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Determination of Glibenclamide in Tablets by Densitometric HPTLC

Shweta S. Havele, Sunil R. Dhaneshwar*

Research and Development Centre in Pharmaceutical Sciences and Applied Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune – 411038, Maharashtra, India.

ABSTRACT

A simple and sensitive, HPTLC method has been developed for the quantitative estimation of glibenclamide in its single component tablet formulation. Glibenclamide was chromatographed on silica Gel 60 F_{254} TLC plate using toluene: ethyl acetate: methanol in the ratio of 8.0:0.5:1 (v/v/v) as mobile phase. Glibenclamide showed R_f value of 0.45±0.07 and was scanned at 229 nm using Camag TLC Scanner 3. The linear regression data for the calibration plot showed a good relationship with r =0.9994. The method was validated for precision and recovery. The limits of detection and quantification were 6 and 20 ng/spot respectively. The developed method was successfully used for the assay of glibenclamide tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

Keywords: Thin layer chromatography, Pharmaceutical analysis, Antidiabetic drug, Glibenclamide Tablet, Bulk drug

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INTRODUCTION

Glibenclamide (glyburide) (Fig. 1) 1[[p-[2-(5-chloro-o-anisamido)ethyl] phenyl] sulfonyl]-3-cyclohexylurea is a sulfonylurea derivative is a potent, second generation oral antidiabetic agent widely used for treatment of hyperglycemia in patients with type-II non-insulin dependent diabetes mellitus [1]. It acts mainly by stimulating endogenous insulin release from beta cells of pancreas [2]. Different HPLC methods coupled with UV detection [3-11], fluorescence detection [1-14], or mass spectrometry [15–18], capillary electrophoresis [19-20], TLC in human serum [21].

But these methods are sophisticated, expensive and time consuming as compared to simple HPTLC method.
Nowadays, HPTLC is becoming a routine analytical technique due to its advantages [22–24].

There is a need for a simple, rapid, cost effective and reproducible method for assay of GLY in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise HPTLC method for the analysis of glibenclamide (GLY) in its tablet formulation. The objective of the current work is, therefore, to develop a simple HPTLC method for analysis of GLY hydrochloride in tablet formulations.

MATERIALS AND METHODS

2. Experimental

2.1 Materials

GLY working standard was a generous gift (Batch No.-000019105). from Ranbaxy, Indore, India. Silica gel 60 F_{254} TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used as a stationary phase. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. Daonil® containing 5 mg of GLY were purchased from sanofi-aventis (T-1) (Batch No.-029079), Semi euglucon 2.5 MG containing 10 mg GLY were purchased from MMC healthcare (T-2)) (Batch No.-EO127810310).

2.2 Instrumentation

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm × 10 cm), Camag winCATS software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on precoated silica gel 60 F_{254} TLC plates (10 cm × 10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

2.3 Preparation of standard solutions

A stock solution of GLY was prepared by dissolving 100 mg in 100 ml methanol (1000 µg/ml). Further standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 20 µg/ml.

2.4 Sample Preparation

Two brands of tablets T-1 and T-2 were selected. Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to one tablet was dissolved in 50 ml methanol. To ensure complete extraction of the drug it was sonicated for 45 min. This solution was filtered through a Whatman no. 41 paper.

2.5 HPTLC method and chromatographic condition

In the proposed HPTLC method, the samples were streaked on the precoated TLC plates in the form of a narrow band 6 mm in length, 10 mm from the bottom and margin and 10 mm apart at a constant flow rate of 150 nl/s by using a nitrogen aspirator. A Camag Twin Trough Chamber was saturated for 20 min at room temperature (25 ± 2 °C) with the mobile phase containing a mixture of toluene: ethyl acetate: methanol in the ratio of 8.0:0.5:1 (v/v/v). After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at 229 nm for all measurements. The slit dimension was kept at 5.0 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. GLY was detected at R_f of 0.45±0.07. The chromatograms were integrated using winCATS evaluation software (Version 1.1.3.0).

2.6 Method validation

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

2.6.1. Linearity and range

From the standard solution 20 µg/ml of GLY, 2 to 10 µl solutions were spotted on HPTLC plate to obtain final concentration of 40- 200 ng/spot for GLY. Each concentration was applied six times on the HPTLC plate. Peak area was recorded for each concentration and a calibration plot was obtained by plotting peak area against concentration

2.6.2 Limit of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

2.6.3 Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (40, 120, 200 ng/spot) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on two different days.

2.6.4 Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for GLY in sample was confirmed by comparing the R_f and spectra of the spot with that of standard. The peak purity of GLY was assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

2.6.5 Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like, toluene: ethylacetate: methanol (8.5:0.5:0.5 v/v/v), (8.0:0.5:1.0 v/v/v) (8.0:0.5:0.2, v/v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5 %. The plates were prewashed by methanol and activated at 60 ^{o}C ± 5 for 2, 5, 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. Robustness of the method was done at three different concentration levels 40, 120, 200 ng/spot for GLY.

2.6.6 Analysis of marketed formulation

Twenty tablets of each brands were weighed their average weight calculated, tablets finely powdered and the powder equivalent to containing 5 mg and 10 mg of GLY from T-1,T-2 respectively and dissolved in 50 ml of methanol. The solution was sonicated for 45 min and then filtered through Whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined. Each of these solutions (1 µl) were spotted on plates and analyzed for GLY in the same way as described earlier.

2.6.7 Recovery studies

Recovery studies were carried out to check the accuracy of the method. Recovery experiments were performed by adding three different amounts of GLY i.e., 80, 100 and 120% of the labeled amount of GLY analyzed from the GLY formulations and the resultant were reanalyzed (n = 6).

RESULTS AND DISCUSSION

3.1 Development of the optimum mobile phase

Initially chloroform: methanol (8.0:2.0 v/v) in varying ratio was tried. Then toluene: ethyl acetate: methanol was tried in different ratios in order to achieve good resolution. The mobile phase of toluene: ethyl acetate: methanol (8.0: 0.5: 1.0, v/v/v) gave good resolution with R_f value 0.45 for GLY and a sharp and symmetrical peak. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature (Fig. 2.). The analytical wavelength, 229 nm, was chosen on the basis of the absorption spectrum recorded in the range 200–800 nm.

3.2. Validation of the method

3.2.1 Linearity

Linearity for GLY was observed in the range of 40−200 ng/spot with a correlation coefficient of 0.999 and the linear regression equation was y = 15.57x + 28.36 (Table 1).

3.2.2. Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 0.91. The results shown in Table 2 revealed intra- and inter-day variation of GLY at three different concentration levels 40, 120, 200 ng/spot. The % RSD for within and day-to-day analysis was found to be <2%

3.2.3 Robustness of the method

The standard deviation of peak area was calculated for each parameter and % R.S.D. was found to be less than 2%. The low values of % R.S.D as shown in Table 3 indicated robustness of the method.

3.2.4 LOD and LOQ

The signal to noise ratios 3:1 and 10:1 were considered as LOD and LLOQ respectively. The LOD and LOQ were found to be 6 and 20 ng/spot respectively.

3.2.5 Specificity

The peak purity of GLY was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.998 and r (middle, end) = 0.9993. Good correlation (r=0.9991) was also obtained between standard and sample spectra of GLY.

3.3.6 Recovery studies

The proposed method when used for extraction and subsequent estimation of GLY from pharmaceutical dosage form after spiking the preanalysed sample with 80, 100 and 120 % of label claim of GLY afforded recovery of 99.39-100.10 % as listed in Table 4.
The data of summary of validation parameters are listed in Table 6.

3.3.7 Analysis of marketed formulations

A single spot at R_f 0.45 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The results, given in Table 5, indicate that the amount of drug in the tablets is within the requirement of 99.82-101% of the label claim.

Table 1 : Linear regression data for the calibration curves[a]

Linearity (ng/spot)	r ± S.D.	Slope ± S.D.	Intercept ±S.D.
40-200	0.999 ± 0.05	28.42± 0.09	15.57± 01.2

[a] n = 6

Table 2: Intra- and inter-day precision of HPTLC method [a]

Inter-day precision		Inter-day precision	
S.D of areas.	% R.S.D.	S.D of areas.	% R.S.D.
0.90	1.09	1.81	0.91

[a] n = 6, Average of three concentrations 40, 80, 120 ng/spot.

Table 3 : Robustness testing[a]

Parameter	SD of peak area	% RSD
Mobile phase composition	0.38	1.14
Amount of mobile phase	0.18	1.89
Temperature	0.60	0.05
Relative humidity	1.26	1.81
Plate pretreatment	0.09	0.02
Time from spotting to chromatography	0.10	0.09
Time from chromatography to scanning	0.07	0.03

[a] n = 6, Average of three concentrations 40, 80, 120 ng/spot.

Table 4: Applicability of the HPTLC method for the analysis of the pharmaceutical formulations

Formulation	Label Claim (mg)	Drug Content (%)	% R.S.D.
T-1	500	99.82	0.98
T-2	850	101.0	1.30

[a] n=6

Table 5: Recovery studies [a]

Formulation	Amount of drug added (%)	Thereotical content (ng)	Amount of MET recovered (ng) mean	% Recovery
T-1	80	18000	18018	100.10
	100	20000	19846	99.93
	120	22000	21995.6	99.98
T-2	80	30600	30544.92	99.82
	100	34000	33874.2	99.63
	120	37400	37171.86	99.39

[a] n = 6

Table 6: Summary of validation parameters

Parameter	Data
Linearity range	40-120 ng/ml
Correlation coefficient	0.9994 ± 0.09
Limit of detection	6 ng/ml
Limit of quantitation	20 ng/ml
Recovery (n = 6) T-1 T-2	 99.82 101.0
Precision (% RSD) Repeatability of application Inter day (n = 6) Intra day (n = 6)	 1.82 1.28 1.91
Robustness	Robust
Specificity	0.9991

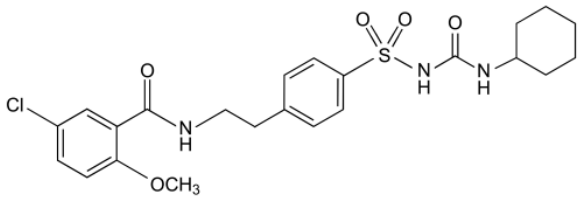

Fig. 1. Chemical structure of Glibenclamide

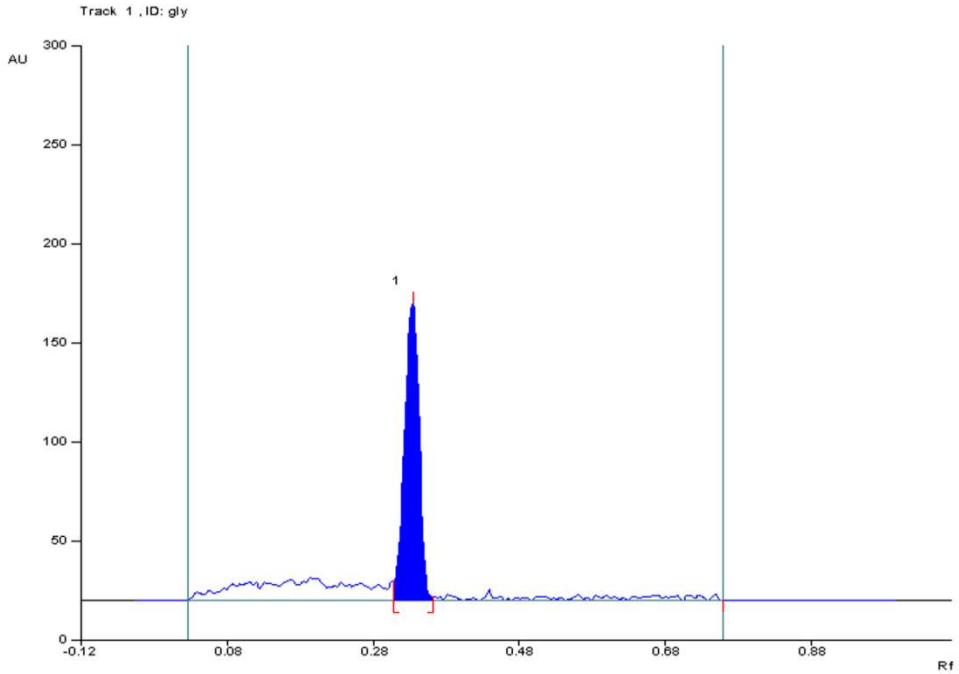

Fig. 2. Densitogram of standard GLY (2000 ng/spot); peak 1 (R_F: 0.45±0.07). toluene: ethyl acetate: methanol (8.0: 0.5: 1.0, v/v/v).

CONCLUSION

A new HPTLC method has been developed for the identification and quantification of GLY in formulations. The method was found to be simple, sensitive, precise, accurate and specific for estimation and can be conveniently employed for the routine quality control analysis of GLY from tablets.

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