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Development and Validation of HPLC Method for Estimation of Gliclazide in Gliclazide Tablets Prepared using Natural Disintegrant

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ABSTRACT

A simple, selective, linear, precise and accurate HPLC method was developed and validated for the estimation of Gliclazide in Gliclazide tablets prepared using natural super disintegrant by direct compression method. Isocratic elution at a flow rate of 1.0 mL/min was employed on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column at ambient temperature using Methanol: Phosphate Buffer 60:40 (v/v) as mobile phase. The UV detection wavelength was carried out at 210nm. The linearity of the developed method was studied over the concentration ranges between 10-30µg/ml. The retention time for Gliclazide was 3.2 min. The developed method was validated as per the ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. The Minimum concentration level at which the analyte can be reliable detected (LOD) and quantified (LOQ) were found to be 0.54 and 1.15 µg/ml respectively. The proposed method can be successfully applied for the estimation of Gliclazide in pharmaceutical dosage forms.

KEYWORDS: *HPLC, Gliclazide, Diabetes, Methanol: Phosphate Buffer*

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INTRODUCTION

Gliclazide is an oral hypoglycaemic agent used in the treatment of type-II diabetes mellitus. 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 β cells to glucose, decreasing hepatic glucose production, and increasing glucose clearance. Gliclazide is present in the market with the brand name of Diamicron containing 30, 60, or 80 mg of gliclazide for oral administration in the form of tablets. [1,2]

The literature survey reveals that various methods has been reported for estimation of Gliclazide by UV spectrophotometric [3], RP-HPLC [4], simultaneous spectrophotometric method [5], HPLC [6], spectrometric method in combination with metformin [7]. Many methods have been reported in the literature for the estimation of gliclazide. [9, 10, 11, 12]

In the early part of this century, colorimetric and spectrophotometric methods were used for drug analysis due to reasons of economy and easy availability. These methods, however, are used to a lesser extent today because they lack specificity, sensitivity and accuracy. For the simultaneous estimation of the drugs present in combination dosage forms, HPLC method is considered to be most suitable since this is a powerful and rugged method.[8]

The aim of presented research is to develop a simple, precise and accurate HPLC method for the estimation of Gliclazide in Gliclazide tablets prepared using natural disintegrant by direct compression method and validated as per ICH guidelines.

Determination of Lambda maximum

Preparation of stock solution of Gliclazide tablet

Gliclazide (100 mg) in a 100mL volumetric flask and 25 mL of methanol to it and it was vortexed (Eltek)

for 2 minutes. This was the main stock accounting for concentrations of 1000 µg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking methanol as blank.

The lambda maximum for Gliclazide tablet was found to be 210.00 nm.

Instrumentation and Chromatographic Conditions
HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Methanol: Phosphate Buffer (60:40 v/v) as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 210nm. All weighing were done on Shimadzu balance (Model AY-120)

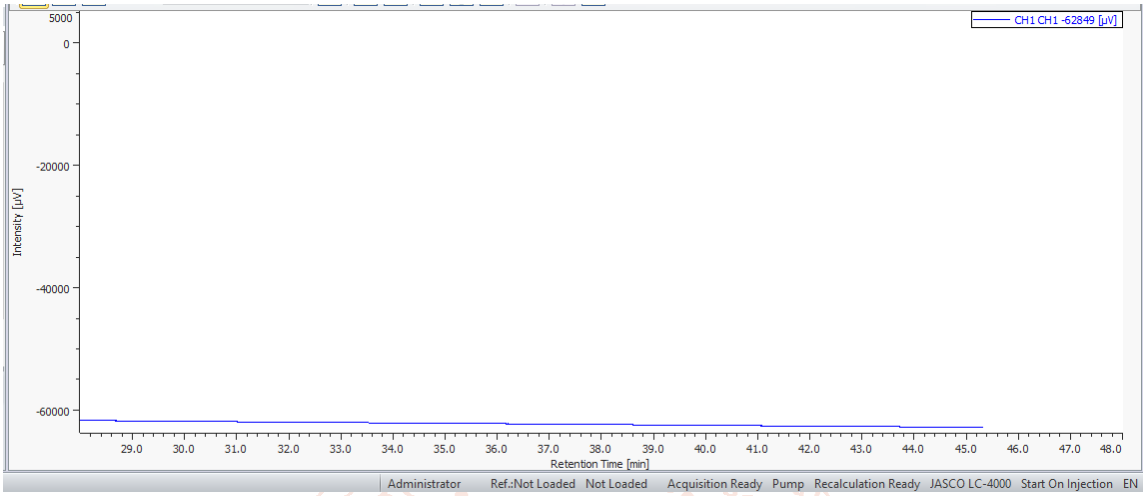

Fig. 1: HPLC chromatogram of blank.

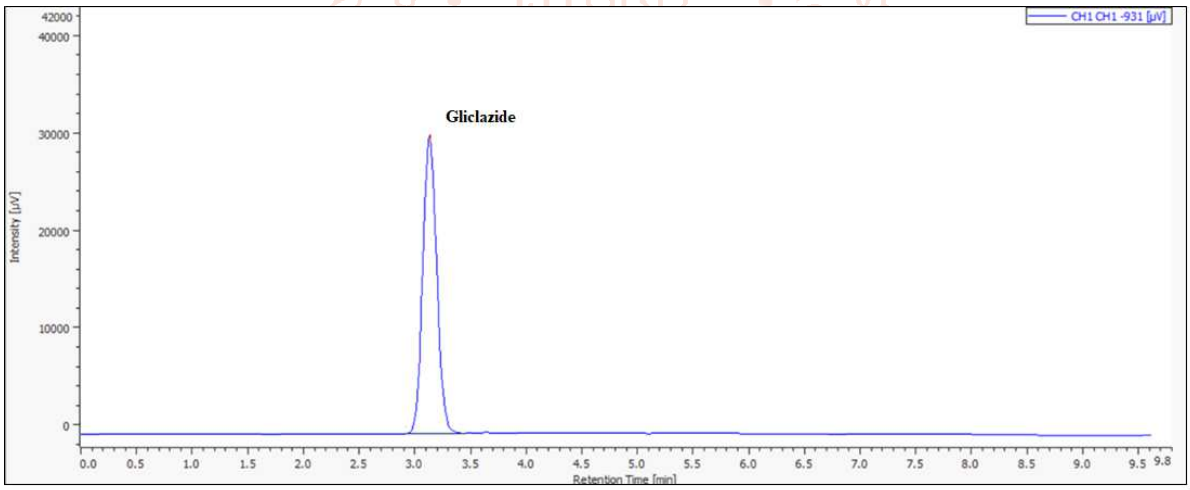

Fig. 2: HPLC chromatogram of standard Gliclazide tablet.

The retention time was found to be 3.2 with distinct peak.

MATERIALS AND METHODS

Material

Gliclazide was procured from Aarti Pharmaceuticals, Mumbai. Chemicals utilized for method development are of HPLC grade includes Methanol, phosphate buffer were purchased from Merck (India) Ltd.

Instrumentation

Chromatographic analysis was performed on Agilent Technologies- 1100 Gradient System equipped with UV (DAD) G13148 Detector controlled by CHEMSTATION 10.1 Software, with auto injector. The column Agilent zorbax eclipse XDB C18 (4.6 x 250 mm, 5.0 µm) is used.

Chromatographic conditions

An Agilent technologies gradient HPLC system with auto sample injector and UV was used for the purpose of separation. The separation was carried out on column Agilent zorbax eclipse XDB C18 (4.6 x 250 mm, 5µm) by using mobile phase vary in the ratio for the development of the HPLC method.

Preparation of mobile phase
The preparation of mobile phase was done by mixing Methanol: Phosphate Buffer (60:40 v/v) in the ratio of 60:40. Removal of gases was carried out in ultrasonic water bath for 15 minutes. Filtered the solution through 0.45µ filter.

Diluent preparation
Mobile phase used as diluents.

Preparation of standard stock solution
100mg of Gliclazide standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10mlvolumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution
100mg equivalent of Gliclazide tablet standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength
It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Gliclazide tablet was carried out under UV ranging from 200-400nm using the standard solution.

Methodology
The optimization of chromatographic conditions was carried out on Agilent zorbax eclipse XDB C18 (4.6 x 250 mm, 5µm) column. The separation was done by utilizing Methanol: Phosphate Buffer (60:40 v/v) ratio, the volume of sample was 20µl. The flow rate was maintained at 1.5ml/min. The detection of drug Gliclazide was done at 210nm.

Method Validation
Linearity:
The linearity of the developed method was studied over the concentration ranges between 10- 30µg/ml. The aliquots of 10, 15, 20, 25 and 30µg/ml were prepared by diluting standard stock solution of 0.2, 0.3, 0.4, 0.5 and 0.6 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Gliclazide was constructed by plotting peak area versus used concentration of Gliclazide tablet. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy
Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Gliclazide, a known amount of standard drug powder of Gliclazide was added to 80, 100, 120% level.

Precision method
By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution were made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation
Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness
Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION
Selection of wavelength maxima
The solution of Gliclazide was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 210nm.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Gliclazide with good resolution, peak shape given in the fig. 3. The mobile phase composed of Methanol: Phosphate Buffer (60:40 v/v) % v/v, at a flow rate of 1.0 ml/min was selected as it gave well resolved peaks of standard Gliclazide. The optimum wavelength 210nm selected for detection and quantitation.

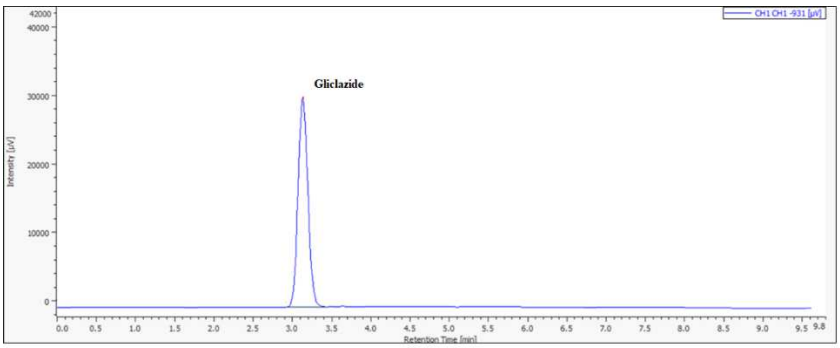

Fig. 3: HPLC Chromatogram with resolved peak of Gliclazide

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 10-30ppm. The standard working curve equation for drug was found to be y = 2199.5x - 10481 with correlation coefficient value $r^2 = 0.9987$. The results of linearity are given in Table. 1 and Fig.4.

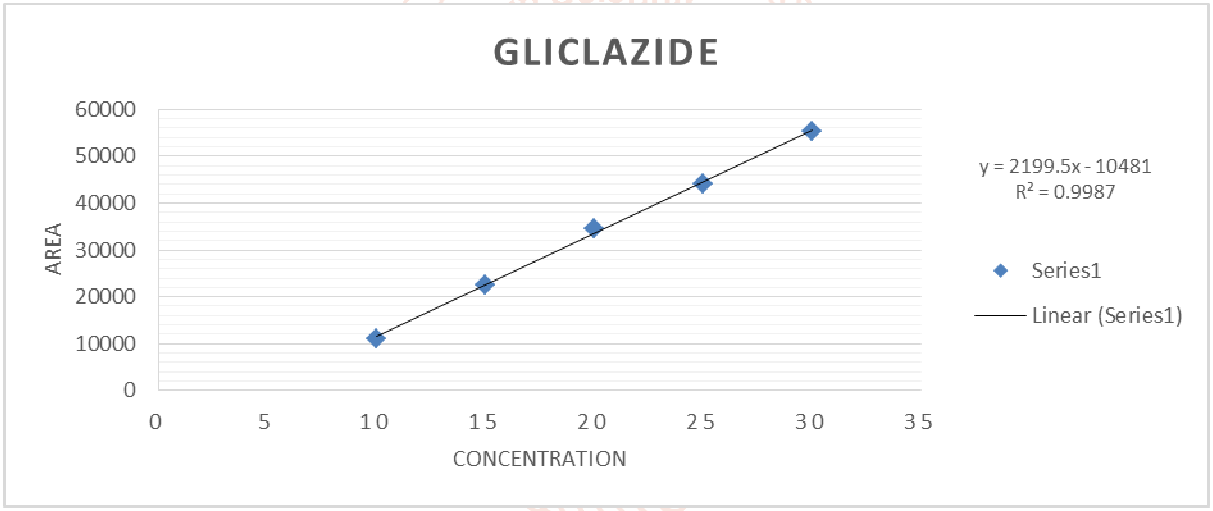

Fig. 4: Linearity curve of standard Gliclazide

Table 1: Linearity data of Gliclazide

Concentration µg/mL	Area
10	11023
15	22589
20	34557
25	44152
30	55230

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Gliclazide given in Table 2. The % recovery at 80, 100, and 120 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table 2: Recovery data of Gliclazide

Level (%)	Drug Conc (mg)	Amt recovered (mg)	% Recovery
100%	10	9.8	98.56
150%	15	19.85	99.85
200%	20	19.80	99.80

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision is given below. The results of precision studies are shown in Table 3.

Table. 3: Precision study (intra- day) of Gliclazide

Conc. µg/mL	Area	AVG	SD	%RSD
10	11457	11516.333	53.8175932	0.46731535
	11562			
	11530			
15	22635	22221.667	359.212101	1.61649487
	21985			
	22045			
20	34559	34380.667	319.331072	0.92881
	34012			
	34571			

Conc. - Concentration; AVG - average; SD - Standard deviation; RSD - Relative standard deviation

Table 4: Precision study (inter-day) of Gliclazide

Conc. µg/mL	Area	AVG	SD	%RSD
10	12459	11766.667	601.785953	5.11432821
	11472			
	11369			
15	22356	22367	225.70113	1.00908092
	22147			
	22598			
20	35601	35099.333	437.045001	1.2451661
	34896			
	34801			

Conc. - Concentration; AVG - average; SD - Standard deviation; RSD - Relative standard deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Gliclazide. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.54 & 1.15 µg/ml respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Gliclazide by changing flow rate 0.9 mL/min and 1.1 mL/min, mobile phase composition Methanol: Phosphate Buffer in the ratio of 59:40 and 61:39, wavelength i.e 209nm and 211nm. The method was found to be robust in the range of deliberate changes made.

Table 5: Robustness study with change in flow rate of Glicla

Flow rate mL/min	Conc. µg/mL	Area	AVG	%RSD
0.9	20	35412	35404	0.84194
0.9		35698		
0.9		35102		
1.1	20	35741	35704.67	0.77384
1.1		35961		
1.1		35412		

Conc. - Concentration; AVG - average; SD - Standard deviation; RSD - Relative standard deviation

Table 6: Robustness study with change in concentration of mobile phase of Gliclazide

Mobile phase (Methanol: 01% OPA)	Conc. µg/mL	Area	AVG	%RSD
59:40	20	34512	34917.67	1.91635
59:40		35690		
59:40		34551		
61:39	20	35621	35207.33	1.54966
61:39		35412		
61:39		34589		

Conc. - Concentration; AVG - average; SD - Standard deviation; RSD - Relative standard deviation

Table 7: Robustness study with change in Wavelength of Gliclazide.

Wavelength nm	Conc. µg/mL	Area	AVG	%RSD
209	20	35102	35170	1.20307
209		35623		
209		34785		
211	20	35698	35226	1.16418
211		34957		
211		35023		

CONCLUSION

A simple, rapid, accurate and precise HPLC method for the analysis of Gliclazide in pure and prepared pharmaceutical dosage form had been developed and validated as per ICH Guidelines. The validation study demonstrates that the developed method is useful for simultaneous determination of Gliclazide from pharmaceutical dosage form, particularly new formulated tablet, and is accurate, quick, precise, reproducible, and affordable with acceptable correlation co-efficient, RSD, and standard deviations. Simplicity of sample preparation and inexpensive reagent costs are two benefits of the developed method. The presented method is easy to use and does not need rigorous sample preparation. It can be utilised for Gliclazide routine analysis either separately or in combination pharmaceutical dosage form.

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