DEVELOPMENT AND VALIDATION OF A TLC DENSITOMETRIC METHOD FOR DETERMINATION OF GLIMEPIRIDE IN TABLETS**PENGEMBANGAN DAN VALIDASI METODE TLC DENSITOMETRI UNTUK DETERMINASI GLIMEPIRIDE DALAM SEDIAAN TABLET**

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ABSTRACT

A simple and valid TLC method has been developed for the determination of glimepiride in tablet formulation. After extraction of the analyte with a mixture of methanol and ammonia 0,2M (1:1, v/v), the extracts were spotted on precoated TLC silica gel F254 plates, which were developed with a mixture of toluene:methanol:ethyl acetate (75:20:5, v/v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 238 nm. The method was validated for specificity, linearity, accuracy and precision. Good linearity was achieved in the concentration range 100–800 ng/spot. The RSD of repeatability and intermediate precision were found to be less than 2%, whereas the mean of the recovery data was 100-101%. The detection limit and quantification limit were 22 and 74 ng/spot, respectively. The method is specific, linear, precise, and accurate; it can be used for the routine quality control testing of marketed formulations.

Keywords: glimepiride, TLC densitometric, validation of pharmaceutical methods, pharmaceutical analysis, antidiabetic drug

ABSTRAK

Sebuah metode Kromatografi Lapis Tipis (KLT) yang sederhana dan valid telah dikembangkan untuk penentuan glimepiride dalam sediaan tablet. Setelah analit dalam sampel diekstraksi dengan campuran metanol dan amonia 0,2 M (1:1,v/v), ekstrak yang terlihat pada lempeng silika gel F254, yang dikembangkan dengan campuran toluen : metanol : etil asetat (75:20:5, v/v/v). Evaluasi kuantitatif dilakukan dengan mengukur reflektansi absorbansi noda analit pada panjang gelombang 238 nm. Metode ini divalidasi meliputi spesifisitas, linearitas, akurasi dan presisi. Linearitas yang baik dicapai pada rentang konsentrasi 100-800 ng / spot. RSD pengulangan dan presisi *intermediate* menunjukkan nilai kurang dari 2 %, sedangkan rata-rata data recovery adalah 100-101 %. Batas deteksi dan batas kuantifikasi adalah 22 dan

74 ng / noda. Metode ini spesifik, linear, tepat, dan akurat, bisa digunakan untuk pengujian kontrol kualitas rutin tablet glimepirid dipasarkan.

Kata kunci : glimepiride, TLC densitometric, validation of pharmaceutical methods, pharmaceutical analysis, antidiabetic drug

INTRODUCTION

Glimepiride (Fig. 1) 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl]phenyl]sulphonyl]-3-*trans*-(4-methylcyclohexyl)-urea is used as an antidiabetic drug in the treatment of type 2 diabetes⁽¹⁾. It is a second-generation sulfonylurea primarily activating specific receptors on pancreatic β -cell membrane. When glimepirides are bound to receptors, ATP-sensitive potassium channels close, thus resulting in an augmented trans-membrane calcium flux and insulin release from β -cells⁽²⁾. Various liquid chromatographic methods have been reported for the determination of glimepiride in human plasma and biological fluids⁽³⁻⁷⁾. In bulk drug and in pharmaceutical preparations, multiple analytical procedures have been reported for the analysis of glimepiride when used as a single active principle or in combined dosage forms⁽⁷⁻¹⁴⁾. Some reported analytical methods involve time and money consuming. There is a need for a simple, rapid, cost effective and reproducible method for assay of glimepiride in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise TLC method for the analysis of glimepiride in its tablet formulation. The objective of the

development of a selective and specific method of analysis.

The proposed TLC system demonstrated that all analyte spots in samples furnished in situ UV spectra that were identical to those of standards ($r \geq 0.9997$). Purity check of the analyte spots using the CATS software also showed that all analyte spots of the extracts were pure. The correlation of the spectra taken at the peak start slope and at the peak maximum ($r_{s,M}$) was 0.9999 and the correlation of the spectra taken at the peak maximum and at the peak endslope ($r_{M,E}$) was 0.9997, demonstrating that the proposed TLC method is highly selective.

Linearity

The linear regression data for the calibration plots ($n = 8$) showed a good linear relationship over a concentration range of 100 – 800 ng/2 μ L spot (concentration of analyte in the spotted solution) for GMP. The slope, intercept, correlation coefficient (r), and relative process standard deviation value (V_{x0}) are shown in Table 1.

Limit of detection and quantification

LOD and LOQ values were found to be 22 and 74 ng/2 μ L spot, respectively, and pointed towards adequate sensitivity of the method.

Precision

The repeatability of sample application and measurement of peak area were expressed in the terms of % CV and found to be 1.91. The results shown in Table 2 revealed intra- and inter-day variation of GMP in six replicates at a concentration of 200 μ g/mL. The % CV for within and day-to-day analysis were found to be less than 5.3%.

Accuracy

The accuracy of this method was confirmed by determining the average percentage recoveries from the sample by applying the standard addition method. Table 3 demonstrates the high accuracy of the proposed method as revealed by percentage of mean recovery data. the mean percentage recoveries of GMP sample was in accordance with fixed limits of 100 up to 101%, indicating the suitability of the developed method in quantifying the concentration of GMP in pharmaceutical tablets.

Analysis of commercial products

A single spot of GMP was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the matrix commonly present in the tablets. The drug content was found to be $99.6\% \pm 2.66$ and $98.1\% \pm 0.45$ for GMP-P and GMP-G respectively. It may therefore be inferred that degradation of GMP had not occurred in the marketed formulations that was analyzed by this method. The low % CV value, given in Table 4, indicated the suitability of this method for routine analysis of GMP in pharmaceutical dosage form.

CONCLUSION

A TLC method has been developed for the analysis and quantification of GMP in tablet formulation. This method was found to be specific, sensitive, precise, and accurate for determination and can be employed for the routine quality control analysis of GMP from tablets.

Figure 2: TLC –Chromatograms obtained from standard solution of GMP in toluene:methanol:ethyl acetate (75:20:5, v/v/v)

Table 1: Linear regression data for the calibration curve

Parameters	GMP
Linearity range (ng/spot)	100 – 800
R	0.9996
Slope	5.67
Intercept	1037
V_{x0}	1.63%

Table 2: Results of precision evaluation

Measurement ^{a)}	RSD value [%] (n = 6) ^{b)}
1	1.91
2	1.75

^{a)}Each measurement was performed by same analyst and on a different plate and different days (intermediate precision).

^{b)}Evaluated by one analysts on one plate (repeatability)

Table 3: Method accuracy results for GMP tablets

Sample	GMP (ng/spot)		% Recovery ^{a)} (mean $\pm$ %RSD)
	Xc	Xf	
GMP-P	770 ^{b)}	a = 774	100.5% $\pm$ 1.55
	782 ^{c)}	b = 782	100% $\pm$ 2.01
	794 ^{d)}	c = 799	101% $\pm$ 0.94

Xc is nominal concentration of analyte in the spotted solution
Xf is measured concentration of analyte in the spotted solution

^{a)}Mean $\pm$ SD (n = 3x3=9)

^{b)} addition 80%

^{c)} addition 100%

^{d)} addition 120%

Table 4. Results of analysis of Glimepiride in Commercial Product

Sample	GMP (ng/spot)		% Recovery ^{a)} (mean $\pm$ %RSD)
	Label claim	Result of measurement	
GMP-P	403	401.5	99.6 $\pm$ 2.67
GMP-G	400	392	98.1 $\pm$ 0.45

^{a)}Mean $\pm$ SD (n = 3)

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