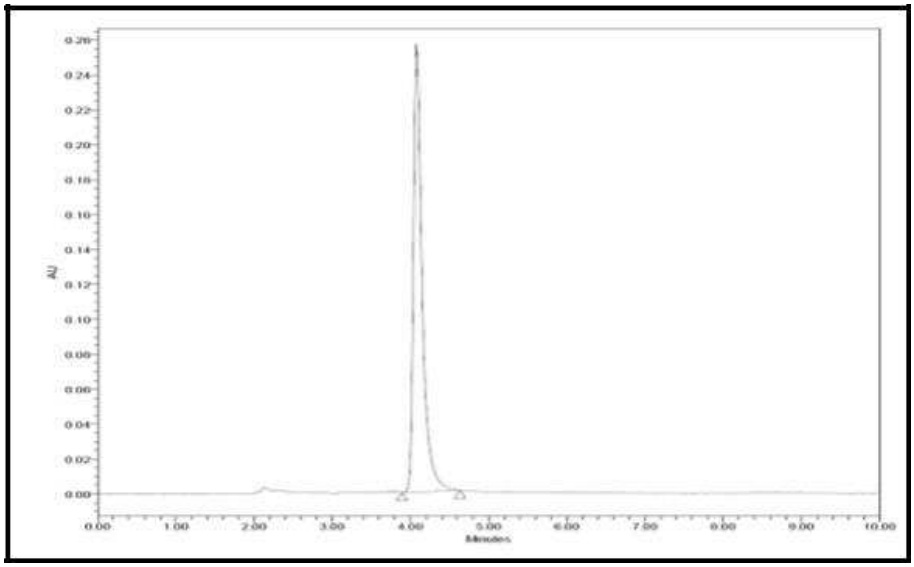

Figure-2: Typical HPLC Chromatogram of Standard Solution of Glibenclamide

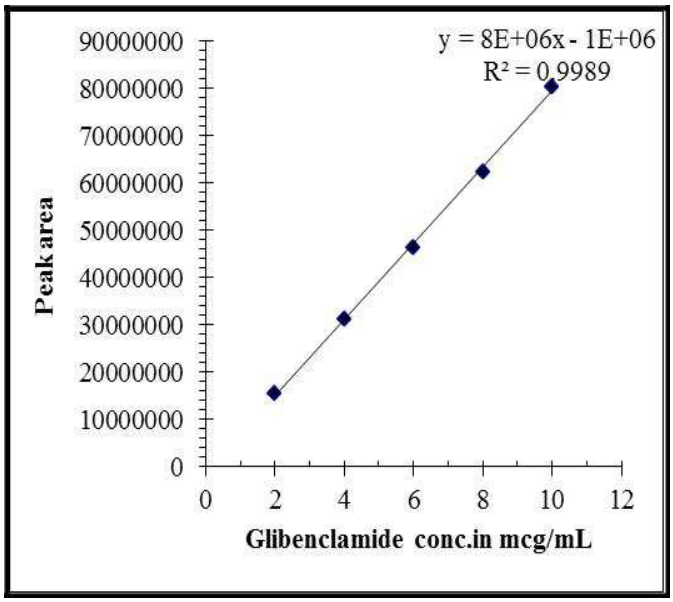

Figure-3: Calibration Curve of Glibenclamide

Precision: The precision of the proposed method was investigated by intra-day and inter-day determinations of Glibenclamide at three different concentrations of Glibenclamide (4, 8 and 12 µg/mL). The intra-day studies were performed in one day (for each level n=5) and inter-day studies in five days over a period of two weeks. The intra and inter-day precisions expressed as relative standard deviation values (RSD %) for Glibenclamide were found to be within 0.87-1.99 % and 0.99-2.03 %, respectively. The data proved good precision for the developed method. The results are shown in Table-3.

Accuracy (Recovery): **T**he accuracy of the proposed RP-HPLC method was accessed with recovery experiments performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure Glibenclamide at three different levels [50, 100 and 150 % of the content present in the tablet powder (taken)] and the total was found by the proposed method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 98.33 and 101.7. Closeness of the results to 100 % showed the fairly good accuracy of the method. The results are shown in Table-4.

Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hrs at room temperature. The results show that for solutions, the retention time and peak area of Glibenclamide remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hrs, which was sufficient to complete the whole analytical process.

Ruggedness and Robustness: Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

Analysis of Pharmaceutical Formulations: Pharmaceutical formulations of Glibenclamide were analyzed by the developed method. The assay of the drug present in the each tablet was calculated by comparing the area of the peak of test with the standard. The assay of Glibenclamide in tablets was found to be 99.60 % and the results were presented in Table-5.

Table-2: Calibration of the RP HPLC for the Estimation of Glibenclamide

Concentration (µg)	Area (mAU)
2.0	15620582
4.0	31241164
6.0	46500000
8.0	62482328
10.0	80500000
Regression equation	Y = a X + b
Slope (a)	8E+06
Intercept (b)	-0.000001
Correlation coefficient	0.9989
LOD	0.0325

Table-3: Results from Intra-day and Inter-day Precision Experiments

Drug	Actual concentration ($\mu g.mL^{-1}$)	Intra-Day			Inter-Day		
		Found* $\mu g.mL^{-1} \pm SE$	% RSD	% Bias	Found* $\mu g.mL^{-1} \pm SE$	% RSD	% Bias
Glibencla-mide	**4**	4.01 ± 20.26	1.99	-0.25	4.03 ± 20.56	2.03	-0.75
	8	8.03 ± 4.42	0.87	-0.37	8.06 ± 5.01	0.99	-0.75
	10	10.01 ± 3.46	1.02	-0.08	10.07 ± 5.58	1.65	-0.58

Table-4: Results of Recovery Study via Standard-Addition Method

Drug studied	GBM in tablet ($\mu g.mL^{-1}$)	Pure GBM added ($\mu g.mL^{-1}$)	Total GBM found ± SD*, $\mu g.mL^{-1}$	% GBM recovered
Glibenclamide	**6.0**	**3.0**	8.95 ± 0.22	98.33
	6.0	**6.0**	11.99 ± 0.17	99.83
	6.0	**9.0**	15.15 ± 0.24	101.7

Table-5: Results of Analysis of Tablet Containing Glibenclamide

PHARMACEUTICAL FORMULATION	AMOUNT OF GLIBENCLAMIDE*		% RECOVERY
	LABELLED	FOUND	
MICRONASE(5.0 mg)	5.0 mg	4.98	99. 60 %

* *Average of three determinations*

CONCLUSION

The reported RP-HPLC method developed by the author for the analysis of Glibenclamide was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the developed RP-HPLC method. The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis determination of Glibenclamide in tablet dosage forms.

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