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Development and validation of TLC densitometry method for the determination of Gliclazide in tablet dosage form

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ABSTRACT

Some tablet pharmaceutical preparations containing Gliclazide as sole active ingredient, are marked now in Indonesia. The determination of the component of preparation both qualitatively and quantitatively is important to ensure quality of the product. This research is aimed to develop a TLC method to analyze gliclazide in its single component tablet formulation. Gliclazide were dissolved with chloroform. Gliclazide was chromatographed on silica Gel 60 F254 TLC plate using toluene: ethyl acetate in the ratio of 1:1 (v/v) as mobile phase. Gliclazide showed Rf value of 0.58 and was scanned at 234nm using Camag TLC Scanner 3. This method validation showed a good linearity with correlation coefficient (r) of 0.999 while the coefficient of variation of the regression function (Vx0) = 2.406%. Specificity showed calculation of purity and identity more than 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) of the method was respectively 28.78 and 86.35 ng/spot respectively. The mean absolute recovery of Gliclazide from the simulation sample was 100.5% ± 0.113 and the method precision was less than 5.3% whereas the mean of the recovery data was 100.3%. The proposed method has been applied to the determination of Gliclazide in commercial tablet formulations and the recovery of label claim were100.494%±0.348 for brand A and 100.111%±1.257% for brand B. The developed method was successfully used for the assay of gliclazide. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

***Key Words*:** Gliclazide, TLC, Validation.

INTRODUCTION

Gliclazide 1- (Hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4-methylphenyl)sulphonyl]urea (Figure 1), is an oral hypoglycemic drug, belonging to second-generation sulphonylureas, used in type-II diabetes (Gunawan *et al.*, 2007). Different analytical methods including HPLC (British Pharmacopoeia Commission, 2009) and spectrophotometric UV-VIS (Revathi *et al.*, 2010; Samina *et al.*, 2011) estimation of gliclazide in dosage forms have been reported for determination of gliclazide. However, no TLC densitometry method is available for quantitative determination of gliclazide in its pharmaceutical dosage forms. Some reported analytical methods are both time and money consuming (Wulandari *et al.*, 2012). There is a need for a simple, rapid, cost effective and reproducible method for assay of gliclazide in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise TLC method for the analysis of gliclazide in its tablet formulation. The objective of the current work is, therefore, to develop a simple TLC method for analysis of gliclazide in tablet formulations.

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EXPERIMENTAL

Materials and Reagents

Gliclazide working standard was a generous gift (Shandong Keyuan Pharmaceutical, Co., LTD; Batch No. 1003-221; Assay 99.2, Expired date: 24 Maret 2014) from Ikapharmindo, Jakarta, Indonesia. Toluen, chloroform (Merck) and ethyl acetate (Riedel-de Haën) were of pharmaceutical grade reagents. Commercial tablet containing 80 mg gliclazide were purchased from local pharmacy in Jember, East Java, Indonesia (April 2012).

Table 1: Optimum condition for analysis of gliclazide.

Parameters	Data
Solute	chloroform
Eluen	toluene: ethyl acetate = 1:1 (v/v)
Stationary phase	silica gel GF 254
λ- max	234 nm
Concentration optimum	400 µg/ml

Table 2: Result of precision evaluation.

Measurement[a]	RSD value [%] (n=6)[b]
1	0.427%
2	1.6%
3	1.21%
Average RSD	1.077%

[a]Each measurement was perfomed by the same analyst and on a different plate and different days
[b]Evaluted by one analyst on one plate (repeatability)

Standard Preparation

Stock standard solution was always freshly prepared by dissolving gliclazide (10 mg) in 25 ml chloroform (400µg/ml).

Sample Preparation

Two brands of tablets A and B were selected. Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to 10 mg of gliclazide was dissolved in 25ml volumetric flask, and approximately 15ml of chloroform was added. The mixture was ultrasonicated for 15 min and diluted to 25ml with the same solvent. This solution was filtered through a Nillon paper.

TLC Method and Chromatographic Condition

Chromatographic was performed on precoated 20cm x 10cm TLC silica gel GF 254 alumunium-backed sheets Merck. A Blaubrand equipped with 2.0 µl glass capillaries. A Camag Twin Trough Chamber was saturated for 30 min with the mobile phase containing a mixture of toluene: ethyl acetate in the ratio of 1:1 (v/v). After chamber saturation, the plates were developed to a distance of 9 cm. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at 234 nm for all measurements. The slit dimension was kept at 6.00 mm × 0.30 mm and a scanning speed of 20 mm/s was employed. Gliclazide was detected at Rf of 0.58. The chromatograms were integrated using winCATS evaluation software (Version 1.4.1.8154).

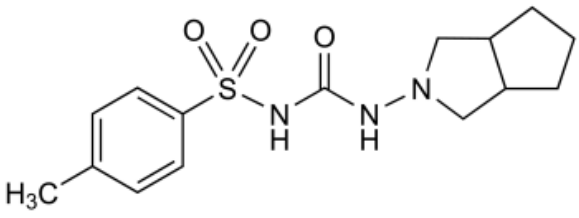

Figure 1: Chemical structure of gliclazide.

Method validation

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

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. Specificity is showed by purity and identity test. The purity and identity of the analyte spots were determined by scanning 200 nm- 400 nm. Calculations for identity checks ($r_{S,S}$ and $r_{S,A}$ where S is spectrum standard and A is spectrum sample and purity checks ($r_{S,M}$ and $r_{M,E}$ where S = start, M = center; and E = end of spectrum)

Linearity

Standard solution were prepared containing 150, 200, 300, 400, 450, 600, 750, 800µg/ml and 2µl of each of these solutions was spotted on the TLC plate. Peak area was recorded for each concentration and a calibration plot was obtained by plotting peak area against concentration.

Limit of Detection and Quantification

Standard solution were prepared containing 50, 75,

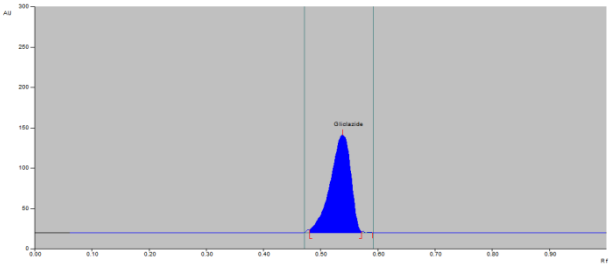

Figure 2: Densitogram of standard gliclazide (800 ng/spot); peak 1 (RF: 0.58).
toluene: ethyl acetate (1.0: 1.0, v/v).

Table 3: Accuracy result of commercial tablets*.

Label claim [%] (mean ± SD)	Added [%]	Recovery
100.3 ± 1.007	30%	100.4%
		100.2%
		100.6%
	45%	100.6%
		100.5%
		100.5%
	60%	100.3%
		100.6%
		100.4%
Average recovery ± SD		100.5%±0.113

*Analysis was performed with Brand A of Gliclazide

100, 150, 200, 225, 250 and 300µg/ml and 2µl of each of these solutions was spotted on the TLC plate. Peak area was recorded for each concentration and a calibration plot was obtained by plotting peak area against concentration. Concentration (ng) were evaluated by software validation method version 1.13.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of one concentrations 800ng/spot) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Accuracy

Recovery studies were carried out to check the accuracy of the method. Recovery experiments were performed by adding three different amounts of pure gliclazide i.e., 30, 45 and 60% of the labeled amount of gliclazide analyzed from the gliclazide formulations and the resultant were reanalyzed (n = 3).

Analysis of marketed formulation

Twenty tablets of each brands were weighed their average weight calculated, tablets finely powdered and the powder equivalent to containing 10 mg of Gliclazide from sample brand A and brand B 25 ml of chloroform. The solution was sonicated for 15 min and then filtered through Nilln paper. Each of these solutions (2 µl) were spotted on plates and analyzed for gliclazide in the same way as described earlier.

Table 4: Results of analysis of Gliclazide in pharmaceutical formulation.

No.	Formulation	% Recovery ± SD
1	Brand A	100.5% ± 0.319
2	Brand B	100.1% ± 1.268

RESULTS AND DISCUSSION

Optimum condition

Table 1 showed optimum conditions for analysis gliclazide using TLC. The mobile phase of toluene:ethyl acetate (1,0 : 1.0, v/v) gave efficiency chromatogram (N value is bigger and H is smallest) with Rf value 0.58 for glicazide and a sharp and symmetrical peak. The analytical wavelength, 234 nm, was chosen on the basis of the absorption spectrum recorded in the range 200-400 nm. Concentration optimum 400 ppm (µg/ml) was chosen on the basis of efficiency chromatogram and % recovery 90%-107% (Huber, 2007) [Figure 1].

Validation of the method

The proposed TLC system demonstrated that all analyte spots in samples furnished in situ uv spectra that were identical to those of standards ($r_{S,S}$ and $r_{S,A} \geq 0.99$). Purity check of the analyte spots using the winCATS software also showed that analyte spots of the extract were pure. The values of $r_{S,M}$ and $r_{M,E}$ were ≥0.99, demonstrating that the proposed TLC method is highly specific. Linearity of gliclazide was achieved from 300-1600 ng/spot (n= 8) obeying the equation Y= 513.956+4.525x with r 0,999. The liniear regression data is presented in Figure 3. The LOD and LOQ (Limit of Detection and Quantification) were found to be 28.78 and 86.35 ng/spot respectively.

All the values of the repeatability and intermediate precision evaluation were less than 5.3% (Table 2). These values were also less than the required values (5.3%; specification range of 90%-107%; n=6) (Huber, 2007) The three measurement were perfomed within one laboratory by same analyst on different plates and different days.

Table 3 demonstrates the high accuracy of the proposed method as revealed by the precentage of mean recovery data (100.5% ± 0.113). To test whether systematic errors had occured, a liniear regression of the recovery curve of Xf (percentage of label claim of the analyte found by proposed

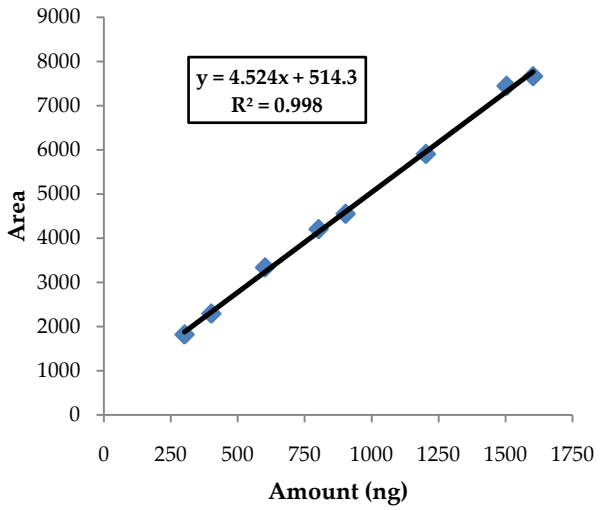

Figure 3: Calibration curve of gliclazide.

Table 5: Validation parameters for standard gliclazide.

Parameter	Value
Specificity	Purity test ≥ 0.99 Identity test ≥0.99
Linierity	r=0.999 Vxo=2.406%
Sensitivity	LOD=28.78 ng/spot LOQ=86.35 ng/spot
Precision	Average RSD 1.077%
Accuracy	Average recovery±SD = 100.5%±0.113

method) againts Xc (nominal precentage of label claim of the analyte after addition with the standards). The data of summary of validation parameters are listed in Table 5.

Analysis of marketed formulations

A single spot at Rf 0.58 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 4, indicate that the amount of drug in the tablets is within the requirement of 95%-105% of the label claim. The percentage recovery values for brand A and brand B were found to be 100.494%±0.348 and 100.111%±1.257% respectively .

CONCLUSION

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

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