METHOD DEVELOPMENT AND METHOD VALIDATION OF GLIMEPIRIDE IN FORMULATION BY HPLC IN REVERSE PHASE METHOD

Maloth Jagadeesh
Department of chemistry, Jawaharlal Nehru Technological University, Kukatpally Housing Board Colony, Kukatpally, Hyderabad, Telangana - 500085.

Geesala Lohi Akshita
RIKI TECH S(RIKI SOLUTIONS (Group of Companies) Plot no 39, 1st Floor, Lavanya Arcade, Jayabheri Enclave, Gachibowli, Hyderabad, Telangana-500032.

Bommella Mathews
Department of chemistry, Jawaharlal Nehru Technological University, Kukatpally Housing Board Colony, Kukatpally, Hyderabad, Telangana -500085.
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Kottakki, Naveen Kumar
RIKI TECH SOLUTIONS (RIKI Group of Companies) Plot no 39, 1st Floor, Lavanya Arcade, Jayabheri Enclave, Gachibowli, Hyderabad, Telangana-500032.
technical@rikiglobal.com

ABSTRACT

The study is focused on developing a modest, quick, validated high performance liquid chromatographic (HPLC) method for glimepiride tablets from their dosage forms. The detection was carried out at 250 nm using Waters HPLC PDA detector system. The accuracy and precision were determined and validated statistically. The linearity was observed in the range of 10-200 µg/ML with a correlation coefficient of 0.999. The limit of detection and the limit of quantification were found to be 10 ng and 20 ng respectively. a Cogent RP-C18 column with a mobile phase consisting of acetonitrile and 0.1 % formic acetic acid (55:45 v/v) was used. The mobile flow rate was 1.000 mL/min, The HPLC method is selective, precise, and accurate and can be used for routine analysis of preparation in pharmaceutical industry quality control laboratories

Keywords: *HPLC, Glimepiride, Tablets, Method development and validation*

INTRODUCTION

Glimepiride is currently used to treat type 2 diabetes. Biochemical/physical Actions Glimepiride is a potent blocker of cardiac K_{ATP} channels activated by pinacidil with an IC_{50} of 6.8 nM. Glimepiride reduces blood glucose levels by stimulating the pancreatic β cells to secrete insulin hormone. It interacts with a 65-kD protein associated with β cells.

FIGURE 1: Chemical Structure of Glimepiride

MATERIALS AND METHODS:

All chemicals and reagents used were HPLC grade. Glimepiride standard was obtained from Merck. 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 Waters HPLC system was utilized, consisting of the following components: quaternary pump, vacuum degasser and a PDA detector Separation was carried out on a MICROSOLV Technology Corporation make Cogent C-18 column (250 x 4.6 mm, particle size 5µm) Part Number: 68318-25P under reversed phase partition chromatographic

conditions. The mobile phase consisted of an aqueous solution containing acetonitrile: 0.1% Formic acid in ratio (55::45 v/v) [1]. The mobile phase was filtered through 0.22 µm membrane filter and degassed by using sonicator. The sample solutions were also filtered using 0.22 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 1 mL/min. The column was maintained at 35ºC temperature. The injection volume was a 20 µL and the total run time was 10 minutes. The detection was carried out at 250 nm [2].

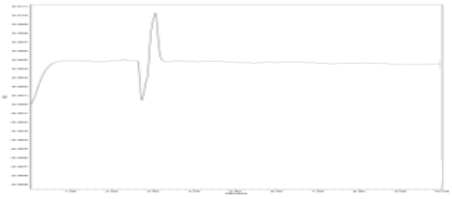
PREPARATION FO STANDARD SOLUTIONS:

About 100 mg of Glimepiride were accurately weighed and transferred into 100 mL volumetric flask and dissolved in mobile phase. The final drug concentration of 100 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 1, 2, 3, & 4 mg/mL Sample Preparation: Twenty tablets were accurately weighed (to obtain the average mass of one tablet) then finely powdered [3,4]. Weight equivalent to 10 mg of Glimepiride (one tablet) was weighed, transferred into a 10.0 mL volumetric flask, and dissolved with about 10 mL mobile phase. The contents were sonicated for 10 minutes. The mixture was made up to 100.00 mL with the same. The solution was filtered through a membrane syringe filter (pore size 0.45 µm) [5]. The sample solution was injected and the peak area was measured for determination of Glimepiride in a tablet formulation.

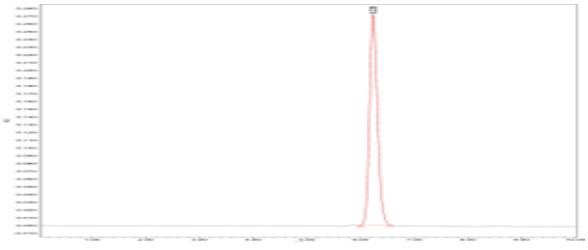
TABLE 1: Result of assay of Glimepiride tablets

Drug	Label	Mean (mg/1 Tablet) (n=6)	Mean % Assay RSD
Glimepiride	1	0.978	99.24±1.866
	2	1.989	99.06±1.854
	3	2.981	99.89±1.909
	4	3.978	99.62±1.899

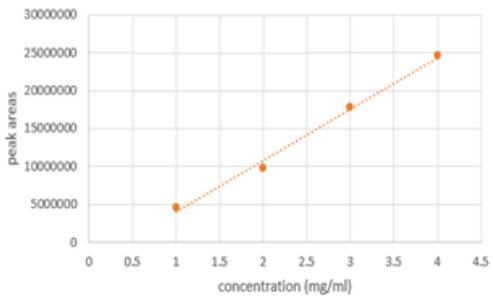
The method was developed and validated by using the ICH guideline 16. The selectivity, limits of detection and quantification, linearity, precision, and accuracy were determined. Determination was carried out using a tablet formulation 3 mg. The presented RP-HPLC method has been proved to be rapid and was successfully used for determination of Glimepiride [6].



Blank - Injection



Standard- Injection



Linearity Curve

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 1-4 mg/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, to estimate the limit of detection and limit of quantification, mobile phase was injected six times, and the noise level was determined [7,8]. 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 σ/s and LOQ 10 σ/s

Where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve [9-11].

TABLE 2: Linearity results, limits of detection (LOD) and Limit of Quantification (LOQ)

Compound	r^2	Calibration Cure Equation	LOQ ng	LOD ng
Glimepiride	0.998	Y=7.0007E7x +49996.5	20	10

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 94% and percentage relative standard deviation is less than 5-6%.

TABLE 3: Recovery studies of Glimepiride

Drug	Taken (mg/1Tablet)	Found (mg/1Tablet) (n=3)	Recovery
Glimepiride	2	1.87	94
		1.9	95
		1.88	94
	3	2.89	96
		2.85	95
		2.81	94
	4	3.78	95
		3.83	96
		3.82	96
Mean			95

SD		0.86603
%RSD		0.91161
%Error		0.288675

CONCLUSION:

The object 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 10 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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