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Development and Validation of RP-HPLC Method for Estimation of Gliclazide in Bulk and Tablet Dosage Form

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ABSTRACT

A simple, selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method for the analysis of Gliclazide in bulk and in tablet dosage form has been developed and validated. Sample was analysed on a Enable C18 (250mm X 4.6 mm i.d, particle size 5µm) column. The mobile phase consist of Methanol: Water (pH 3.5) in the ratio of 85:15*v/v* which was sonicated to degased and delivered at a flow rate of 1ml/min at ambient temperature. The retention time of Gliclazidewas 3.7±0.02 minutes. Studies were performed using an HPLC system equipped with a UV detector; the response was monitored at 230 nm.The calibration curve was linear over the concentration range of 20-70 µg/ml (r^2=0.999). The limit of detection for Gliclazide was found to be 0.2438 µg/ml and the limit of quantification limit was about 0.7388 µg/ml. The accuracy of the method was established based on the recovery studies. The proposed method can be applied to the routine analysis of Gliclazide in bulk and in tablet dosage form.

Keywords: Gliclazide, ICH, RP-HPLC, Validation.

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INTRODUCTION

Gliclazide is an oral hypoglycaemic agent, which lowers the blood glucose level by stimulating the pancreatic β-cells to secrete insulin. Gliclazide is 1-(hexahydrocyclopenta [c]pyrrol-2(1H)-yl)-3-[(4-methylphenyl) sulphonyl] urea.Gliclazide is official in Indian Pharmacopoeia[1]. Literature survey reveals that some methods have been developed for their determination by HPLC[2-8], HPTLC[9] or spectrophotometry[10] either alone or in combination. However, overall cost of analysis of reported HPLC method is more. In this view, an economical HPLC method has been developed for estimation of Gliclazide in bulk and pharmaceutical dosage form.

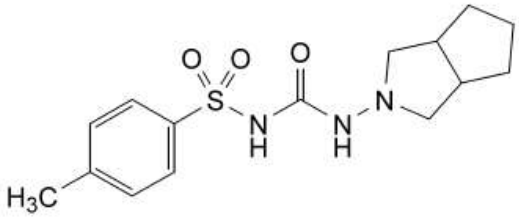

Figure 1: Chemical Structure of Gliclazide

MATERIALS AND METHOD

Chemicals and Reagents

Gliclazide standard was provided by Bal Pharma Limited, Bangalore, India. "Glychek-40 mg" tablets were procured from local market. HPLC grade of solvents used for this study were purchased from Merck Pvt. Ltd., Mumbai. Methanol (HPLC grade) was purchased from Loba Chemie Pvt Ltd., Mumbai, Water (HPLC grade).

Instruments

Shimadzu LC2010 with UV detection system, Elga (UHQ-II) water purification system, Shimdazu AY-120 Balance, Enable C_{18} Columns, LC solution Software were used.

Chromatographic Conditions

The mobile phase consisting of methanol: water (adjust the pH 3.5 with o-phosphoric acid) in the ratio of 85:15*v/v*, was filtered through 0.45µm nylon filter, sonicated and was pumped from the solvent reservoir. The flow rate of mobile phase was maintained at 1ml/min and the response was measured at 230nm with a run time of 10min. The volume of injection loop was 20µl.The separation was done at ambient temperature.

Selection of detection wavelength

Detection wavelength for both methods was selected on the basis of wavelength maxima of UV spectrum. For this study 100 µg/ml solution of drug was prepared in methanol and scanned in

range of 200-400 nm. The recorded spectrum showed maximum absorbance at 230 nm. Hence, the wavelength 230 nm was selected for further studies as shown in Figure 2.

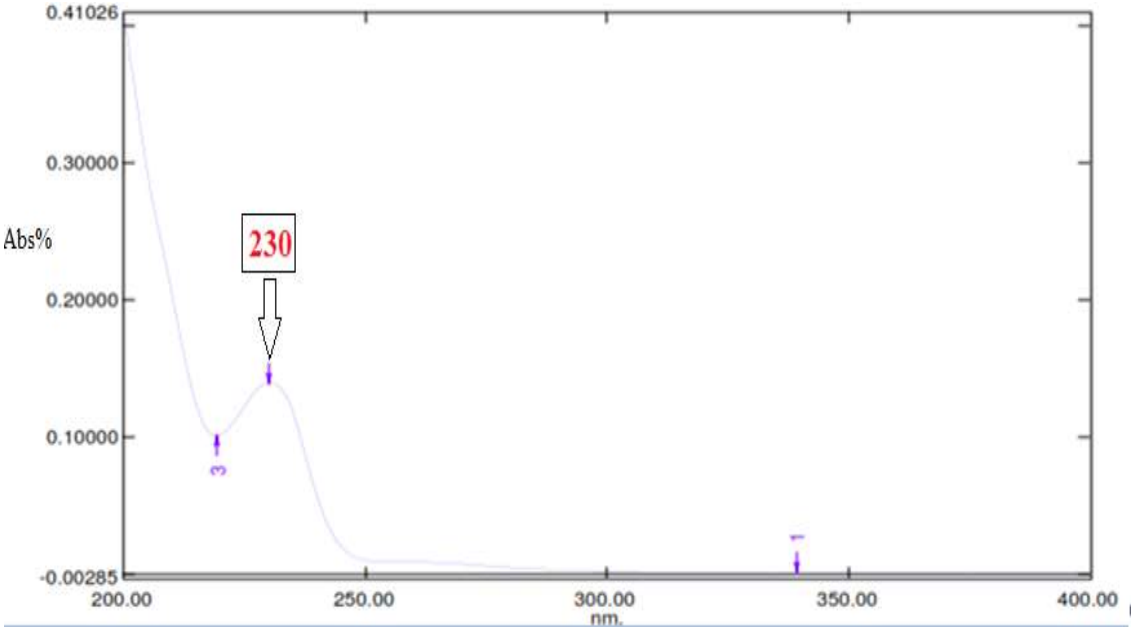

Figure 2: ***UV- Spectrum of Gliclazide in Methanol (100µg/ml)***

Optimization of Chromatographic Conditions:

The HPLC method was optimized with a view to develop assay method for Gliclazide. Initially, different columns, different combinations of mobile phases such as methanol: water, and acetonitrile: water in different proportions were tried at different flow rates. Finally, Methanol: Water (85:15 %v/v, pH 3.5) at flow rate of 1.0 ml/min gave acceptable retention time and system suitability parameters. The mobile phase and samples prepared for RP-HPLC analysis were filtered using 0.45 µm nylon filter and 0.45µm syringe filter, respectively and sonicated for 15 minutes before injecting into the HPLC system.(figure 3& Table 1)

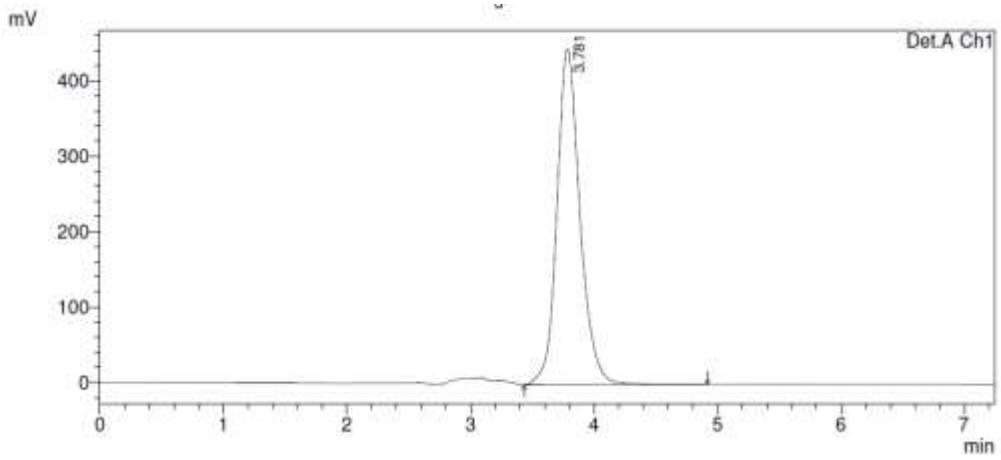

Figure 3: Chromatogram of Gliclazide

Table 1: Optimized chromatographic conditions

Parameters	Details
Mobile phase	Methanol:Water (85:15 % v/v) (pH 3.5 adjusted with Orthophosphoric acid)
Column	Enable C18, 250mm x 4.6mm, 5 µm
Flow Rate	1.0 ml/minute
Detection	UV at 230 nm
Column oven Temperature	Ambient
Injection Volume	20 µl
Run time	10 minutes
Retention time (min.)	3.7±0.02
Diluent	Methanol:Water (85:15 % v/v) (pH 3.5)

Preparation of standard stock solutions and calibration curves

Gliclazide (100 mg) were dissolved in 100 ml of diluent. This standard stock solution was further diluted to obtain six different concentrations. 20 µl volume of each solution was injected into HPLC under the optimized chromatographic conditions. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations (n = 6) were computed for Gliclazide. The Beer-Lambert's law was obeyed in the concentration range of 20-70 µg/ml. The prepared calibration curves was used to calculate the amount of Gliclazide in pharmaceutical dosage form. (Figure 4)

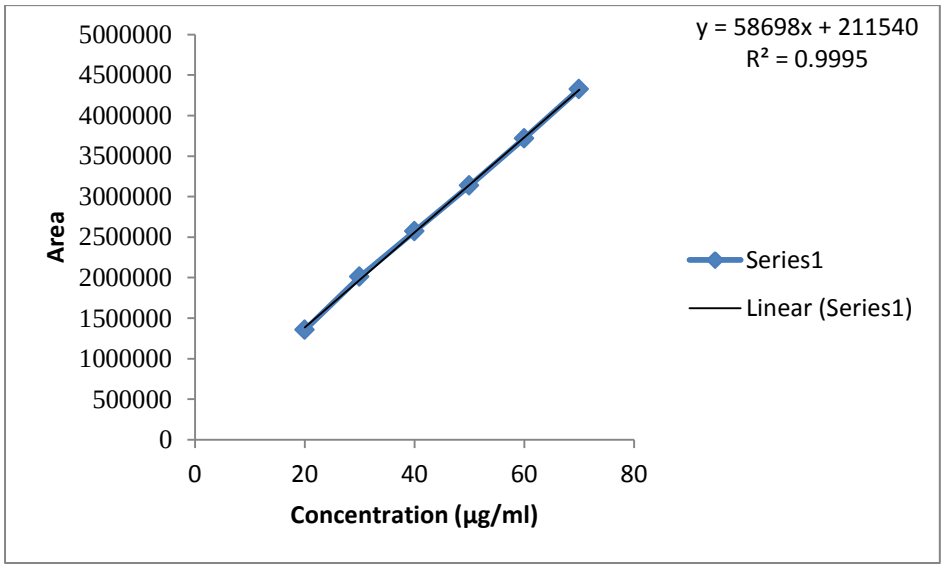

Figure 4: Calibration Curve of Gliclazide

Assay of Tablet Formulation

"Glychek-40 mg" tablets were procured from local market. Tablet of Gliclazide equivalent to 100 mg of Gliclazide was accurately weighed and transferred into 100 ml volumetric flask. About 70 ml of diluent was added to dissolve the components present in tablet. The contents were sonicated

for 15 minutes with intermittent shaking and diluted up to the mark with the diluent. This solution was filtered through 0.45 µm nylon filter, discarding first five ml of filtrate. The sample solution was prepared in triplicate and 20 µl volume of each sample solution was injected into the sample injector of RP-HPLC under the optimized chromatographic conditions (Table 2).

Table 2: Results of assay of Gliclazide

Sr. No.	Sample solution concentration (µg/ml)	Sample solution area	Mean Sample solution area	% Drug Found
1	70	4142730	4144062	100.3
2	70	4139066		
3	70	4150391		

Method Validation[11]

Validation of optimized RP-HPLC method was done according to ICH guideline Q2 (R1) for following parameters.

Linearity and Range

Linearity was evaluated for a set of six standard solutions containing 20-70 µg/ml, for Gliclazide. The relationship between peak area (as a dependant variable) and concentration of drug in the solution (as an independent variable) were established by simple linear regression method. The regression equation was obtained as calibration curve (Table 3).

Table 3: Linearity Results of Gliclazide

Concentration (µg/ml)	Area
20	1354459
30	2010666
40	2572016
50	3136265
60	3718751
70	4325645

Table 4: Linear regression data for calibration curve

Parameters	Gliclazide
Linearity range (µg/ml)	20-70 µg/ml
r^2± SD*	0.999±0.00083
Slope ± SD*	59816.8± 1259.72
Intercept ± SD*	15490.8± 4419.67
Y= mx + c	y = 58698x + 21154

Table 5: LOD and LOQ of Gliclazide

Drug	LOD	LOQ
Gliclazide	0.2438 µg/ml	0.7388 µg/ml

Table 6: Results for method precision (Repeatability)

Precision	Amount (µg/ml)	Area	Mean Area ± SD*	%RSD*
Repeatability	60	3718751	3722573 ±3289.592	0.088
	60	3723842		
	60	3728243		
	60	3720456		
	60	3721457		
	60	3722687		

n=6

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

Five sets of known concentrations (20-70 µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as

$$LOD = 3.3\frac{SD}{S}$$

$$LOQ = 10\frac{SD}{S}$$

Where, SD = Standard Deviation of response

S = Average of the slope of the calibration curve

Precision

Method Precision (Repeatability)

In repeatability, six standard solutions were prepared each having concentration of 60µg/ml of Gliclazide. The response of each of these solutions was measured and percentage relative standard deviation (% RSD) was calculate. The obtained results are given in table 6.

Table 6: Results for method precision (Repeatability)

Precision	Amount (µg/ml)	Area	Mean Area ± SD*	%RSD*
Repeatability	60	3718751	3722573 ±3289.592	0.088
	60	3723842		
	60	3728243		
	60	3720456		
	60	3721457		
	60	3722687		

n=6

Intermediate Precision

For intraday precision nine different solutions were prepared across the intended range (50, 60 and 70 µg/ml) with three replicates of each and their area was measured on the same day(Table 7). For interday precision nine different solutions were prepared across the intended range (50, 60 and 70 µg/ml) with three replicates of each and their corresponding area was measured on three

subsequent days. The results were reported in terms of relative standard deviation (RSD). The obtained results are given in table 8.

Table 7: Intraday precision studies

Precision	Amount (µg/ml)	Area	Mean Area ± SD	%RSD*
Intra-day (n=3)	50	3156336	3155777 ± 19238.59	0.6
	50	3174730		
	50	3136265		
	60	3718751	3723612 ± 4750.178	0.12
	60	3723842		
	60	3728243		
	70	4142730	4144062 ± 5778.862	0.13
	70	4139066		
	70	4150391		

Table 8: Inter-day precision studies

Precision	Amount (µg/ml)	Area	Mean Area ± SD	%RSD*
Inter-day (n=3)	50	3156894	3184995 ± 24464.27	0.76
	50	3201548		
	50	3196542		
	60	3766541	3755708 ± 36102.76	0.96
	60	3715429		
	60	3785154		
	70	4158496	4166301 ± 6873.847	0.16
	70	4168954		
	70	4171453		

Accuracy: Recovery studies were performed at 80.0%, 100.0% and 120.0% levels using standard addition method. Standard drug solution of Gliclazide was spiked at 80.0%, 100.0% and 120.0%. For each level three replicates were prepared and injected into column under optimized HPLC conditions. The calculation for total recovery of Gliclazide was performed & results are expressed in terms of % recovery ± S.D and % R.S.D values. The values are summarized in table 9.

Table 9: Results of recovery study for Gliclazide

Spike level	Amount added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Mean % recovery ± S.D.	% R.S.D.*
80%	36	2156621	36.38	101.05	101.25±0.7697	0.76
		2147405	36.22	100.6		
		2179567	36.77	102.1		
100%	40	2597463	43.89	109.7	109.96±0.3055	0.27
		2612661	44.83	110.3		
		2586117	43.69	109.9		
120%	44	2800127	47.34	107.5	106.1±1.22	1.14
		2749579	46.48	105.6		
		2739537	105.2	105.2		

Robustness

Robustness of the above method was carried out by purposefully varying some chromatographic parameters. These parameters include change in the flow rate (0.8ml and 1.2ml), temperature (23^0C and 27^0C) and wavelength (229nm and 231nm) etc. The results obtained by changing all these conditions are indicated in terms of % RSD values. These studies were carried out on concentration of 70µg/ml of drug and obtained results are presented in table 10.

Table 10: Robustness data in terms of retention time for Gliclazide

Factor	Level	Gliclazide (t_R in min)
A: Change in Flow Rate		
0.8	-2	4.614
1	0	3.778
1.2	+2	3.241
	%RSD	1.8
B: Change in temperature		
23^0 C	-1	3.781
25^0 C	0	3.778
27^0 C	+1	3.783
	%RSD	0.078
C: Change in Wavelength		
229 nm	-1	3.786
230 nm	0	3.778
231 nm	+1	3.784
	%RSD	0.11

RESULTS AND DISCUSSION

The Calibration curve was plotted of Gliclazide peak area v/s Concentration. The generated regression equation was y = 58698x + 21154 (R^2= 0.999). The R^2 value as 0.999 indicates that developed method was linear. The calibration curve was obtained in the range of 20-70 µg/ml. The proposed method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80%, 100% and 120% levels 101.25 $\pm$ 0.7697, 109.96$\pm$0.3055, 109.96$\pm$0.3055showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 0.2438 µg/ml and 0.7388 µg/ml respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Gliclazide in tablet dosage form.

CONCLUSION

A new, accurate, economical and precise RP-HPLC method had been developed for the Gliclazide in bulk and Tablet dosage form. The standard deviation and % RSD (<2 %) is within limit,

indicating high degree of precision of the methods. The results of the recovery studies performed shows the high degree of accuracy of the proposed method. Hence, it can be concluded that the developed RP-HPLC method for Gliclazide is accurate and precise.

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