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ORIGINAL ARTICLE **OPEN ACCESS**

Optimization and Validation of RP-HPLC Stability Indicating Method for Determination of Gliclazide in Pure and Bulk Drug

S.Pulla Reddy[1], J. Sai Chandra[2], K. S. Yoganand[3], G. Kanthimathi[4] and K.V.L.N. Murthy[5]
[1]Dept. of Chemistry, Anurag Engg.College, Kodad, Telangana, INDIA- 508206.
[2]Dept. of Chemistry, JNTUH College of Engg. Sultanpur, Sangareddy Dist. Telangana, INDIA-502273.
[3]Dept. of Chemistry,SRM Institute of science and technology, Ramapuram campus, Chennai, INDIA-600089.
[4]Dept. of Chemistry,Ramco Institute of Technology, Rajapalayam, Tamilnadu, INDIA-626117.
[5]Dept. of Chemistry, SVR Degree College Macherla, Andhra Pradesh, INDIA– 522426.

ABSTRACT
A simple, accurate and economical reverse phase high performance liquid chromatography (RP-HPLC) was developed for the estimation of gliclazide (GLZ) in pure and dosage form the method was carried out on hypersil BDS C-18 column (250mm x4.6mm, particle size) column with mobile phase composition of Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) at flow rate 1.0 mL/min. With this mobile phase gliclazide eluted at a retention time of ~ 3.65 minutes (Fig.1.12). with an injection volume of 20 and UV detection at 235nm. The developed method was validated according to ICH guidelines.
Key Words: *gliclazide, RP-HPLC, accuracy, tablets.*

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INTRODUCTION

Gliclazide (1-(3-azabicyclo [3.3.0] oct- 3- yl) - 3- ptolylsulfonylurea or 1-(hexahydrocyclo -penta[c]pyrrol-2(1H)-yl)-3-(p-tolylsulfonyl) urea is an oral hypoglycemic agent used in the treatment of type-II diabetes mellitus (Fig.1.01) [1-5]. It belongs to the sulfonylurea class which act by stimulating β cells of the pancreas to release insulin. It reduces blood glucose levels by correcting both defective insulin secretion and peripheral insulin resistance, increasing the sensitivity of ßcells to glucose, decreasing hepatic glucose production, and increasing glucose clearance. It also has anti-platelet adhesive activity and reduces levels of free radicals, thereby preventing vascular complications. It also has been reported to reduce plasma cholesterol and triglyceride levels after repeated administration.

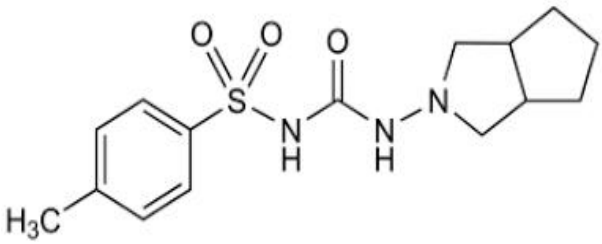

Fig.1.01.MOLECULAR STRUCTURE OF GLICLAZIDE

It has a molecular weight 323.4, and exists as a white or almost white crystalline powder, odorless, tasteless, M.P. 165-170^{0}C. It is official in British Pharmacopoeia 2007. It is marketed in the brand name of Azukon (label claim 80mg) manufactured by torrent pharmaceutical Ltd used for the treatment of type-II diabetes mellitus.

A survey of literature has revealed few analytical methods [6-20] were reported for the estimation of gliclazide in human serum and in pharmaceutical formulation. In view of its therapeutic importance the author developed and validated few analytical methods using HPLC technique.

A detailed literature survey revealed few RP-HPLC methods for the determination of assay of gliclazide in bulk and in dosage forms [9-20]. In the view of the above importance of stability testing, the author therefore, "developed and validated a stability-indicating RP-HPLC assay method for gliclazide in pure

and in pharmaceutical dosage form as per ICH guidelines**"**. The author's investigations and the experimental work carried were incorporated in this present chapter.

MATERIAL AND METHODS

Chemicals and solvents:

Gliclazide standard (99.9%pure) was obtained as gifted sample from Torrent Pharmaceuticals Ltd, Hyderabad. Tablets of gliclazide [AZUCON;80mg**]** were purchased from local pharmacy. HPLC grade Acetonitrile (Qualigens), orthophosphoric acid of AR grade were obtained from Sd. Fine Chemicals Ltd, and HPLC grade water was obtained from a Milli-QRO water purification system.

Instrumental apparatus:

The HPLC analysis of gliclazide was carried out on HPLC system with Waters 2695 alliance equipped with binary HPLC pump, Thermo Hypersil BDS C-18 column (250mmx4.6mm, particle size) and Waters 2998 PDA detector. The output signal was monitored and processed using the built-in Empower 2 software. Electronic analytical balance (DONA) and Micro pipette (In labs, 10-100µl)were employed in the present analysis. All the glassware employed in the present analysis were cleaned with hot water and dried in hot air oven whenever required.

Mobile phase preparation: Acetonitrile, water and phosphoric acid inthe ratio of 80:15:5 (v/v/v) were employed as a mobile phase in the present assay. Before use, this mobile phase was degassed and filtered through 0.45µ membrane filter.

Diluent preparation:

Mobile phase is used initially as diluents for extracting the drug and consequent dilutions were made with mobile phase.

Preparation of standard solution:

Accurately weighed about50.0mg of gliclazide and transferred into a 100mL volumetric flask then, add 10mL of methanol and sonicated to dissolve. Cool the solution to room temperature and diluted to mark with methanol [stock solution]. Daily working standard solutions of gliclazide were prepared with mobile phase containing gliclazide at a concentration of 5.0-15.0µg/mL and each of these dilutions (20µl) was injected six times in to the column, with flow rate of 1.0 mL/min and peak area of each of the drug concentrations, retention times were recorded.

Analysis of marketed sample (dosage forms):

Ten tablets of market formulations [AZUKON;Label claim -80mg of gliclazide] were purchased from local pharmacy and their average weight was determined. An accurately weighed portion of the powder, equivalent to about 50mg of gliclazide was transferred to a 100mL volumetric flask followed by the addition of 10mL of methanol. The solution was sonicated at controlled temperature for 30min and diluted to volume with methanol and mixed thoroughly. Filter the solution through 0.45µm membrane filter. Further, different concentrations that obey within the linearity limits was prepared by transferring of different aliquots of this solution into a series of 10mL volumetric flask and diluting the mark with the same mobile phase. These prepared dilutions were injected six times into the column to obtain the respective chromatograms. From that peak area of the chromatograms, the content of gliclazide in the capsules was quantified.

Method development

In the present study the development of a new stability indicating RP-HPLC method for gliclazide involved the optimization studies of various chromatographic conditions (i.e, using different column, different buffer and different mode of HPLC run).Initially the method development was started with the use of two different columns C_8 and C_{18}. Of the two columns [C_8 and C_{18}], Thermo Hypersil BDS C-18 column (250mmx4.6mm, particle size) gave satisfactory resolution at 3.65mins run time. Secondly a study on selection of mobile phase and flow rate basing on peak parameters (height, area, tailing, theoretical plates, capacity factor and resolution) was extensively carried in the development of the proposed method.

The mobile phase composition of Acetonitrile and water in the ratio of 80:15(v/v) at void volume eluted gliclazide with long retention time. The best results were obtained when the mobile phase composition of Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) at a flow rate 1.0mL/min. With this mobile phase gliclazide eluted at a retention time of ~ 3.65 minutes (Fig.1.12). Finally, a flow rate of 1.0 mL/min with an injection volume of 20µL and UV detection at 235nm was found to be best for analysis of gliclazide. The chromatogram of gliclazide standard using the proposed method is shown in (Fig,1.12). System suitability results of the proposed methods are presented in Table,1.06.

HPLC Conditions:

The injection volume of sample was 20µL. An isocratic mobile phase containing Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) was carried out with the flow rate of 1.0mL/min at

ambient column temperature. Before the analysis, the mobile phase was degassed and filtered through a 0.45µm membrane filter. The photodiode array UV-detector was set to a wavelength of 235nm for the detection and chromatographic runtime was 7minutes. The entire HPLC system was equilibrated before making each injection. The work was carried out in an air-conditioned room maintained at temperature 25±2^{o}C.

Procedure

Prior to the assay the column was equilibrated with the mobile phase for about 30 min at a flow rate of 1.0 mL/min. With the above optimized chromatographic conditions, a steady base line was recorded. 20µL of daily working standard and formulation sample solutions of gliclazide were prepared with mobile phase containing gliclazide at a concentration of 5.0-15.0µg/mL were injected separately(six times) into the column the HPLC system at a flow rate of 1.0 mL/min and peak area of each of the drug concentrations, retention times were recorded.

RESULTS AND DISCUSSION

Method validation: The developed RPHPLC method extensively validated for assay of gliclazide using the following parameters.

a) **Specificity: Blank and Placebo Interference:** A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution (Figure not given) showed no peaks at the retention time of gliclazide peak revealing that the diluent solution used in sample preparation do not interfere in the assay of gliclazide in dosage forms. Similarly, chromatogram of placebo solution (Figure not given) showed no peaks at the retention time of gliclazide peak indicating that the placebo used in sample preparation do not interfere in the assay of gliclazide in dosage forms.

b) **Forced Degradation: Control Sample:** Weighed and finely powdered not fewer than 20 tablets. Accurately weigh and transfer powder equivalent to 50mg of gliclazide into a 100mL volumetric flask, containing 70mL of methanol, sonicated for 30minutes with intermittent shaking at controlled temperature and finally diluted to the mark with methanol and mixed. Filtered the solution through 0.45µm membrane filter. Transfer 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with the same diluent.

Acid Degradation Sample: Accurately weigh and transfer powder equivalent to 50mg of gliclazide into a 100mL volumetric flask, containing 70mL of methanol, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 10mL of 5N acid to the same flask and refluxed for 30min at 60°C, then cooled to room temperature, and neutralized with 5N NaOH and finally diluted to volume with methanol and mixed. Filtered the solution through 0.45µm membrane filter. Transferred 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with diluent (Fig.1.13a).

Base Degradation Sample: Accurately weigh and transfer powder equivalent to 50mg of gliclazide into a 100mL volumetric flask, containing 70mL of methanol, and sonicated for 30minutes with intermittent shaking at controlled temperature. Then added 10mL of 5N base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralized with 5N Acid (HCl) and diluted to volume with methanol and mixed. Filtered the solution through 0.45 µm membrane filter. Transferred 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with diluent (Fig.1.13b).

Peroxide Degradation Sample: Accurately weighed and transferred equivalent to 50mg of gliclazide into a 100mL volumetric flask, added about 70mL of methanol and sonicated for 30minutes with intermittent shaking at controlled temperature. Then added 2.0mL of 30% peroxide, refluxed for 30min at 60°C, then cooled to room temperature and diluted to volume with methanol and mixed. Filtered the solution through 0.45µm membrane filter. Transferred 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with diluents (Fig.1.13c).It was observed from these studies that there was no marked degradation in the obtained chromatograms revealing the good stability indicating of the proposed method.

c) Linearity & detector response (LOD &LOQ):

Linearity was evaluated by analysis of working standard solution of gliclazide at seven different concentrations. The range of linearity was from 5.0-15µg.mL^{-1}. The peak areas obtained {Fig.1.14.a-e &Table.1.05} with respect to their concentration of gliclazide was subjected to regression analysis to calculate the calibration equations and correlation coefficient. The calibration plot and the regression data result (Slope, intercept and correlation coefficient [r^2]) so obtained for gliclazide are represented in Fig.1.14f&Table.1.07. These results showed that within the concentration range mentioned above,

there was an excellent correlation between peak areas and concentration of gliclazide respectively. The LOD and LOQ of gliclazide were experimentally determined by six replicate injections. The LOD and LOQ values of gliclazide were found to be 0.095µg/mL and 0.317µg/mL respectively.

d) **Precision:** The precision of the method was demonstrated by inter day and intraday variation studies. In present study the intraday studies were made, six repeated injections of standard and sample solutions and the response factor of drug peaks (Fig.1.15.a-f) and percentage RSD were calculated. The results of the above precision studies of gliclazide are summarized in Table.1.09 indicted that developed RP-HPLC method is precise.

e) **Accuracy:** The accuracy of the method was determined by analyzing a known quantity of drug substance corresponding to 50%, 100% and 150% of the working concentration of gliclazide was added. Each set of addition were repeated three times The accuracy was expressed as the percentage of analyte recovered by the assay. The Validative chromatograms obtained at each accuracy level were represented in Fig.1.16(a-c) respectively. Table.4.09 listed the recoveries of the gliclazide from a series of spiked concentrations and the percentage recoveries were found in the range of 99.66 to 99.94%. These results indicated that the proposed RP-HPLC method is highly accurate for the assay of gliclazide in dosage forms.

f) **Robustness:** Robustness of the method was determined by making slight changes in the chromatographic conditions (change in flow rate and column temperature). No marked changes in the chromatograms demonstrated that the HPLC method developed is robust (Table.1.11).

g) **Ruggedness:** The ruggedness of the proposed RP-HPLC method was evaluated by a different analyst and different analyst and different instrument in the same laboratory. The % RSD for peak areas of gliclazide was calculated and the experimental data are shown in Table.1.10. These results revealed that the %RSD was within the limits indicating that the developed RP-HPLC method was found to be rugged.

h) **Analysis of Marketed Formulation:** Analysis of marketed formulations (AZUCON-80mg) of gliclazide was carried out by using the proposed method under the above described optimized HPLC conditions. The % drug content of tablets obtained by the proposed method for gliclazide was found to be 99.95%, respectively. The results are given in Table.1.12.

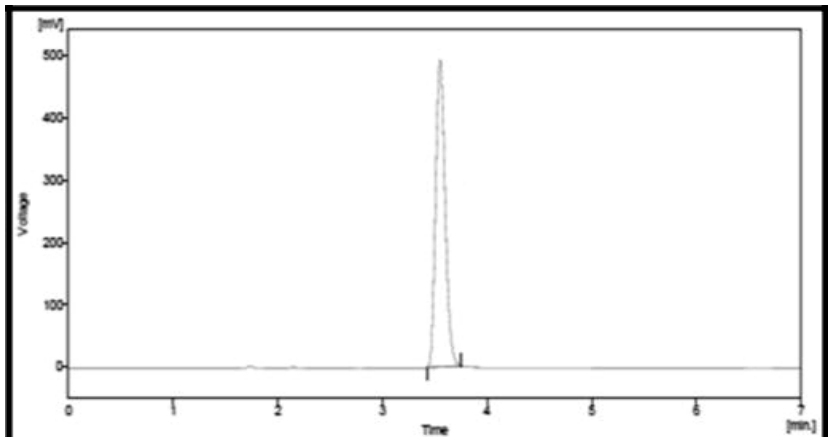

Fig.1.12: Validative Chromatogram of Gliclazide

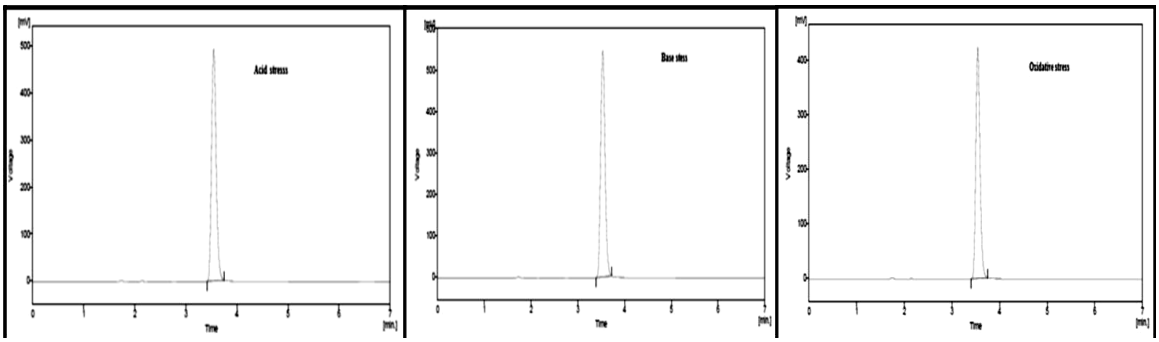

Fig.1.13(a),1.13(b) and 1.13(c): Validative Chromatogram of Gliclazide in Acid Stress, Base Stress and Oxidative Stress

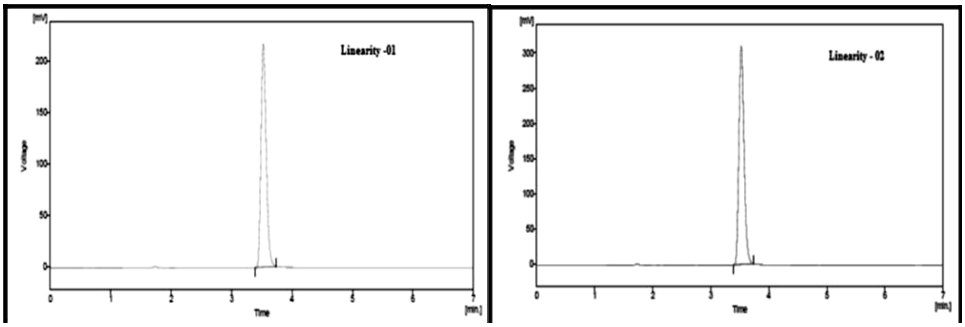

Fig.1.14(a) ad (b):):Linearity Chromatogram of Gliclazide AT 5.0µg/mL and 7.5µg/mL

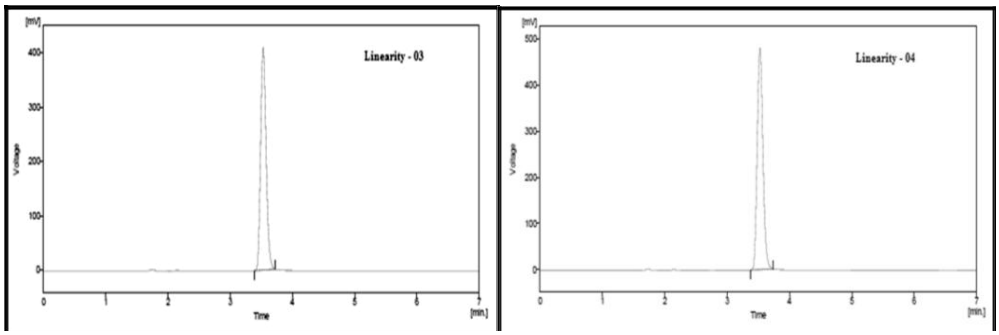

Fig.1.14(c) and (d)Linearity Chromatogram of Gliclazide AT 10.0µg/mLand 12.5µg/mL

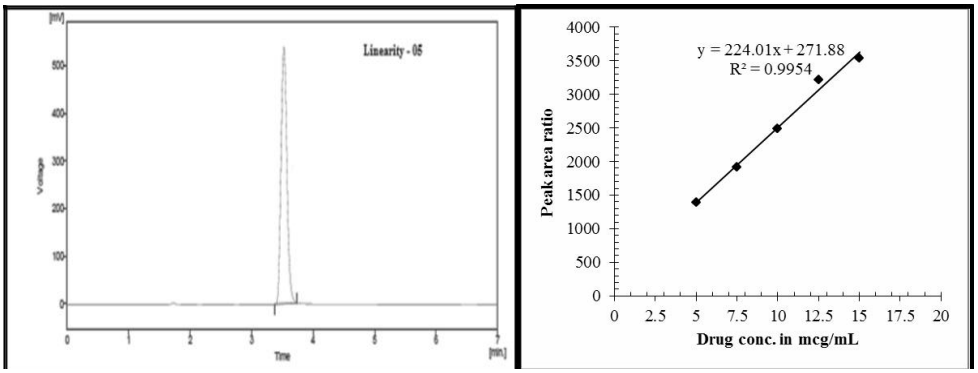

Fig.1.14(e): Linearity Chromatogram of Gliclazide at 15.0µg/mL
Fig.1.14(f): Linearity Curve of Gliclazide

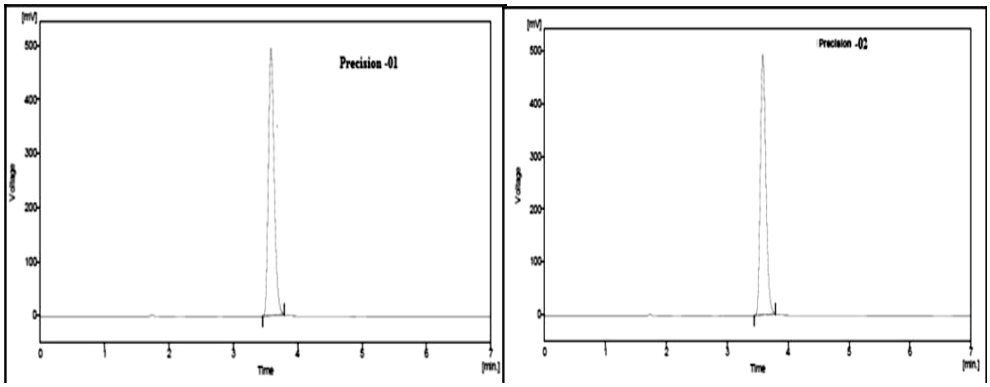

Fig.1.15(a) and (b): Precision Chromatogram of Gliclazide (SET-1) and (SET-2)

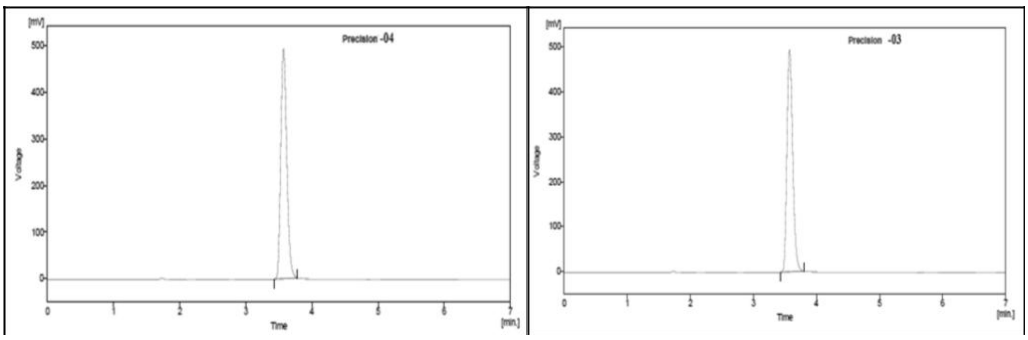

Fig.1.15(c) and (d)Precision Chromatogram of Gliclazide (SET-3) and SET-4)

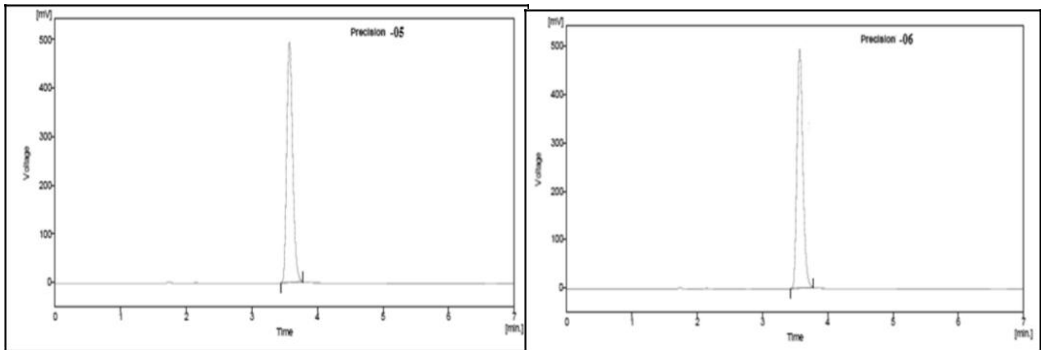

Fig.1.15(e) and (f): Precision Chromatogram of Gliclazide (SET-5) and (SET-6)

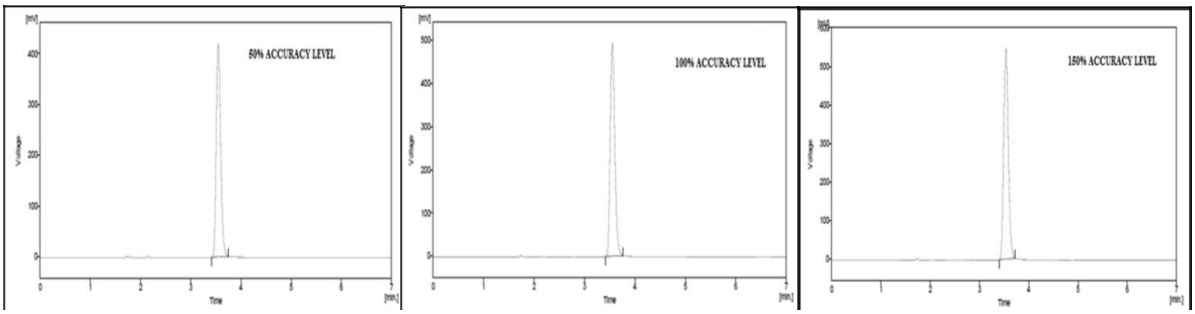

Fig.1.16(a), (b) and (c): HPLC chromatogram of gliclazide at 50%, 100 % and 150% Accuracy Level

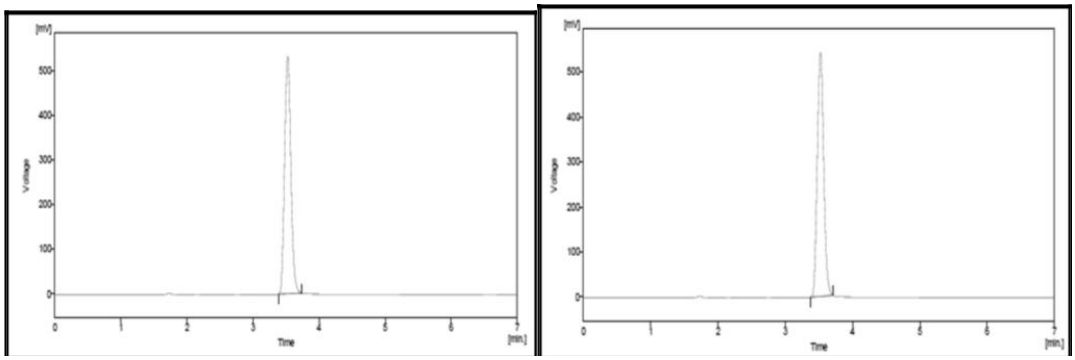

Fig.1.17(a) and (b)Validative Chromatogram of Gliclazide at Flow Rate (0.8mL/min) and (1.2mL/min) Gliclazide

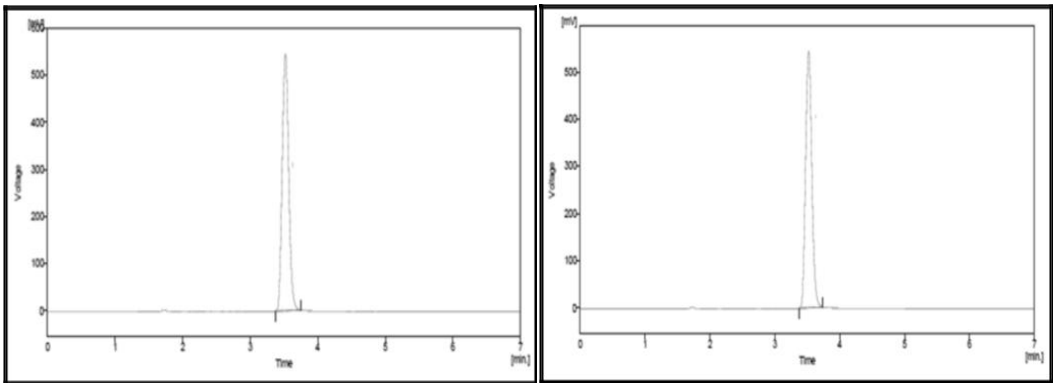

Fig.1.18(a) and (b)Validative chromatogram of gliclazide at column Temperature at 40°C and 45°CGliclazide

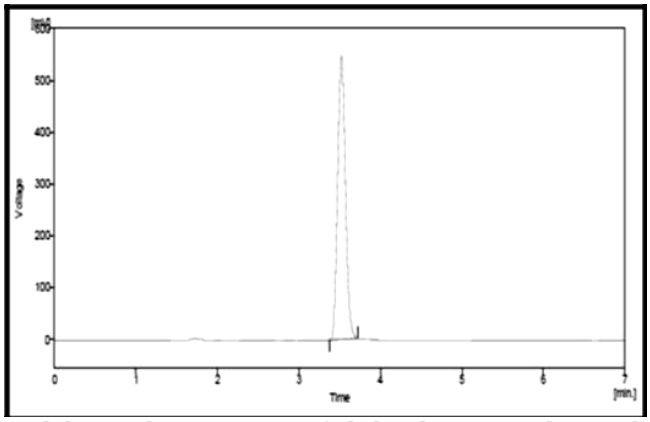

Fig.1.19: Validative Chromatogram of Gliclazide in Formulation Gliclazide

TABLE.1.06:System Suitability Parameters

PARAMETERS	GLICLAZIDE
Retention time	3.65
USP Plate count	7235
USP Tailing	1.266
Linearity Range (µg/mL)	5.0-15.0
Limit Of Detection (LOD) (µg/mL)	0.095
Limit Of Quantitation (LOQ) (µg/mL)	0.317

TABLE.1.07:Calibration Of The RP-HPLC for the Estimation of Gliclazide

Concentration (µg.mL)	Area (mAU)
5.0	1388.875
7.5	1922.393
10.0	2492.523
12.5	3212.154
15.0	3544.178
Regression equation; Intercept (a)	271.878
Slope (b)	224.015
Correlation coefficient	0.9954
Standard deviation on intercept (Sa)	7.12
Standard deviation on slope (Sb)	12.355
LOD	0.095
LOQ	0.317

TABLE.1.08: Results of Method Precision

S No	Name	Area
1	Injection-1	3212.154
2	Injection-2	3234.221
3	Injection-3	3256.432
4	Injection-4	3241.274
5	Injection-5	3322.723
6	Injection-6	3196.354
Avg*		3243.86
Std Dev*		44.13415
% RSD*		1.36

TABLE.1.09: Recovery Studies of The Proposed RP-HPLC Method

Level	Gliclazide in tablet (µg/mL)	Pure drug Added(µg/mL)	Drug found* (µg/mL)	% Recovery
50%	10	2	11.96	99.66
100%	10	4	13.98	99.85
150%	10	8	17.99	99.94

*All the values are the averages of three determinations

TABLE.1.10: Evaluation Data of Ruggedness Study

No of injections	Ruggedness	
	Analyst -1	Analyst-2
	Area	Area
Injection-1	3212.154	3232.254
Injection-2	3234.221	3267.127
Injection-3	3256.432	3156.432
Injection-4	3241.274	3249.341
Injection-5	3322.723	3222.569
Injection-6	3196.354	3274.167
AVG*	3243.86	3233.648
STDEV*	44.13415	42.65185
%RSD*	1.36	1.31
*All the values are the averages of six determinations		

TABLE.1.11: Evaluation Data of Robustness Study

ROBUST CONDITIONS		GLICLAZIDE	
		RT	PEAK AREA
Flow rate	0.8 mL/min	3.882	3689.524
	1.2 mL/min	3.520	3645.521
Temperature	40^0C	3.761	3648.497
	45^0C	3.623	3685.563

TABLE.1.12:Results of Analysis of Tablet Containing Gliclazide

Pharmaceutical Formulation	Amount of Gliclazide*		% Recovery
	Labelled	Found	
AZUCON	80 mg	79.96	99. 95 %
* Average of three determinations			

CONCLUSIONS

In conclusion, a simple, accurate and stability indicating RP-HPLC method has been developed and validated for the analysis of gliclazide. Based on peak purity results, obtained from the analysis of force degradation studies, it can be concluded that the absence of coeluting peak along with the main peak of gliclazide indicated that the developed method is specific for the estimation of gliclazide in presence of degradation products. The developed method gave a linear calibration curve ranging from 5.0-15.0µg/mL.The % RSD for precision and accuracy was found to be less than two, which revealed that the results obtained are within acceptance criteria. Finally, it is concluded that "the proposed RP-HPLC

method is precise, economical, reproducible and stability indicating and can be applicable for the analysis of gliclazide in pure and in Pharmaceutical formulations".

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