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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR DETERMINATION OF GLICLAZIDE IN API AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The aim of present work was to develop a simple and sensitive, HPTLC for the quantitative estimation of Gliclazide in its single component tablet formulations (40 mg). Gliclazide was chromatographed on silica Gel 60 F254 TLC plate using Toluene:Chloroform: methanol (4:4:2 v/v) as mobile phase. Gliclazide in methanol was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm. Gliclazide showed Rf value 0.35 and scanned at 230 nm using Camag TLC Scanner. The method was validated in terms of linearity (4-14 ng/ml), precision (intra-day variation 2.164, inter-day variation 2.614), accuracy (86.34to 92.20%) and specificity. The limit of detection and limit of quantification for Gliclazide were found to be 0.5 ng/spot and 1.53 ng/spot, respectively. It can be concluded from the results that the proposed method was accurate, precise and consistent the determination of Gliclazide in tablet dosage form. This method was validated as per ICH guideline Q2 (R1). Results suggest that this method can be used for routine estimation of Gliclazide in bulk and pharmaceutical dosage forms.

KEY WORDS: Gliclazide, Toluene, Methanol, Chloroform, HPTLC.

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 HPTLC method is more. In this view, an economical HPTLC method has been developed for estimation of Gliclazide in bulk and pharmaceutical dosage form.

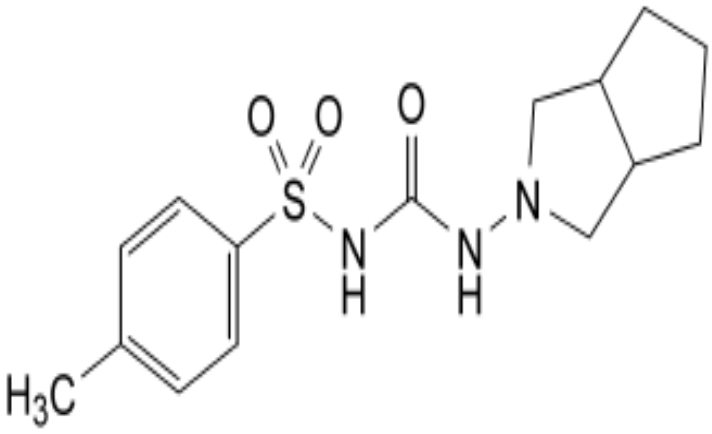

Fig. 1: Chemical Structure of Gliclazide

MATERIALS AND METHODS

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

Preparation of standard solution

A standard stock solution of Gliclazide was prepared by dissolving 10 mg of standard API in 10 ml of methanol to get concentration of 1000 µg/ml. This solution was further diluted to get 100 µg/ml solution of Gliclazide as working standard.

Selection of wavelength for Detection

The working standard of Gliclazide in methanol was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm. Wavelength 230 nm was selected for detection of obtained spectrum. (Figure 2&3)

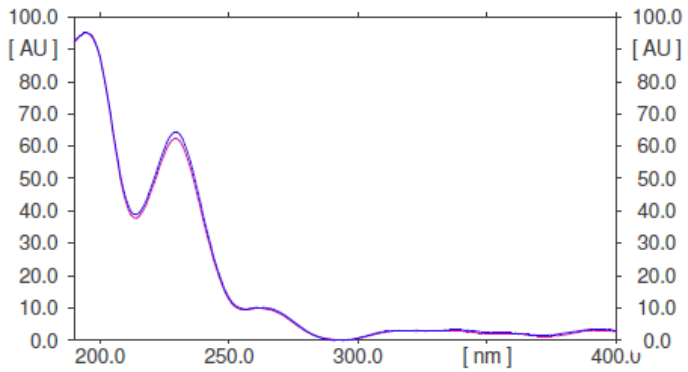

Fig. 2: The overlain UV spectra of 1000 ng/ml Gliclazide (API and sample) between 200 and 400 nm

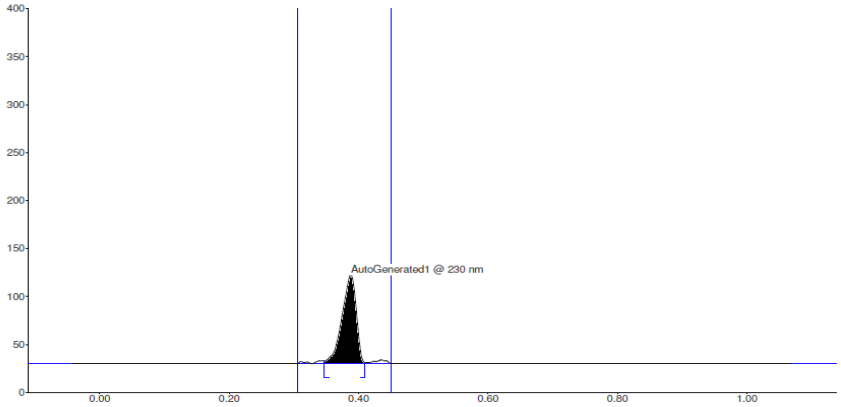

Fig 3: Chromatogram of 100 µg/ml solution of Gliclazide

Chromatographic Conditions

This analysis was performed on Camag HPTLC system (Switzerland). It is equipped with a Linomat-5 applicator, 100 µl sample syringe (Hamilton, Switzerland) and Camag TLC scanner-4. On the basis of trial and error method using different solvent system, following chromatographic conditions were chosen for analysis. Pre-coated silica gel 60 F_{254} TLC (E-Merck, Germany) plates (10x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110°C for 10 min prior to application. The standard samples of Gliclazide were spotted on pre-coated TLC plates In the form of bands of length 4 mm using 100 µl sample syringe with a Linomat-5 applicator. The chromatographic development was carried using Toluene: Chloroform: Methanol (4:4:2 v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanners at 230 nm, operated by win CATS Software (Version 1.4.3, Camag).

Preparation of Calibration Curve

Different concentrations of the working standard solution were applied on the TLC plate, corresponding peak areas were recorded (Figure 3) and linear regression was done between the peak area vs concentration. Finally, 400-1400 ng/sopt range was selected for preparation of calibration curve and linear regression equation was obtained in this range (Figure 4).

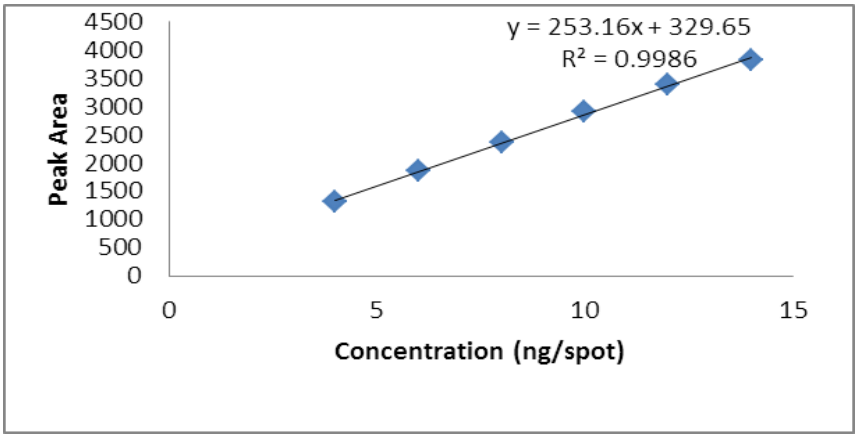

Fig 4. Calibration Curve of Gliclazide

ASSAY OF TABLET FORMULATION

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to average weight of Gliclazide was accurately weighed, transferred to a 100 ml of volumetric flask and diluted up to mark with methanol. The solution was filtered with Whatmann filter paper No. 41 and the first 5 ml of filtrate was discarded. This solution was further diluted with same solvent and subjected for HPTLC study. This procedure was repeated in triplicate (Table 1).

Table 1. Assay of marketed formulation of Gliclazide

Sr. No.	Sample solution concentration (ng/spot)	Sample solution area	Mean Sample solution area	% Drug Found
1	800	2306.07	2340.44	99.26
2	800	2332.06		
3	800	2383.19		

METHOD VALIDATION [11]

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity & Range, Precision, Limit of Detection (LOD) & Limit of Quantitation (LOQ) and Accuracy according to ICH Q2 (R1) guidelines.

LINEARITY AND RANGE

The linearity was determined by using working standard solutions between 400-1400ng/sopt. The spectrums of these solutions were recorded and area in wavelength 230 nm. Calibration curve of peak area *v/s* Concentration was plotted after suitable calculation and simple linear regression was performed (Figure 3). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation (Table 2).

Table 2. Linearity and Range of Gliclazide

Concentration (ng/sopt)	Area
400	1307.48
600	1882.76
800	2370.12
1000	2910.15
1200	3381.69
1400	3826.37

PRECISION

Repeatability

The precision of the method was checked by repeatedly injecting (n= 8) standard solutions of Gliclazide (8 ng/spot). Area of each curve of these solutions was measured in the 230 nm. Relative standard deviation (%RSD) was calculated (Table 3).

Table 3. Repeatability study of Gliclazide

Concentration (ng/spot)	Area	Mean area	%RSD
800	2338.17	2405.454	2.518
800	2447.51		
800	2487.31		
800	2319.15		
800	2387.77		
800	2380.25		
800	2411.93		
800	2470.84		

***n=8, % RSD = % Relative Standard Deviation.**

Reproducibility

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days of same

concentrations of 8 ng/spot of Gliclazide. The results were reported in terms of percentage relative standard deviation (%RSD). The results were tabulated in (Table 4).

Table 4. Intraday and Interday Precision study of Gliclazide

Drug	Concentration (ng/spot)	% RSD*	
		Intraday	Interday
Gliclazide	800	2.514	2.607
	800	2.164	2.870
	800	2.53	2.67

***n=3**

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

Five sets of known concentrations (400-1400ng/spot) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation (Table 5) and following formulae as

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

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

Where,

SD is standard deviation of y-intercept of the calibration curves

S is mean slope of five calibration curves.

Table 5. LOD and LOQ of Gliclazide

Drug	LOD	LOQ
Gliclazide	50.50 ng/spot	153.01 ng/spot

Accuracy

To check the accuracy of the method, recovery studies were carried out by over spotting standard drug solution to pre-analyzed sample solution at three different levels 80%, 100% and 120 %. Basic concentration of sample chosen was 100 ng/spot. The areas were noted after development of plate. The drug concentration was calculated by using regression equation (Table 6).

Table 6. Accuracy of Gliclazide

Concentration taken in ng/spot (A)	Standard addition in ng/spot (B)	Total drug Concentration (ng/spot) (A+B)	Area	Average	% Recovery
800	200	1000	3029.90 3075.30 3090.40	3065.20	86.34
1000	200	1200	3588.60 3667.90 3661.40	3639.30	87.34
1200	200	1400	4224.90 4433.80 4419.70	4359.47	92.20

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf and spectra of the spot with that of standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on Win CATS software 5.

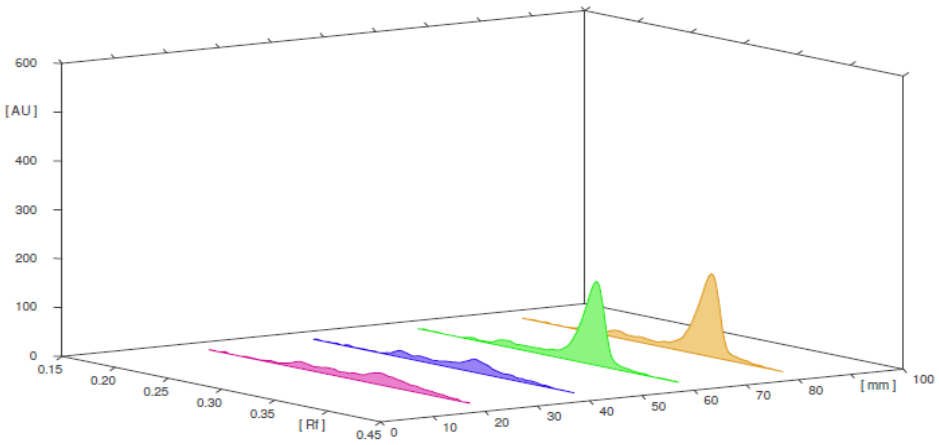

Fig 5: Specificity curve of Gliclazide

RESULTS AND DISCUSSION

The Calibration curve was plotted of Gliclazide peak area v/s Concentration. The generated regression equation was y = 2.531x + 329.6 (R^2= 0.998). The R^2 value as 0.998 indicates that developed method was linear. The calibration curve was obtained in the range of 400-1400 ng/spot. 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

86.34%, 87.34%, 92.20% showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 50.50 ng/spot and 153.01 ng/spot 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

It can be concluded from the results that the proposed method was accurate, precise and consistent the determination of Gliclazide in tablet dosage form. This method was validated as per ICH guideline Q2 (R1). Results suggest that this method can be used for routine estimation of Gliclazide in bulk and pharmaceutical dosage forms.

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