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A RP-HPLC Method Development and Validation for the Estimation of Gliclazide in bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Gliclazide in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.2 ml min -1 was employed on a symmetry C_{18} column at ambient temperature. The mobile phase consisted of methanol: phosphate buffer 50:50 (V/V). The UV detection wavelength was at 210 nm. Linearity was observed in concentration range of 1-100 µg/mL. The retention time for Gliclazide was 3.25 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Gliclazide in pharmaceutical dosage forms.

INTRODUCTION

Gliclazide (1-(3-azabicyclo [3.3.0] oct- 3- yl) - 3- p-tolylsulfonylurea or 1-(hexahydrocyclopenta [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).

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 ß-cells 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 (Brunton *et al.*, 2006, Tripathi., 2004).

From the literature survey, it was found that Gliclazide was estimated by analytical methods such as Spectrophotometry methods (Dhable *et al.*, 2010, Ketan *et al.*, 2011), HPLC methods (Rouini *et al.*, 2003, Talari *et al.*, 2011, Damanjeet *et al.*, 2009) and HPLC methods in combination with other drugs (Bhanu *et al.*,2006, Gandhimathi *et al.*,2003, Gayatri *et al.*,2004) .

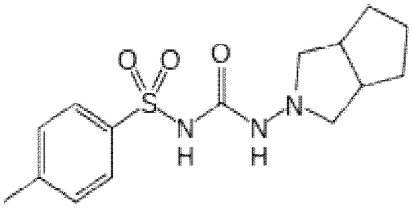

Fig1: Structure of Gliclazide.

The aim of the study is to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Gliclazide in pharmaceutical dosage form as per ICH guidelines.

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MATERIALS AND METHOD

Instrumental and analytical conditions

The HPLC analysis were carried out on YOUNGLIN ACME 9000 (Korea) with Autochrome 3000 integrater and UV Visible detector. The column used is Xbridg-Extend C_{18} (250 × 4.6 mm, packed with 5 µm). UV detection was performed at 210 nm. The injection volume of sample was 20µl. An isocratic mobile phase containing methanol and 0.02 M potassium dihydrogen orthophosphate 50:50(V/V) at the PH 3.5 was carried with the flow rate at 1.2ml/min with 8 minutes run time. The mobile phase was filtered through 0.4µm membrane filter and degassed before use.

Reagents and chemicals

Gliclazide working standard was kindly gifted by Dr Reddy's laboratories, Hyderabad. Tablets were purchased from local pharmacy manufactured by Cadila pharmaceuticals (Glyloc). Ultra pure water was obtained from a millipore system. HPLC grade methanol was obtained from Merck (India) limited. All other chemicals used were AR grade. The optimum chromatographic conditions were summarized in table-7.

Preparation of mobile phase

Dissolved 2.7218g of Potassium Di hydrogen orthophosphate in 1000 ml of water and mixed, pH adjusted to 3.5 with orthophosphoric acid followed by degassing of the buffer.

Transferred 500 volumes of Methanol and 500 volumes of buffer (0.02M) into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 mins for degas the mobile phase.

Preparation of Standard Solution

Accurately weighed about 10 mg working standard Gliclazide and transferred into a 10ml volumetric flask and 5 ml of methanol was added and kept in an ultrasonic bath to ensure the complete solubilization and the volume was adjusted with methanol to get stock solution of 1000 µg/ml. Then 0.5 ml of stock solution was transferred into 10 ml volumetric flask and make up to volume with mobile phase and mix. This yielded solution of 50µg/mL concentration.

METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of Gliclazide in the concentration of 1, 5, 10, 25, 50, 75, and 100µg/mL were prepared. The solutions were injected using 20µl injection volume in to the chromatographic system at the flow rate of 1.2 ml/min and the effluents were monitored at 210 nm, chromatograms were recorded given in table 1. Calibration curve was obtained by plotting the peak area ratio versus the applied concentrations of Gliclazide. The linear correlation coefficient was found to be 0.999and shown in Fig 2.

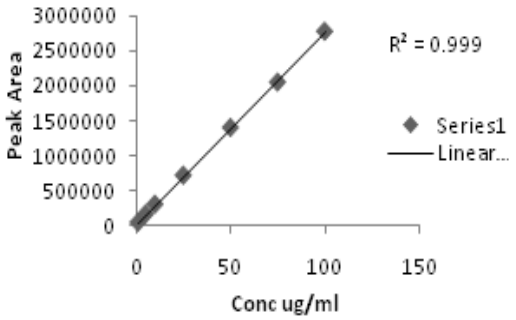

Fig. 2: Linearity curve of Gliclazide.

Table . 1: Linearity parameter for Gliclazide.

Conc.(µg/ mL)	Area
1	37402
5	157311
10	297076
25	714376
50	1401829
75	2054242
100	2783953

Precision

Repeatability of the method was checked by injecting replicate injections of 50µg/mL of the solution for six times on the same day as intraday precision study of Gliclazide and the % RSD was found to be 0.01 as shown in table 2.

Table. 2: Precision parameter of Gliclazide**.**

Injections	Area
I.P-1	1401712
I.P-2	1401921
I.P-3	1402135
I.P-4	1401822
I.P-5	1402126
I.P-6	1402067
Mean	1401964
SD	174.1326
% RSD	0.012421

Accuracy

Gliclazide reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50, 100 and 150µg/mL). At each level, samples were prepared in triplicate and the recovery percentage was determined and given in table 3.

Specificity

Spectral purities of Gliclazide chromatographic peaks were evaluated using the UV spectra recorded by a UV detector. In addition, a solution containing a mixture of the tablets excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks.

Table . 3: Accuracy parameter for Gliclazide.

Conc.		inj-1	inj-2	inj-3	Mean	% Recovery	STD	% RSD
25µg/mL	50%	691544	691283	691387	691404.7	98.64322	131.3938	0.019004
50µg/mL	100%	1400673	1401278	1401183	1401045	99.94405	325.3588	0.023223
75µg/mL	150%	2108939	2109567	2109735	2109414	100.3172	419.568	0.01989

Table. 4: Robustness parameter for Gliclazide.

Parameters	Adjusted to	Avg. Area	RT	SD	%RSD
	1.1 mL/min	1835126.7	3.26	9505.82	0.52
Flow rate as per method 1.2mL/min	As it is	1402041.5	3.22	416.03	0.03
	1.3ml/min	1062340.33	3.28	4341.03	0.41
Mobilephase	Buffer: Methanol (45:55)	1719049.83	3.26	3096.83	0.18
composition(Buffer:Methanol,	As it is	1402081.5	3.22	293.07	0.02
50:50)	Buffer: Methanol (55:45)	1183250.00	3.25	3340.39	0.28

Robustness

To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which shown in table 4.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and quantitation limits

According to the determined signal-to-noise ratio, Gliclazide presented limits of detection of 0.1 µg/mL and limits of quantitation of 0.5µg/mL, where the compounds proportion found in the sample solutions injected onto the chromatograph. However, the objective of the method is the quantitation of Gliclazide, so that the values obtained for Gliclazide should be considered as the limit of method sensitivity.

System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Gliclazide and it was calculated by determining the standard deviation of Gliclazide standards by injecting standards in six replicates at 6 minutes interval and the values were recorded and given in table 5.

Assay of Gliclazide tablet

Three different batches of Glyloc were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. The tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Gliclazide was transferred to a 100 ml volumetric flask followed by the addition of 25 ml of methanol. The solution was sonicated for 3 minutes then filtered through Whatman filter paper (No.41) and volume adjusted with the mobile phase. Further dilutions were made to get the final concentration equivalent to 50 µg/ml of Gliclazide. The results were presented in table 6.

All the analyzed batches presented Gliclazide were very close to the labeled amount. The content in the tablets samples varied from 99.25 to 100.20%.

Table. 5: System Suitability for Gliclazide.

Concentration	Injection	Area	R_t
50µg/mL	Inj-1	1402212	3.25
	Inj-2	1401521	3.24
	Inj-3	1402235	3.24
	Inj-4	1402422	3.26
	Inj-5	1402526	3.25
	Inj-6	1402267	3.24
Statistical Analysis	Mean	1402197	3.246667
	SD	352.8203	0.008165
	% RSD	0.025162	0.251488
	Tailing Factor	1.0167	
	Plate Count	7108.6	

Table 6: Contents of Gliclazide in tablets (n=6)

Sample tablet	Batch	Content of Gliclazide (%) + S.D.
Glyloc	*1*	*99.25± 0.25*
	2	99.55 ± *0.32*
	3	100.20± *0.45*

S.D=Standard Deviation

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Gliclazide preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and buffer were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase methanol: buffer in the ratio of 50:50 (v/v).The retention time of Gliclazide was found to be 3.2 min, which indicates a good base line.

Table. 7: Developed Chromatographic Conditions.

Parameters	Method
Stationary phase (column)	Xbridg-Extend C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	50:50 (Methanol : Phosphate Buffer)
pH	3.5 ± 0.02
Flow rate (mL/min)	1.2
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	210
Drugs R_t(min)	3.2

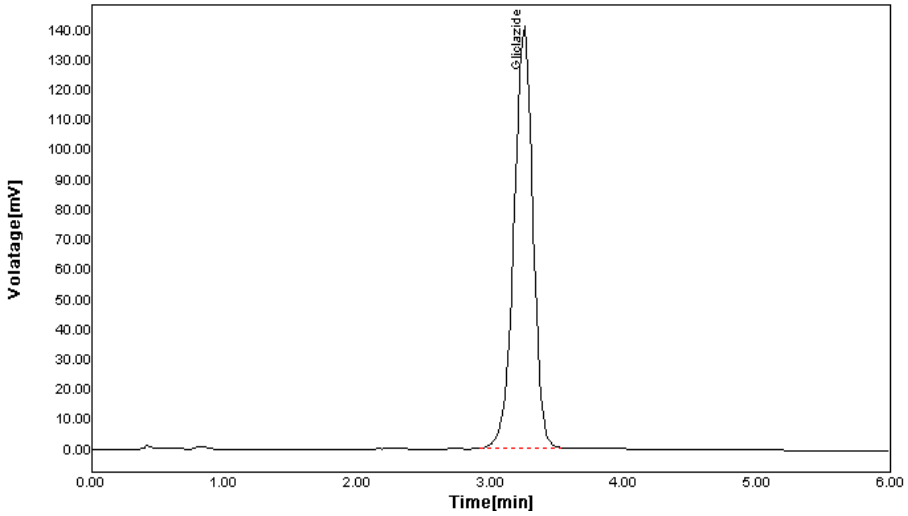

Fig. 3: Standard Chromatogram of Gliclazide.

The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Gliclazide in tablet formulation which shown in table 7. A typical chromatogram showing the separation of Gliclazide is shown in figure 3.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Gliclazide in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Gliclazide in pharmaceutical dosage form.

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