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

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ABSTRACT

The determination of the active agent both qualitatively and quantitatively is important to ensure a quality of the product. The present work is aimed to develop and validate a simple, economic and sensitive High-Pressure Thin Layer Chromatography method to analyse Gliclazide in bulk as well as in its single component tablet formulation. GLZ was chromatographed on Silica Gel 60 F254 precoated TLC plates and Toluene: Acetonitrile: Methanol (7:2:1 v/v) was selected as a mobile phase. GLZ dissolved in methanol was scanned over the range of 200 to 400 nm by Camag TLC scanner 4. GLZ showed Rf value 0.55 at 231 nm. The developed method was validated in terms of linearity, range, precision, accuracy, robustness as per the International Conference on Harmonization guidelines Q2 (R1). It showed a significant calibration coefficient 0.993 with a linearity of concentration (40-280ng/spot).The limit of detection (LOD) and the limit of quantification (LOQ) of the method was found to be 1.64 and 4.97 ng/spot respectively.

KEYWORDS: Gliclazide, High-Pressure Thin Layer Chromatography, Method developeent, Method validation, ICH guidelines.

INTRODUCTION

Gliclazide is a potential second generation oral hypoglycemic agent widely used for the treatment of noninsulin-dependent diabetes mellitus (NIDDM). The literature of prior research work revealed that it has good general tolerability, low incidence of hypoglycemia and low rate of secondary failure. In addition, it has potential for slowing the progression of diabetic retinopathy. Hence, gliclazide can be considered as a drug of choice in long-term sulfonylurea therapy for the control of NIDDM.[1]

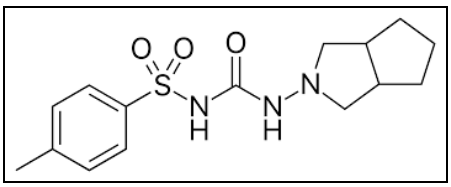

Figure 1: Chemical structure of Gliclazide

Analytical methods such as UV spectrophotometric methods,[3] Reverse Phase High Performance Liquid Chromatography,[4] LCMS,[5] Thin Layer Chromatography,[6] High Pressure Liquid Chromatography,[7] High-Pressure Thin Layer Chromatography,[8] methods have been reported for the analysis of GLZ. Nowadays, high performance thin layer chromatography (HPTLC) has become a routine analytical technique due to its advantages of reliability in quantitation, analysis at microgram and even in nanogram levels and cost effectiveness. The large number of samples can be simultaneously subjected to analysis. The amount of solvent required in comparison to HPLC is very less. This reduces the time and cost of analysis. HPTLC also facilitates repeated detection of the chromatogram with same or different parameters. Simultaneous assay of several components in a multicomponent formulation is also feasible. Hence, the present work was undertaken to develop and validate a simple, rapid, accurate, precise and specific HPTLC method for determination of GLZ in bulk as well as in pharmaceutical dosage form.[9]

MATERIALS AND METHODS

Materials and reagents

1. Gliclazide was obtained from Cipla, Mumbai.
2. Marketed tablet 'GLIZIDE' was obtained commercially with labelled amounts of 30 mg of GLZ.
3. Reagents: Toluene, Methanol, Acetonitrile of AR grade were used as solvents for the preparation of

mobile phase (S.D. FINE CHEMICALS, Mumbai, Maharashtra, India).
4. Silica Gel 60 F254 TLC plates (MERCK) as a stationary phase.

Experimentation

Preparation of standard solution

A standard stock solution of GLZ was prepared by dissolving 10 mg of standard drug in 10 ml of methanol which obtains 1000 µg/ml concentration of GLZ. This solution was further diluted to get different required concentrations of GLZ. 100 µg/ml solution of GLZ was selected as working standard.

Sample Preparation

Ten tablets were weighed and the average weight was calculated. The tablets were then finely powdered and an amount equivalent to 10 mg of GLZ was dissolved in a 10ml volumetric flask, and sufficient amount of methanol was added to dissolve the drug. The mixture was ultra-sonicated for 15 min and diluted to 10ml with the same solvent. The solution was double filtered, first through 0.45μm Whatman filter paper and after that through 0.45μm syringe filter in order to get a clear solution.

Selection of wavelength for detection

The prepared working standard of GLZ in methanol was scanned by UV visible detector over the wavelength range 200 to 400 nm to check wavelength of API. The wavelength of 231nm was selected for further detection of samples and the resulted chromatogram is showed in (Figure 2).

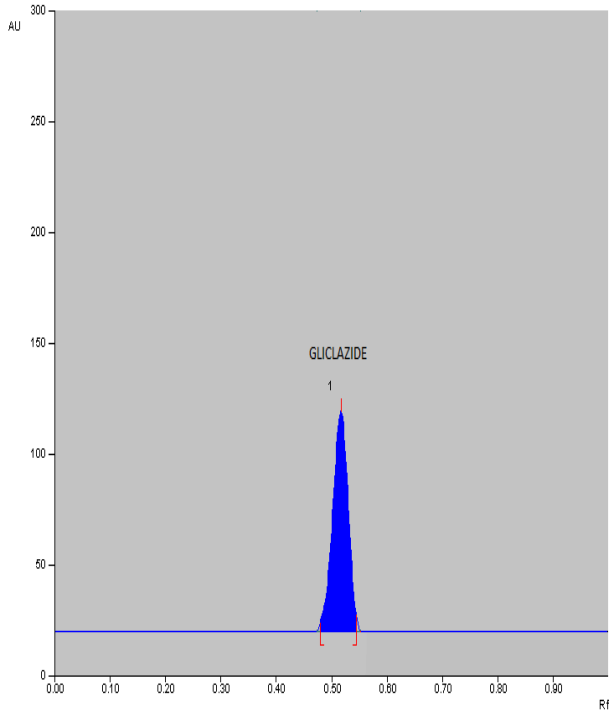

Figure 2: Chromatogram of 100 µg/ml solution of Gliclazide.

Chromatographic conditions

- HPTLC was performed on pre-coated silica gel 60 F254 TLC (E-Merck, Germany) plates (10x10 cm). TLC plates were prewashed with methanol and activated at 110°C for 10 min prior to application. The 20 µl standard samples of GLZ were spotted on pre-coated TLC plates in the form of bands of 6 mm width under a stream of nitrogen gas using a 100 µl sample syringe with a Camag Linomat sample applicator. The space between two bands was fixed at 8 mm.
- On the basis of trial and error method, toluene: acetonitrile: methanol (7:2:1 v/v) was selected as mobile phase composition which resulted in well-defined and sharp peaks of GLZ with RF=0.55 ±0.02. Chamber was saturated with the mobile phase for 20 minutes in Camag twin trough chamber at room temperature. The mobile phase is allowed to run at migration distance of 90 mm.
- The developed TLC plate was air dried and densitometric analysis was carried out using Camag TLC Scanner 3 using the WinCATS software. The absorbance mode was set at 231 nm for all measurements. The slit dimension was kept at 5.00 mm × 0.45 mm.

Preparation of Calibration curve

Different concentrations of the working standard solution 40, 80, 120, 160, 200, 240 and 280 ng/spot were applied on the TLC plate, corresponding peak areas were recorded and the graph of peak area vs. concentration was extrapolated.

Method validation

The purpose of validation of an analytical method is to signify whether the procedure is appropriate for its intended purpose. The developed 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.

Range and linearity

The linearity of an analytical procedure is its ability to produce test results which are directly proportional to the amount of analyte in the test sample. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the method is determined by analyzing standard GLZ and GLZ extracted from tablets.

Precision

The precision of an analytical procedure indicates the closeness of agreement between a series of measurements obtained from multiple sampling of the

same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, interday precision and intraday precision.

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

The detection limit of an analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision.

The values of LOD and LOQ were calculated based on the standard deviation of the response and the slope of calibration curve. The detection limit and quantitation limit is expressed as follows

LOD= 3.3 * SD/S

LOQ= 10 * SD/S

Where,

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

S is a mean slope of five calibration curves.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy should be measured as percent recovery by the assay of known amount of analyte added in the sample. 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 %.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate changes in method parameters and provides an indication of its reliability during normal use. The % RSD values less than 2 indicate robustness of method.

RESULTS AND DISCUSSION

Linearity and Range

Standard solutions of 2, 4, 6, 8, 10, 12, 14 ppm were prepared and 20µl of each of these solutions were spotted on the TLC plate. The calibration plot was obtained by plotting peak area against concentration to find out Regression equation and correlation coefficient as shown in Figure 3.

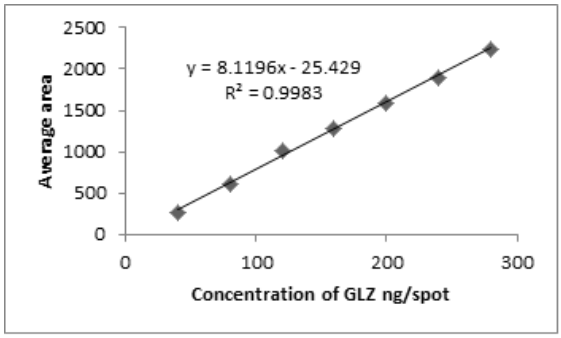

Figure 3: Calibration Curve of a Gliclazide.

Specificity

The specificity of the method was ascertained by analyzing standard GLZ and GLZ extracted from tablets. The spot for the drug in a sample was confirmed by comparing the Rf and spectra of the spot with that of standard drug spot (Figure 4).

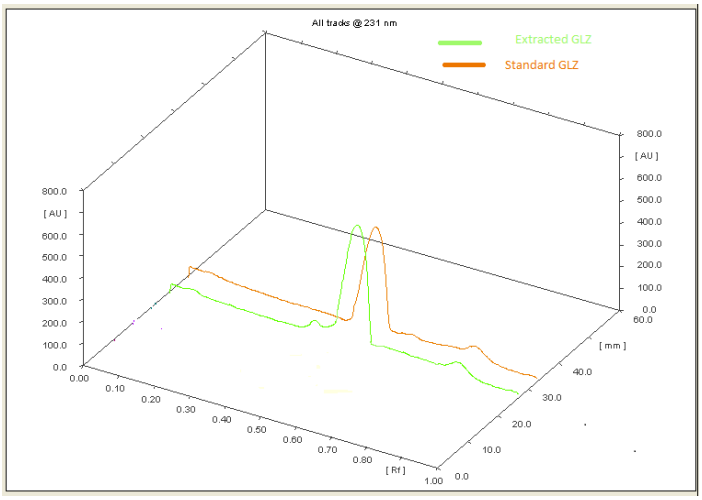

Figure 4: Spectra of standard Gliclazide and Gliclazide extracted from tablet.

Precision

The precision of the method was verified by repeatability, intraday and interday precision studies.

Repeatability

Repeatability studies were performed by analysis of one concentration (160ng/spot) of the drug six times on the same day. The peak area of each spot is measured and Relative standard deviation (%RSD) was calculated which were found to be within acceptable limits as shown in Table 1.

Table 1: Data for repeatability study.

Sr. no.	Concentration (ng/spot)	Area	% RSD
1	160	1289	0.78
2	160	1280	
3	160	1293	
4	160	1301	
5	160	1279	
6	160	1274	

Intra-day precision

The intraday precision of the method was performed by analyzing standard solutions of GLZ at three different concentration levels covering low (40ng/spot), medium (160ng/spot), high (260 ng/spot) for three times on the same day. The peak areas obtained were used to calculate % RSD for intra-day precision studies which were found to be within acceptable limits as shown in Table 2.

Table 2: Intraday Precision study of Gliclazide.

Conc. levels	Conc. (ng/spot)	Average peak area			S.D.	% RSD
		Session 1	Session 2	Session 3		
Low	40	287	284	279	283.33	1.42
Mid	160	1295	1280	1291	1288.66	0.60
High	280	2253	2248	2269	2256.66	0.48

Inter-day precision

The inter-day precision of the method was assessed by analyzing standard solutions of GLZ at three different concentration levels covering low (40ng/spot), medium (160ng/spot), high (260 ng/spot) for three different days. The peak areas obtained were used to calculate % RSD for inter-day precision studies which were found to be within acceptable limits as shown in Table 3.

Table 3: Interday Precision study of Gliclazide.

Conc. levels	Conc. (ng/spot)	Average peak area			S. D.	% RSD
		Day 1	Day 2	Day 3		
Low	40	287	290	280	285.66	1.79
Mid	160	1295	1274	1285	1287	0.56
High	280	2253	2240	2285	2252.33	0.53

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

The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated based on the standard deviation of the response and the slope of calibration curve. The obtained results are shown in Table 4.

Table 4: Results for Limit of detection and Limit of Quantification.

Sr. no.	Parameters	Results (ng/spot)
1	LOD	1.64
2	LOQ	4.97

Accuracy

For recovery studies the basic concentration of sample chosen was 200 ng/spot. The drug concentration was calculated using regression equation. The recovery of the method was determined by comparing the determined concentration of spiked samples to the theoretical concentrations. The average percentage recovery was calculated at each concentration level and obtained results are tabulated as in Table 5.

Table 5: Recovery studies of GLZ from a marketed formulation.

% level	Amount present in extract (ng/spot)	Amount present in std sample (ng/spot)	Total	Average peak area	% Recovery	Average % Recovery
80	200	160	360	2811	97.03	97.93
100	200	200	400	3197	99.21	
120	200	240	440	4728	97.57	

Robustness

Robustness of the method was determined by making small changes in the mobile phase composition and chamber saturation time. The % RSD values calculated and were found to be below 2 % which indicated the robustness of the method. The results are given in Table 6.

Table 6: Results for Robustness parameter.

Robustness parameters	Changed parameters for robustness	Average peak area	% RSD
Mobile phase Composition	Toluene: Acetonitrile: Methanol (7.2: 2.2: 1.2)	1178.33	1.62
	Toluene: Acetonitrile: Methanol (6.8: 1.8: 0.8)	1180	1.32
Saturation time	22min	1286.23	0.29
	18min	1283.66	0.32

CONCLUSION

A unique HPTLC method has been developed for the identification and quantification of GLZ. It can be concluded that the developed method of HPTLC for GLZ is precise, accurate, as %RSD values are within limits. According to % RSD values, current developed method can be considered as specific, reproducible, and robust. It also suggests that this method can be used for routine estimation of GLZ in bulk as well as in pharmaceutical dosage forms.

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