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DEVELOPMENT, ESTIMATION AND VALIDATION OF GLIMEPIRIDE IN PHARMACEUTICAL FORMULATION BY HPLC METHOD

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ABSTRACT: The study is focused on developing a simple, rapid, validated High Performance Liquid Chromatographic (HPLC) method for Glimepiride Tablets from their dosage forms. The detection was carried out at 230 nm using Shimadzu UV - Visible detector HPLC system. The accuracy and precision were determined and validated statistically. The linearity was observed in the range of 15-120 µg/mL with a correlation coefficient of 0.999. The limit of detection and the limit of quantification were found to be 4 ng and 10 ng respectively. A Lichrosorb® (RP-18 column with a mobile phase consisting of acetonitrile – water – glacial acetic acid (550:450:0.6 v/v)) was used. The flow rate was 1 mL/min. The HPLC method is selective, precise and accurate and can be used for routine analysis of preparations in pharmaceutical industry quality control laboratories.

INTRODUCTION: Glimepiride 1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulphonyl]-3- *trans*-(4-methylcyclohexyl) urea is a Sulfonyl Urea derivative. It is widely used in type-2 diabetes. It is an oral Anti Diabetic with prolonged effect and it maintains a more physiological regulation of insulin secretion during physical exercise, which suggests that there may be less risk of hypoglycemia [1]. Glimepiride is one of the third generation sulfonylurea, antidiabetic drug which stimulates insulin release [2]. The drug shows low, pH dependent solubility. In acidic and neutral aqueous media, Glimepiride exhibits very poor solubility at 37°C (<0.004 mg/mL).

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In media pH>7, solubility of drug is slightly increased to 0.02 mg/mL. This poor solubility may cause poor dissolution and unpredicted bioavailability [2-4]. Glimepiride is a white powder and is insoluble in water, soluble in dimethylformamide, and slightly soluble in methylene chloride and methanol [5]. The chemical structure of the molecule is presented in Figure 1. Literature survey revealed HPLC methods developed for the estimation of glimepiride in tablets and biological fluids [6-11].

Capillary electrophoresis [12], spectrophotometry [13], polarography [14], liquid chromatography-electro-spray ionization tandem mass spectrometry[15] methods were also developed for the estimation of glimepiride in biological fluids. The aim of the present study was to develop a simple, economical and accurate analytical method for the estimation of Glimepiride in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC-UV method was validated in

accordance with the International Conference on Harmonization (ICH) guidelines [16], by assessing its selectivity, linearity, accuracy, precision, and limits of detection and quantitation.

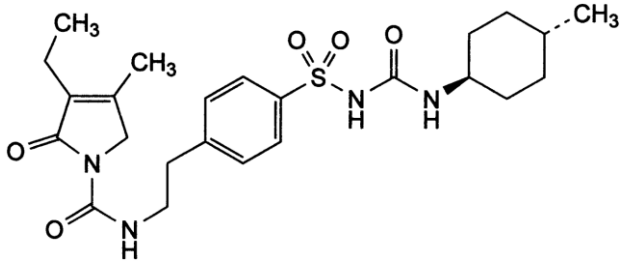

FIGURE 1: CHEMICAL STRUCTURE OF GLIMEPIRIDE

MATERIALS AND METHODS:

Reagents and Chemicals: All chemicals and reagents used were HPLC grade. Glimepiride standard was obtained from Sigma Aldrich. Tablet formulation containing 1, 2, 3 and 4 mg Glimepiride was obtained commercially. HPLC grade Acetonitrile was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation and Chromatographic Conditions: A Shimadzu HPLC system was utilized, consisting of the following components: quaternary pump LC – 20 AD, vacuum degasser unit DGU – 20 A_5 and a UV/VIS variable detector SPD – 20 A. Separation was carried out on a LiChrosorb C 18 column (125 x 4 mm, particle size 5µm) under reversed phase partition chromatographic conditions. The mobile phase consisted of an aqueous solution containing acetonitrile: water: glacial acetic acid in ratio (550:450:0.6). The mobile phase was filtered through 0.45 µm membrane filter and degassed by using sonicator for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 1 mL/min. The column was maintained at 25ºC temperature. The injection volume was a 20 µL and the total run time was 7 minutes. The detection was carried out at 230 nm.

Preparation of the Standard Solution: About 30 mg of Glimepiride were accurately weighed and transferred into 200 mL volumetric flask and dissolved in mobile phase. The final drug concentration of 30 µg/mL was obtained by dissolving the appropriate amount from this standard stock solution in the above said mixture. Calibration standards of Glimepiride were prepared by making serial dilutions of the stock solution at concentrations of 15.0, 30.0, 60.0, 90.0, 120.0 µg/mL.

Sample Preparation: Twenty tablets were accurately weighed (to obtain the average mass of one tablet) then finely powdered. Weight equivalent to 3 mg of Glimepiride (one tablet) was weighed, transferred into a 100 mL volumetric flask and dissolved with about 50 mL mobile phase. The contents were sonicated for 10 minutes. The mixture was made up to 100 mL with the same.

The solution was filtered through a membrane syringe filter (pore size 0.45 µm). The sample solution was injected and the peak area was measured for determination of Glimepiride in a tablet formulation.

RESULTS AND DISCUSSION: After equilibration of column with the mobile phase indicated by a stable baseline, aliquots of sample (20 µL) were injected. The typical chromatogram is shown in **Figure 2**. The amount of Glimepiride present in the tablets was calculated using single point analysis method and results are shown in **Table 1**.

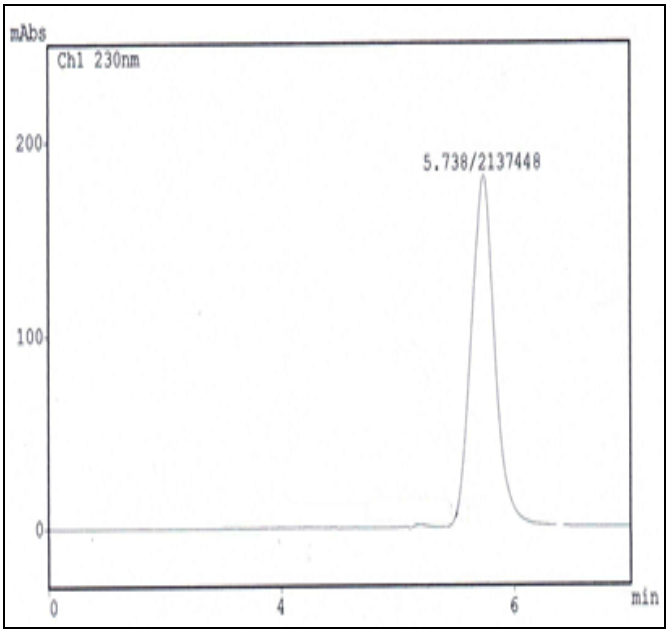

FIGURE 2: CHROMATOGRAM OF GLIMEPIRIDE

TABLE 1: RESULTS OF ASSAY OF GLIMEPIRIDE TABLETS

Drug	Label claim (mg/1 tabl)	Mean amount found (mg/1 tabl) (n=6)	Mean % Assay RSD
Glimepiride	1	0.980	99.41±1.799
	2	2.01	100.5±1.141
	3	2.98	99.67±0.521
	4	3.99	99.89±0.539

The method was developed and validated by using the ICH guideline [16]. The selectivity, limits of detection and quantification, linearity, precision, and accuracy were determined. Determination was carried out using a tablet formulation 3 mg. The presented RP-HPLC method has been proved to be rapid and was successfully used for determination of Glimepiride.

Linearity: The linearity for Glimepiride was determined by plotting a calibration graph of the ratio of drug's peak area to concentration. The linearity of this method was found to be in the concentration range 15 - 120 µg/mL for Glimepiride. Y=6.9687E7x +50926.5 which is linear regression equation with correlation coefficients of 0.999 was determined from linearity curve (**Table 2**).

Limit of detection and limit of quantification: In order to estimate the limit of detection and limit of quantification, mobile phase was injected six times, and the noise level was determined. The limit of detection was calculated to be three times the noise value and ten times the noise, which gave limit of quantification, and was also cross-checked, by formulas given below (Table 2).

$LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$

Where σ is the standard deviation of the lowest standard concentration and *S* is the slope of the standard curve.

TABLE: 2. LINEARITY RESULTS, LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Compound	r^2	Calibration curve equation	LOQ ng	LOD ng
Glimepiride	0.999	Y=6.9687E7x +50926.5	10	4

Accuracy/Recovery: Accuracy of the developed method was confirmed by performing a recovery study as per ICH norms at three different concentration levels (50%, 100%, 150%) by replicate analysis (n = 3). The results obtained (**Table 3**) indicate that recovery is good, not less than 98% and percentage relative standard deviation is less than 2%.

TABLE 3: RECOVERY STUDIES OF GLIMEPIRIDE

Drug	Taken (mg/1 tabl)	Found (mg/1 tabl)	Recovery (%)
Glimepiride		1.49	99.34
	1.50	1.48	98.66
		1.50	100.0
		3.01	100.3
	3.00	2.98	99.33
		2.99	99.67
		4.48	99.55
	4.50	4.51	100.2
		4.50	100.0
Mean			**99.67**
SD			**0.519**
% RSD			**0.521**
% Error			**±0.399**

Precision: The precision of the method was determined by repeatability, intermediate precision (intra-day, inter-day) and was expressed as % relative standard deviation (% RSD). Intra-day precision was determined by performing analysis of triplicate injections of two different concentrations of Glimepiride on the same day at different time intervals and on two different days for inter-day precision. The % RSD of the study was found to be less than 2% as shown in **Table 4**.

TABLE 4: RESULTS OF PRECISION

Sample #	Concentration (μg/mL)	RSD (%) Intra-day (n=3)	RSD (%) Inter-day (n=3)
Glimepiride	30	1.0385	1.4123
	60	0.7543	1.6638
	90	0.6934	1.2371

CONCLUSION: The aim of the present research work was to achieve highest precision in quantitative estimation of Glimepiride in tablet dosage form. The method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification. The developed method has a simple procedure for the preparation of the samples and shorter run time for chromatographic analysis (less than 6 min). Hence the proposed RP-HPLC method can be considered as simple, rapid, suitable and easy to apply for routine analysis of Glimepiride in pharmaceutical dosage form.

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