WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 788-799. Research Article ISSN 2277– 7105

METHOD DEVELOPMENT AND VALIDATION OF GLIMEPIRIDE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

A. Chowdeswari[1], B. Sivagami*[1], R. Sireesha[1], R. Chandrasekar[2] and M. Niranjan Babu[2]

[1]Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy.

[2]Department of Pharmacognosy, Seven Hills College of Pharmacy.

Venkataramapuram, Tirupati-517561, Andhra Pradesh, India.

Article Received on
30 May 2018,

Revised on 20 June 2018,
Accepted on 10 July 2018

DOI: 10.20959/wjpr201814-12869

***Corresponding Author**
B. Sivagami
Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Venkataramapuram, Tirupati-517561, Andhra Pradesh, India.

ABSTRACT

A simple, precise, rapid, newer, specific and accurate method has been made for the estimation of Glimepiride in formulation by RP – HPLC method. Standard substance was dissolved in Methanol and this solution was scanned in the UV region from 200-400nm range. Spectra was recorded and the spectrum shows that λ_{max} of Glimepiride was 228 nm. Reverse phase chromatographic technique was selected by using C_{18} column with 150 x 4.6 mm i.d. and 5 µm particle size as a stationary phase with Acetonitrile : Disodium hydrogen o- phosphate (70 : 30, v / v, pH 4) was selected as mobile phase for the analysis. Mobile phase flow rate was maintained at 1.0 ml / min. 228 nm was selected as detection wavelength. The developed method was validated in terms of accuracy, precision, specificity, system suitability, linearity, and robustness, limit of detection and limit of quantification. With the optimized chromatographic conditions, the drug was linear in the concentration range of 20-120 µg/ ml. The correlation coefficient was found to be 0.9991. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification, robustness and stability. The proposed method can be used for the routine estimation of drugs in pharmaceutical dosage forms.

KEYWORDS: Glimepiride, Method Validation, RP-HPLC, Stability.

INTRODUCTION

Glimepiride, like glyburide and glipizide, is a "second-generation" sulfonylurea agent. Glimepiride is used with diet to lower blood glucose by increasing the secretion of insulin from pancreas and increasing the sensitivity of peripheral tissues to insulin. Chemically glimipride is known as 3-ethyl-4-methyl-2-oxo-N-(2-{4-[({[(1r,4r)-4-methylcyclohexyl]-C hydroxycarbonimidoyl}amino)sulfonyl] phenyl}ethyl)-2,5-dihydro-1H-pyrrole-1-carboximidic acid.

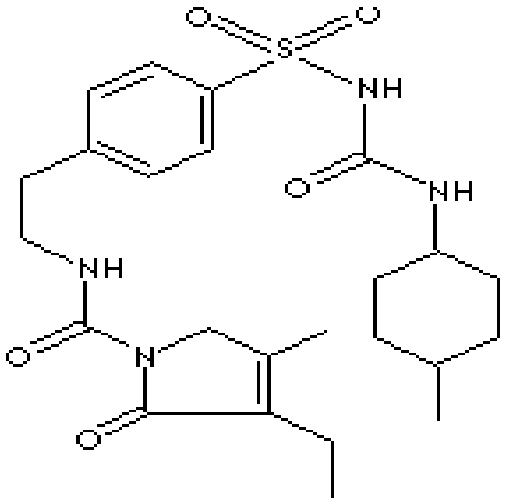

Figure No. 1: Structure of Glimepiride.

The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin.

The are many methods developed for glimepiride by UV and HPLC methods in API and combination drugs. This method seems to be economic than other methods. The main aim of this method is to develop a new method and validate Glimepiride in tablet dosage forms.[1,5]

MATERIALS AND METHODS

Apparatus and software

The liquid chromatographic system consisted of following components: Shimadzu HPLC model containing LC-20AT (VP series) dual pump, variable wavelength programmable UV

/VIS detector SPD-20A (VP series) and Hamilton syringe (705 NR, 20 µL). Chromatographic analysis was performed using Chromtech N-2000 software on a phenomenex luna C-18 column with 150 x 4.6 mm i.d. and 5 µm particle s i z e .

Chemicals and Reagents

Glimepiride working standard (99.3%), Di sodium hydrogen ortho-phosphate, ortho phosphoric acid, Glacial acetic acid, salicylic acid, methanol and acetonitrile all the chemicals were of analytical grade obtained from Sigma Aldrich Mumbai Pvt ltd.

Method Development

Preparation of mobile phase

Accurately 0.14 g of Di sodium hydrogen ortho-phosphate was weighed and dissolved in 500 ml of HPLC water. The solution was sonicated for 5 min to dissolve the buffer completely. Then the pH was adjusted to 4 using ortho phosphoric acid. This solution was filtered through Millipore vaccume filter (0.22 µm). From this 300 ml of buffer solution was mixed with 700 ml of Acetonitrile and shake well. Finally the solution was sonicated for 15 min.

Preparation of Stock solution

A stock solution of Glimepiride was prepared by accurately weighing 25 mg of drug, transferring to 25 ml volumetric flask, dissolving in 25 ml of mobile phase and sonicated for 5min. Appropriate aliquot of this solution was further diluted to 100 ml with mobile phase to obtain final standard solution of 100 µg / ml of Glimepiride and the resultant solution was filtered through Whatman filter paper.

Preparation of Standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 100 mg pure drug in 100 ml of diluents. This will become 1000 mcg / ml solution, which was taken as stock solution.

Selection of the Chromatographic method

A RP C-18 column equilibrated with mobile phase Acetonitrile: Disodium hydrogen o phosphate (70: 30, v / v, pH 4) was used. Mobile phase flow rate was maintained at 1.0 ml / min. Detection wavelength 228 nm was selected by scanning standard drug over a wide range of wavelength 200 nm to 400 nm in U.V Spectroscopy. The sample was injected through 20 µl fixed loop, and the total run time was adjusted for 15 min.

Preparation of Mobile Phase

Required weight of buffer with molarity 0.002M was taken and is dissolved in 100 ml of HPLC Grade water. Sonicate the solution for 10 min. and adjust the pH to 4 using orthophosphoric acid and filter the solution using vacuum filter and add it to reagent bottle. To this solution add acetonitrile. Mix well and sonicate the mixture for 20 min. The ratio of the mobile phase (ACN: Buffer) was kept as 70:30.

RESULTS AND DISCUSSIONS

Linearity

Appropriate aliquots of standard Glimepiride stock solutions (100 µg / ml) were taken in different 10 ml volumetric flask and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 20-120 µg / ml. These solutions were injected into chromatographic system. The chromatograms were obtained and peak area was determined for each concentration of drug solution the data are given in table 1.

Table No 1: Linearity data of Glimepiride by RP-HPLC method.

Sl. no.	Concentration (µg / ml)	Retention time (min)	Peak area (mv)
1	20	6.115	683545.500
2	40	6.117	1446235.875
3	60	6.105	242963.250
4	80	6.095	2976456.500
5	100	6.112	3653019.000

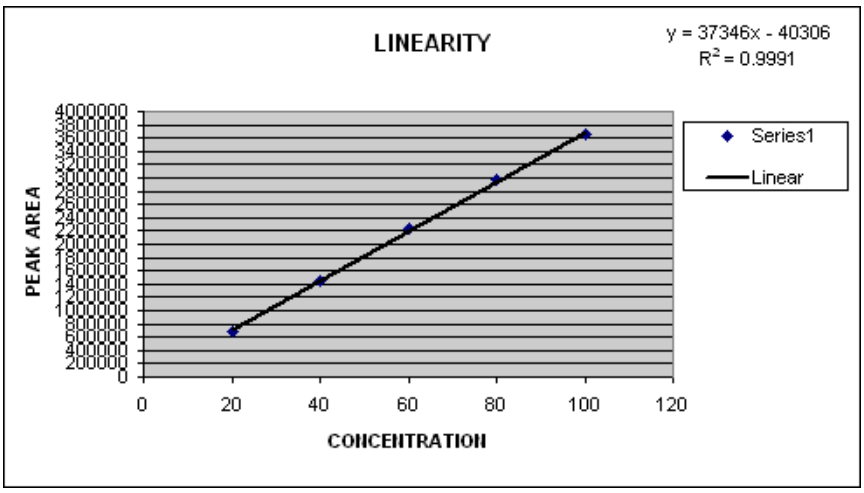

Figure 2: Linearity of Glimepiride at concentration range of 20-120 µg / ml.

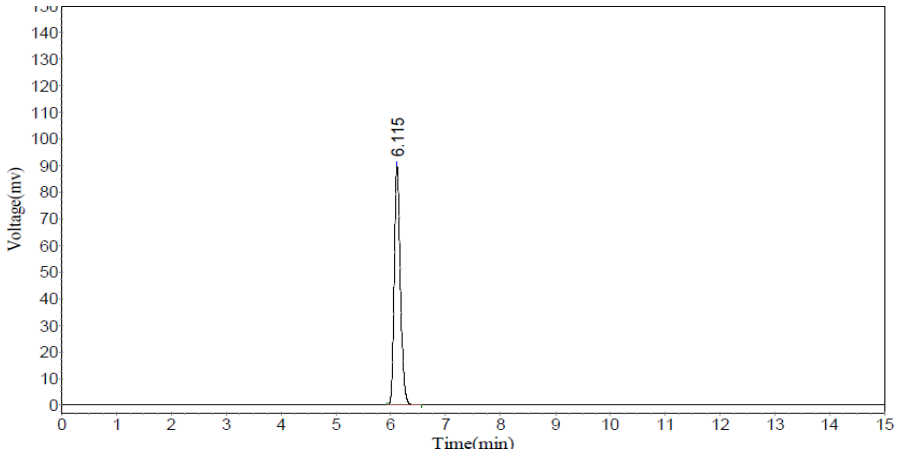

Figure 3: Linearity Graph of Glimepiride at 20µg/ml.

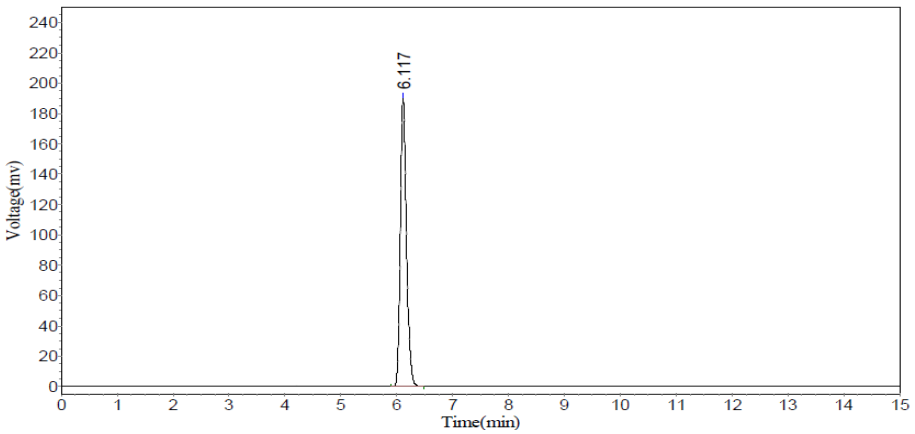

Figure 4: Linearity Graph of Glimepiride at 40µg/ml.

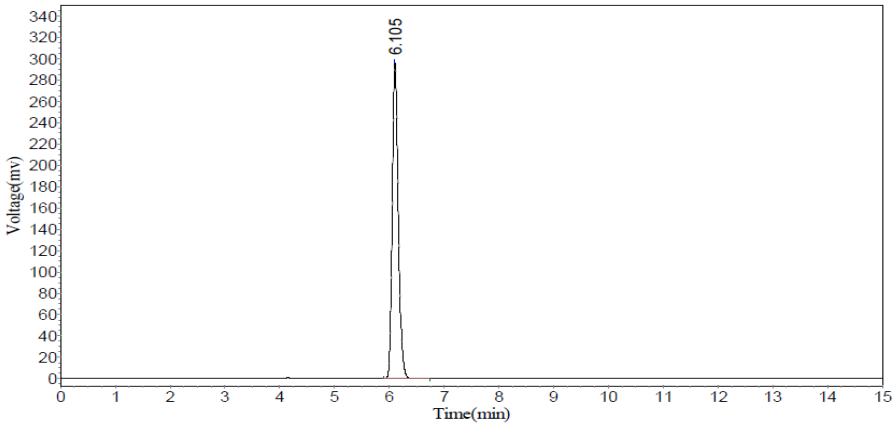

Figure 5: Linearity Graph of Glimepiride at 60µg/ml.

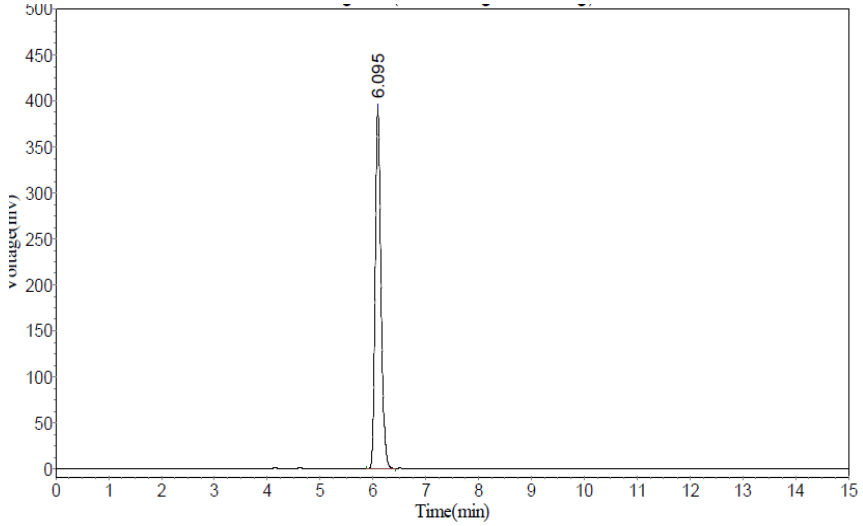

Figure 6: Linearity Graph of Glimepiride at 80µg/ml.

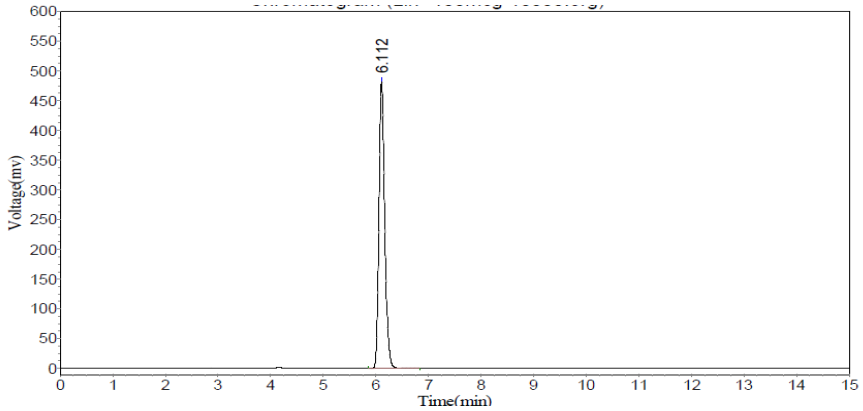

Figure 7: Linearity Graph of Glimepiride at 100µg/ml.

Precision

System Precision (Injection repeatability) was measured by using six replicates of the same band containing 1000 ng pure Glimepiride and % RSD of the replicate injections was calculated. The precision of the method was determined by spotting six replicates of the sample solution of Glimepiride such that each band containing 1000 ng of Glimepiride and % RSD of the replicate injections was calculated. Both the system precision and method precision were subjected to intra-day and inter-day variation. The results of the system precision and method precision studies are shown in the table 2.

Table No 2: Precision results for Glimepiride.

Sl. no.	Concentration (µg / ml)	Retention time (min)	Intraday precision (Area)
1	60	6.125	2263635.75
2	60	6.107	2270039.25
3	60	6.107	2269668
4	60	6.1	2255107.75
5	60	6.098	2248836.25
Mean		6.1074	2261457.4
Std. Dev		0.010644247	9292.722865
%RSD.		0.174	0.41

Accuracy

The accuracy of the method was determined by use of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution of Glimepiride was prepared, 80%, 100% and120% of the standard drug solution was added to the solution, and the recovery [%] was determined. Values were found to be within the limits. The results are shown in Table 3.

Table No 3: Accuracy results for Glimepiride.

Brand used	Label claim (mg/ml)	% accuracy	Amount recovered (µg/ml)	Recovery ± SD* (%)
Amaryl	4	80	7.95	99.4
		100	10.06	100.6
		120	11.97	99.77

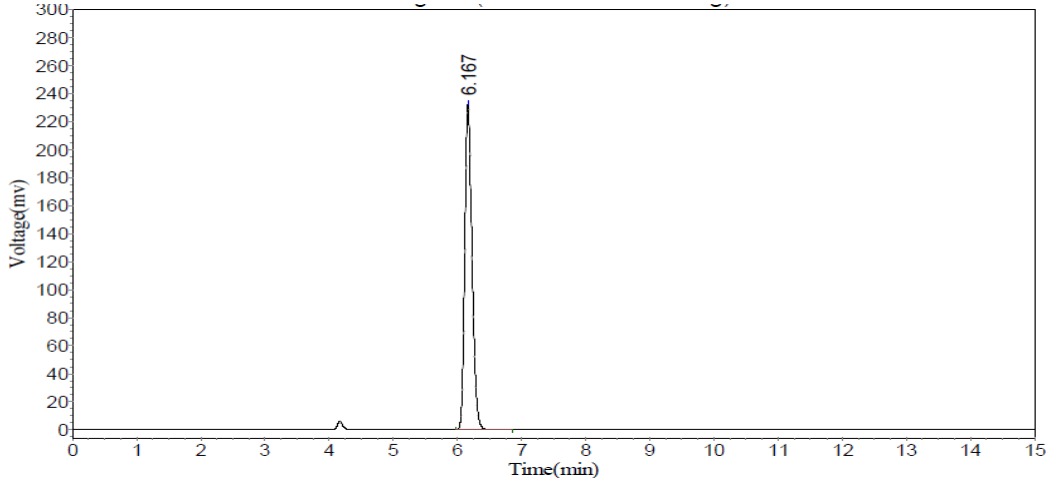

Figure 8: Accuracy Graph of Glimepiride at 80%.

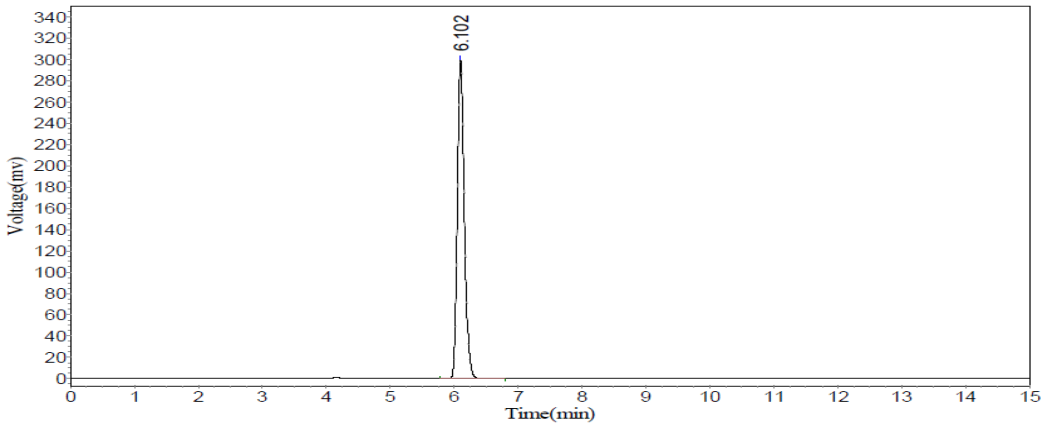

Figure 9: Accuracy Graph of Glimepiride at 100%.

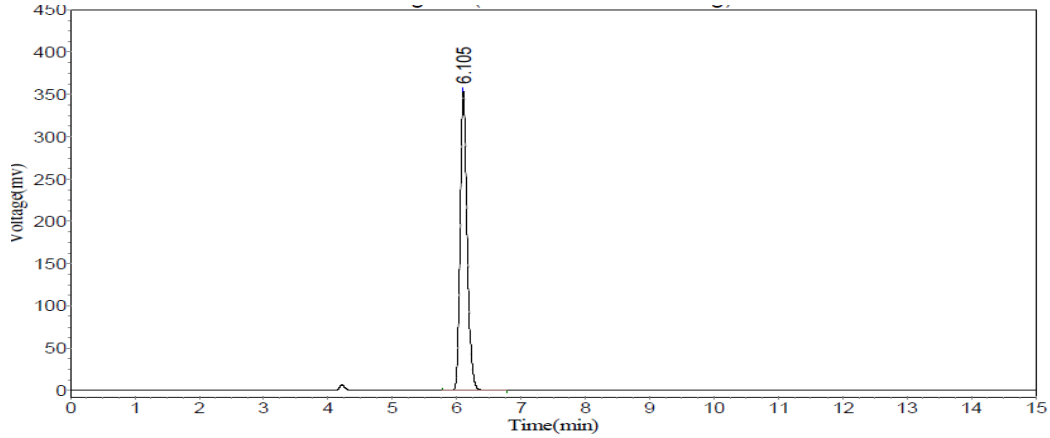

Figure 10: Accuracy Graph of Glimepiride at 120%.

Robustness

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of mobile phase ratio (68:32 v / v). The variation in mobile phase pH by ± 0.2 units (pH 4.2). The results are given in table 4.

Table No 4: Robustness studies of Glimepiride.

Sl No	Parameters	Std. Area	Area	% RSD
	Change in Flow Rate			
1	0.9ml	2255108	2257357	0.07051
2	1.1ml	2255108	2252833	0.07135
	Change In pH			
1	3.8	2255108	2249356	0.18057
2	4.2	2255108	2263209	0.25356

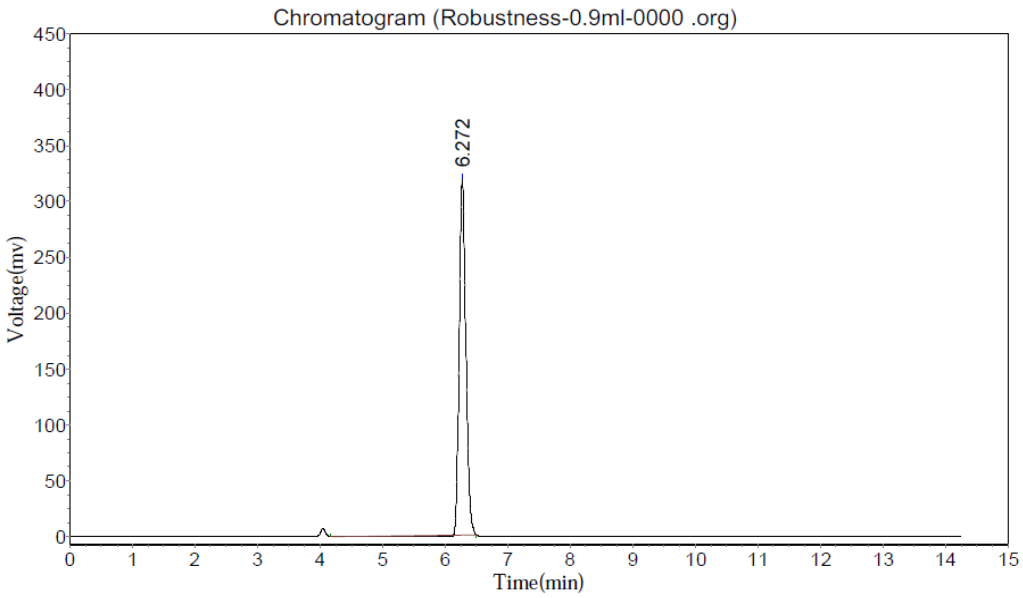

Figure 11: Robustness graph of Glimepiride at 0.9 ml flow rate.

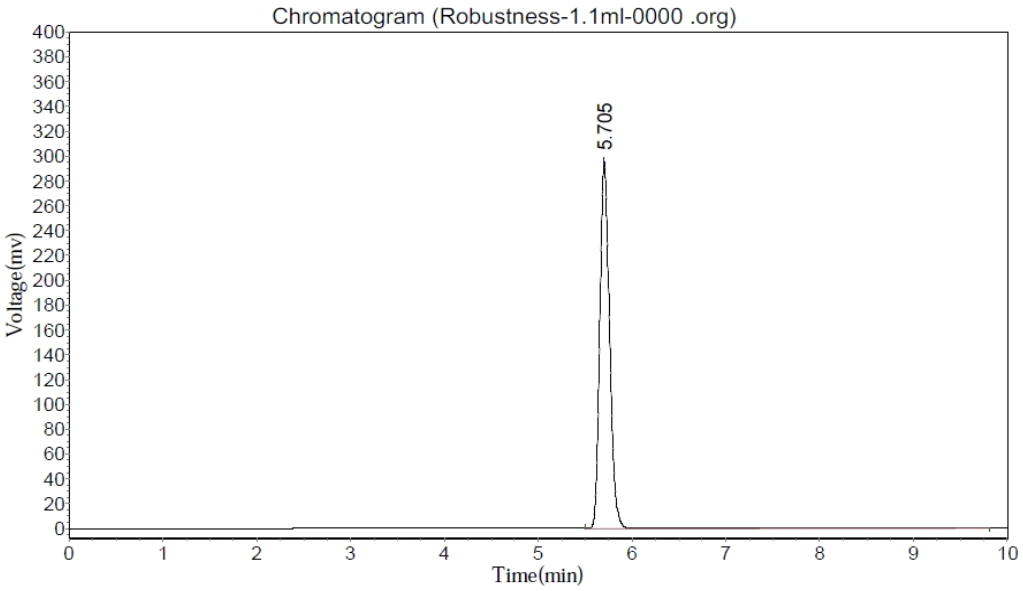

Figure 12: Robustness graph of Glimepiride at 1.1ml flow rate.

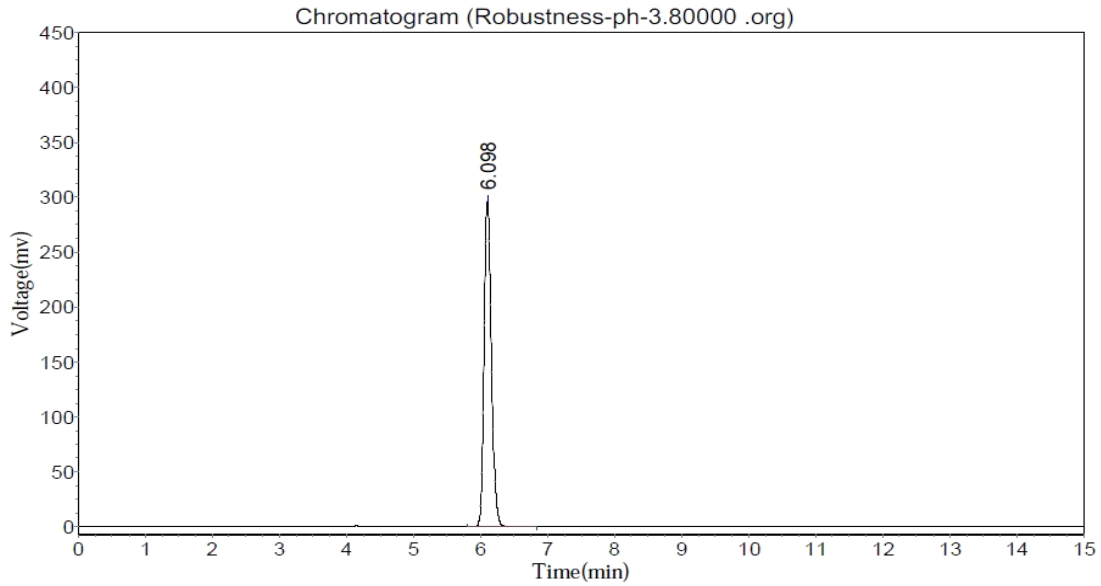

Figure 13: Robustness graph of Glimepiride at 3.8 pH.

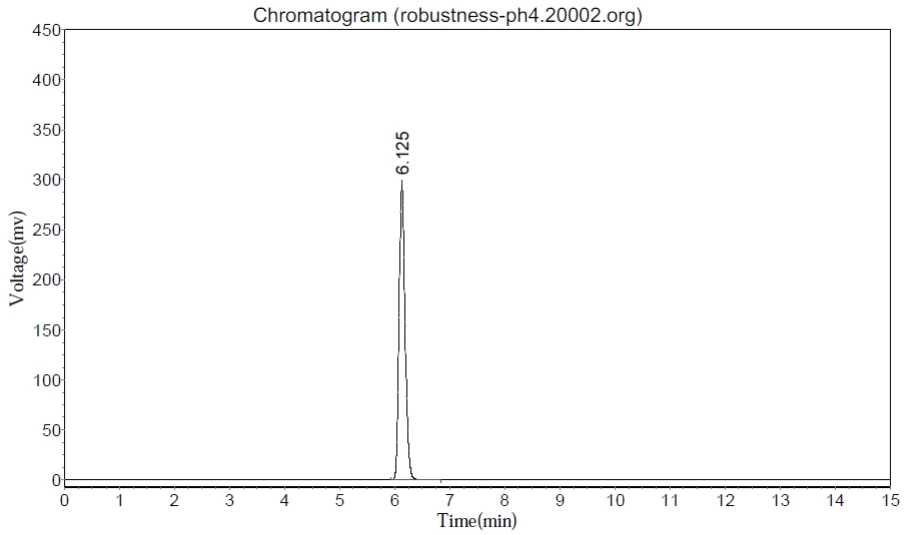

Figure 14: Robustness graph of Glimepiride at 4.2 pH.

Limit of Detection

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The Limit of detection was found to be 500ng for Glimepiride the results of LOD were shown in Table 5.

Table 5: LOD of Glimepiride.

Sl. No	Y	X
1	37346	40306
2	36990	27857
3	37289	38735
Mean	37208.33	35632.67
Standard deviation	191.218	6779.584

Intercept LOD= 3.3X Standard deviation/Average of slope

LOD = 3.3 X 6779.584/37208.3

=0.60 ng/ml.

Limit of Quantitation

Limit of quantitation is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably Quantitate. The LOQ can also be calculate based on the LOD strength (500 ng / ml, standard solution), the LOD values were multiplied by three times to get LOQ.

Table 6: LOQ of Glimepiride.

Sl. No	X	Y
1	37346	40306
2	36990	27857
3	37289	38735
Mean	37208.33	35632.67
Standard deviation	191.218	6779.584

Intercept LOQ = 10 X Std. Dev. of intercept/ Avg. of slope

LOQ = 10 X 6779.584/ 37208.33

= 1.822 ng/ml

Degradation Studies

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali, and oxidative, photolytic, thermal and neutral analysis.

Table 7: Stress Degradation results of Glimepiride.

Sl.No	Stressed area	Area	% degradation
1	Acid hydrolysis	2936539	61.60
2	Alkali hydrolysis	2098402.5	72.56
3	Oxidative stress	559065.5	92.68
4	Water stress	487979	93.61
5	Standard	7647435	-

CONCLUSION

In the present study, an attempt was made to provide a newer, simple, sensitive, precise, accurate stability and low cost HPLC method for the effective quantitative determination of Glimepiride as an active pharmaceutical ingredient as well as in pharmaceutical preparations without the interferences of other constituent in the formulations. The method is rugged and robust as observed from insignificant variation in the results of analysis on changes in mobile phase composition ratio and analysis being performed by different analysts and on different days respectively. In all the above cases the recovery is found to be within the limit of 99%. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision and accuracy, suitable for chemist-to-chemist and day-to-day for routine analysis as well as for stability analysis.

REFERENCES

1. Vinay Pandit, Roopa S. Pai, Kshama Devi, Gurinder Singh, Satya Narayana, and Sarasija Suresh Development and validation of the liquid chromatographic method for simultaneous estimation of metformin, pioglitazone, and glimepiride in pharmaceutical dosage forms Pharm Methods., 2012; 3(1): 9–13.
2. K. Neelima, Y. Rajendra Prasad Analytical Method Development and Validation of Metformin, Voglibose, Glimepiride in Bulk and Combined Tablet DosageForm by Gradient RP-HPLC Pharmaceutical Methods, 2014; 5(1): 27-33.
3. Magda Mohamed Ibrahim. Development and Validation of a RP-HPLC Method For The Simultaneous Determination of Carvedilol, Glimepiride or Glibenclamide In Binary Combinations; And Its Application For In Vitro - Interaction Studies. Indo American Journal of Pharm Research, 2015; 5(08): 2791-2802.
4. Gadapa Nirupa, and Upendra M. Tripathi RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms Journal of Chemistry, 2013; 1-8.
5. M. Suchritra, D. Sunitha, C. Parthiban, B. Siddartha, C. Madhavi, Methid Development and Validation of Metformin, Glimipride and Pioglitazone in Tablet Dosage form by RP-HPLC method International Research Journal of Pharmacy, 2013; 4(8): 250-254.