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Research Article

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Development and validation of thin layer chromatography-densitometry method for determination of glimepiride in tablets

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ABSTRACT

A simple, selective and accurate thin layer chromatography (TLC)-densitometry method has been developed and validated for analysis of glimepiride in tablets. Glimepiride assay was performed by TLC-densitometry using silica gel 60 F_{254} plates as the stationary phase and a mixture of chloroform : methanol (9 : 1) as the best mobile phase. Standard solution of glimepiride in the range of 100-500 ppm resulted in a regression equation y = 1221.03 + 17.9959x with r = 0.9973. Glimepiride detection limit was 44.10598 ppm and the limit of quantification of glimepiride was 133.6545 ppm. Accuracy was obtained percent recovery for glimepiride was 101.19 % ± 2.67 % for Metrix® (PT Kalbe Farma) and 100.86 % ± 1,83 % for generic tablet (PT Dexa Medica). Precision intraday and interday had good repeatability as RSD ≤ 2 %. The analysis showed levels of glimepiride on a generic tablet of 104.68 % ± 0.50 % and glimepiride tablets under the trade name of 104.49 % ± 0.60 %. The levels glimepiride obtained have suitably qualified Indonesian Pharmacopoeia edition V, i.e. 90-110 %.

Keywords: Glimepirida, thin layer chromatography, densitometry, validation

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INTRODUCTION

Glimepiride (Fig. 1) is a derivative sulfonylurea class of drugs, with the chemical name 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3pyrolin-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methyl-cyclohexyl) urea [1]. Metformin-glimepiride tablets resulted in significantly greater reductions in glycosylated hemoglobin and fasting plasma glucose compared with metformin plus glibenclamide in patients with type 2 diabetes mellitus [2]. Glimepiride monotherapy markedly improved a rapid homeostatic model assessment (HOMA-R) with moderate insulin stimulation, which may account for the difference in macro vascular disease development as compared with the group receiving glibenclamide [3].

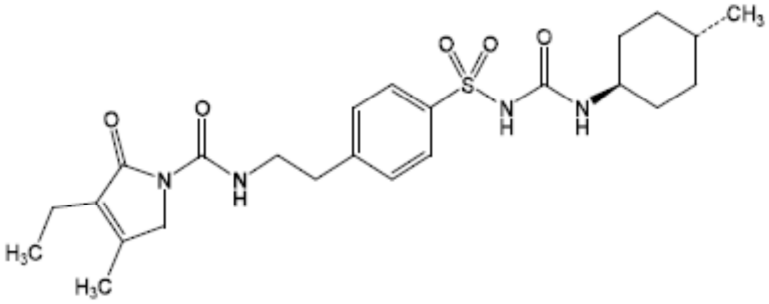


Fig. 1: Chemical structure of glimepiride

Later this as an antidiabetic drug use is increasing, because of the benefits that a low therapeutic dose and risk of hypoglycemia effect is smaller compared to other sulfonylurea class. Glimepiride has a long-term action with a half-

life of 5 hours, allowing once-daily dosing. Therefore patient compliance using this medicine will be increased so that the use of this drug is very profitable. This drug becomes the first choice in patients with type II diabetes mellitus [4].

The assay of glimepiride in tablets is usually carried out by high performance liquid chromatography as Indonesian Pharmacopoeia [1]. Literature survey revealed that several methods were used to analysis of glimepiride in tablets. These methods either in single or multiple components include ultraviolet-visible spectrophotometer [5, 6, 7, 8, 9, 10, and 11], derivative ultraviolet spectrophotometer [12, 13], thin layer chromatographic–densitometry assay [14], and high performance liquid chromatography [15, 16].

The aim of this study is performing very simple method of TLC-densitometry in terms of mobile phase and program to analysis glimepiride in tablet, and validation of method in according to ICH guideline [17].

EXPERIMENTAL SECTION

Materials, chemicals and equipment

The materials used in this study were the glimepiride raw material obtained from PT Tatarasa Primatama. Generic tablet (PT Dexa Medica, No. Batch 4406377) containing 4 mg glimepiride and glimepiride tablets under the trade name Metrix® (PT Kalbe Farma, No. Batch BN 524020) containing 4 mg glimepiride were procured from local market. Methanol, chloroform, acetone and potassium hydroxide were procured from Merck Indonesia. The tools used in this research were the UV lamp 254 and 366 nm (Camag), TLC Scanner 4 with software Wincat (Camag), Capillary pipette 5 µL size (Camag), Twin Chamber size 20 x 20 cm (Camag), silica gel plate 60 F_{254} 250 µm (Merck) size 20 x 20 cm, sonicator, vacuum desiccators, digital analytical balance (ABJ 220-4M type), and a filter paper (Whatmann No. 41).

Preparation of standard solution

A total of 100 mg of glimepiride was weighed carefully and put in a 100 mL volumetric flask, then dissolved in methanol while stirring and added methanol to the mark. The glimepiride solution contains 1 mg/mL or 1,000 ppm.

Method Development

Glimepiride solution was prepared using chloroform as solvent. The TLC plates were pre washed with methanol and activated by keeping at 115 °C for about 30 minutes. Solutions of 5.0 µL were applied on the TLC plates as using Camag Nanomat 4. Application positions were at least 10 mm from the sides and 10 mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left to saturate with mobile phase vapor for 15 minutes before each run. Mobile phase components were listed in Table 1.

Table 1: Component of mobile phase used in TLC of glimepiride analysis

Component of mobile phase	Ratio	Rf
Chloroform : Methanol, plate was sprayed with KOH 0.1 N	9 : 1	0.28
Chloroform : Methanol	9 : 1	0.60
Chloroform : Methanol	8 : 2	0.86
Chloroform : Methanol	7 : 3	0.94
Acetone	10	0.78
Methanol	10	0.86

Development of the plates was carried out by the ascending technique to a migration distance of 8 cm. The plates were dried by hair dryer. Densitometry scanning was done in absorbance mode at 320 nm using a deuterium lamp. The slit dimensions were set at 6 x 0.30 mm, the scanning speed at 20 mm/s and data resolution at 100 m/step. Single wavelength detection was performed because we are dealing with main component analysis and not impurities determinations where scanning at the individual λ values would be preferred. These conditions were transferred to the TLC system and the results were evaluated with the aim of achieving an optimum separation between spots ($Rs \geq 2$) and a migration of spots with Rf values between 0.2 and 0.8 in order to ensure separation reproducibility [18, 19].

Sample Preparation

The samples used were trademarks Matrix® tablets and generic glimepiride that each of them contain glimepiride 4 mg. Twenty tablets were weighed and then crushed and weighed an amount equivalent to 1 tablet, put in a 10 mL flask, then dissolved with methanol. The solution was vibrated by the sonicator for 15 minutes at a temperature of 30 °C, added volume to the mark, in order to obtain a solution of 400 ppm glimepiride.

Qualitative analysis of sample solution

TLC plate 10 x 4 cm was prepared and created a line of mark 1 cm from the bottom edge and 1 cm from the top. Standard solution, the generic samples and Metrix® each with a concentration of 400 ppm were spotted by 2 µL with 3 spots on the start line with a distance spotting each 1 cm. The plate was inserted into the chamber that had been saturated with mobile phase. Chamber was closed and left so that the mobile phase moves until it reaches the top line. Chamber was opened, the TLC plate was taken and dried by the wind. Then the value of Rf was determined by using UV lamp at 254 nm.

Quantitative analysis of sample solution

Test solution with a concentration of 400 ppm of each sample was spotted as many as three spots with a volume of 5 µL at the start line with a spot distance of 1 cm from each other. The plates were put in a chamber that has been saturated with mobile phase. The chamber was closed and left so that the mobile phase moves until it reaches the top line. The chamber was opened; the TLC plate was taken and dried. Spotting was observed under 254 nm UV lamps. Then spotting was scanned with tools Camag TLC Scanner 4 with a wavelength of 228 nm in order to get the data area under curve of the test compound. The area was included in the regression equation, and then obtained compound content.

Method Validation

Linearity

The glimepiride standard solution was pipette sequentially respectively 1, 2, 3, 4, and 5 mL in 10 mL volumetric flask and added methanol to mark boundaries in order to obtain a concentration of 100, 200, 300, 400, and 500 ppm. Glimepiride solutions were applied as much as 5 µL on a TLC plate silica gel 60 F_{254}, then eluted with an eluent to mark boundaries above and dried at room temperature. The plates were analyzed using a densitometer, so that would be obtained area under curve (AUC) for each concentration. Linearity was determined by processing the concentration data (x) and wide area (y) of the calibration curve obtained using the linear regression equation, in order to obtain the value of the correlation coefficient. The regression equation can be used if the correlation factor of $0.99 \leq r \leq 1$.The limits of detection and limits of quantization were calculated from a calibration curve statistically through linear line of the standard curve.

Precision

Testing was done by testing the repeatability as a variation in a day. The levels used in testing precision were 200, 400, and 500 ppm spotted on silica gel 60 F_{254} plates with a volume of 5 µL and eluted with eluent and dried. Spotting the silica plate and then analyzed by a densitometer. AUC data obtained were then calculated the average value, standard deviation (SD) and the relative standard deviation (RSD). The precision was tested with intra-day precision for one day in the morning, noon and afternoon, while the precision inter-day was checked by repeating the research for three consecutive days. Glimepiride concentration in the sample was calculated by regression equation obtained from the calibration curve.

Accuracy

Recovery studies were performed to check the accuracy of this method. This sample contained 400 ppm glimepiride. Recovery experiments were performed by adding three different amounts of glimepiride, i.e. 80, 100 and 120 %. These levels were expected to represent the lowest and highest levels of standard curve used. Samples were spotted on a silica gel 60 F_{254} plates each 3 times application with application volume 5 µL and eluted with the eluent. Spotting the silica plate was then analyzed by densitometry and the data will be obtained in the form of AUC values of samples that have been added to the standard.

RESULTS AND DISCUSSION

The experimental results showed the best mobile phase that can be used for analysis of glimepiride was chloroform : methanol (9 : 1), because once used as a mobile phase for 3 consecutive days Rf value obtained was stable and enter the range of 0.60 (Table 1). Qualitative analysis of samples showed that glimepiride contained in the two samples (Fig. 1).

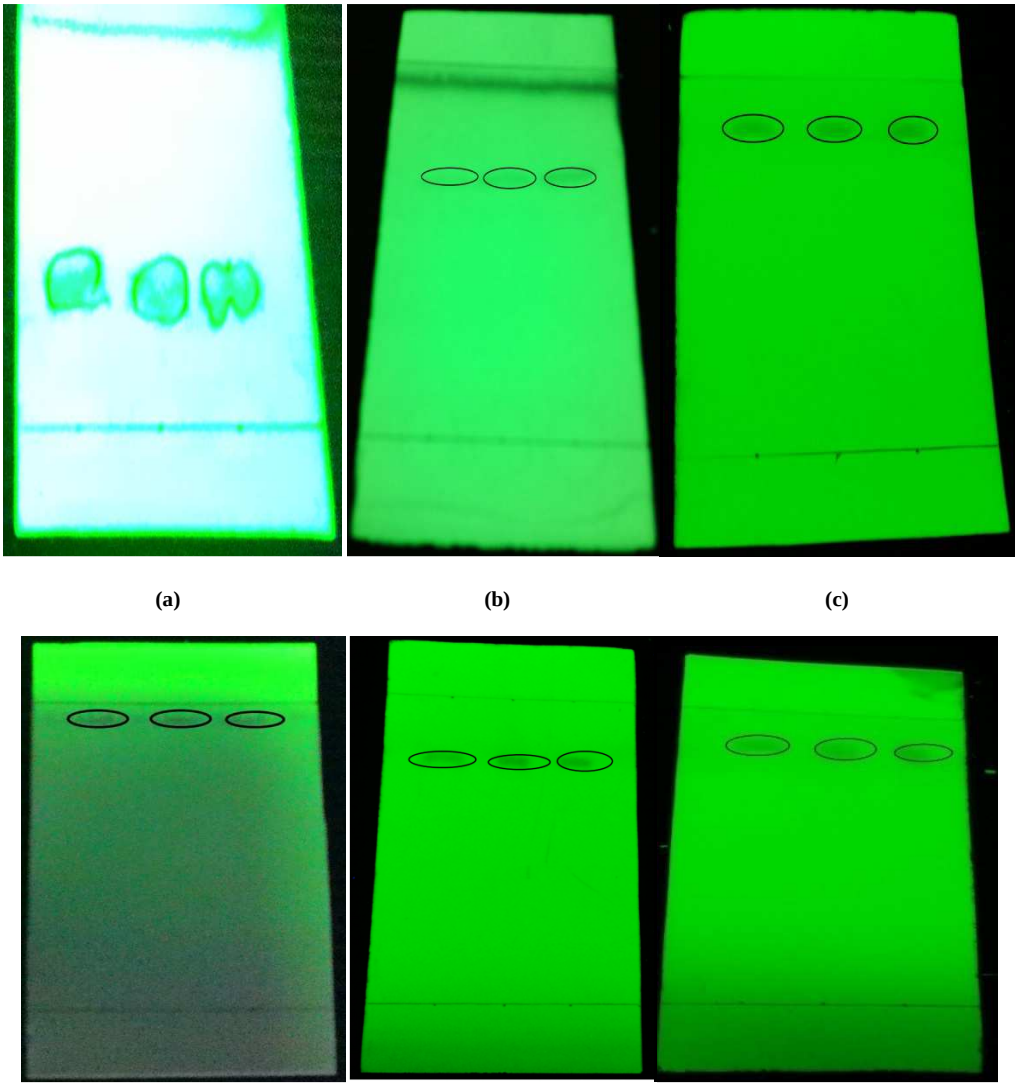


Fig. 1: Thin layer chromatogram of glimepiride with the mobile phase (a) chloroform : methanol (9 : 1, plate was sprayed with KOH 0.1 N); (b) chloroform : methanol (9 : 1); (c) chloroform : methanol (8 : 2); (d) chloroform: methanol (7 : 3); (e) methanol (10); (f) acetone (10) seen under UV light at 254 nm. Left = Standard glimepiride; middle = Tablets generic glimepiride and right = glimepiride tablets under the trade name Metrix®

Validation of the method in this study, the correlation coefficient showed a linear (Fig. 2), because it meets the acceptance criteria that the correlation coefficient (r) ≤ 1 [17]. LOD and LOQ values can be determined from regression equations and standard deviation (Table 2). RSD value of precision obtained was about equal to 2 %, it can be said that this method has good repeatability value (Table 3 and Table 4).

Table 2: Results of method validation

Parameter	Glimepiride
Linearity range	100 – 500 ppm
Correlation coefficient	0.997331
Regression Equation	Y = 1221.03 + 19.9959 X
LOD	44.10598 ppm
LOQ	133.6545 ppm

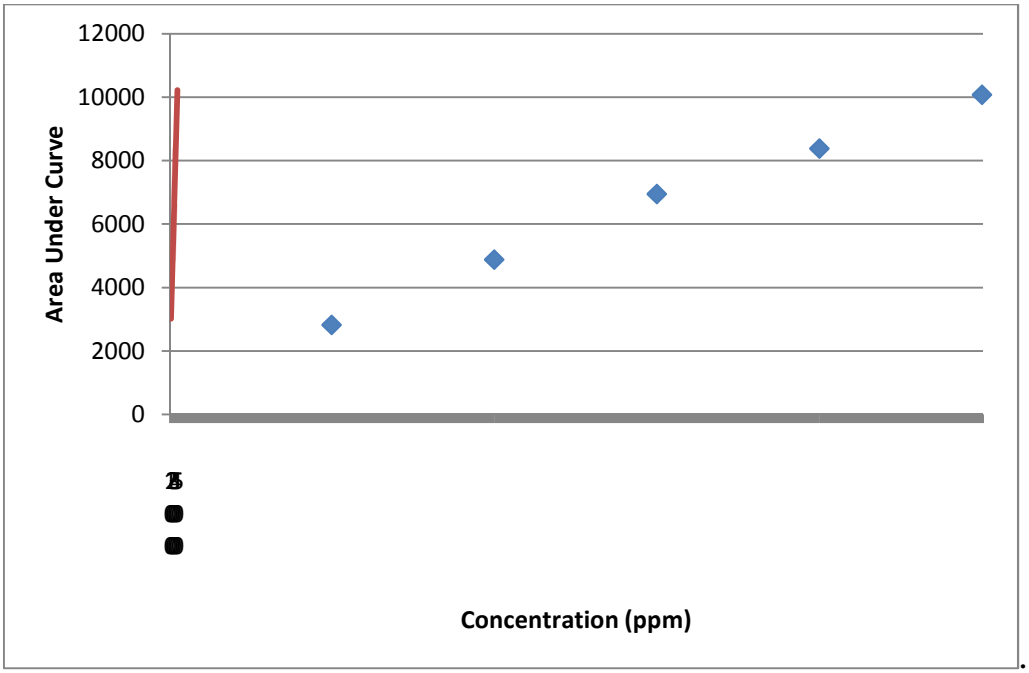


Fig. 2: Calibration curve of glimepiride

Table 3: Evaluation of intra-day precision of glimepiride

Time	AUC	Concentration (ppm)	Average (ppm)	SD	RSD
Morning	4892.8	204.03	202.37	3.36	1.66
	4902.6	204.58			
	4793.3	198.50			
	8190.5	387.28	391.29	5.29	1.35
	8370.6	397.29			
	8227.0	389.31			
	10143.5	495.81	496.09	1.54	0.31
	10178.5	497.75			
	10123.8	494.71			
Noon	4988.4	209.35	208.81	1.38	0.66
	4950.6	207.25			
	4997.3	209.84			
	8225.0	389.20	387.84	1.18	0.31
	8186.1	387.04			
	8190.5	387.28			
	10744.7	529.21	533.39	7.54	1.41
	10738.5	528.87			
	10976.6	542.10			
Afternoon	4755.3	196.39	196.33	2.36	1.20
	4796.0	198.65			
	4711.1	193.94			
	8229.2	389.43	390.01	1.26	0.32
	8265.7	391.46			
	8223.9	389.14			
	10478.9	514.44	516.09	1.72	0.33
	10505.9	515.94			
	10540.7	517.88			

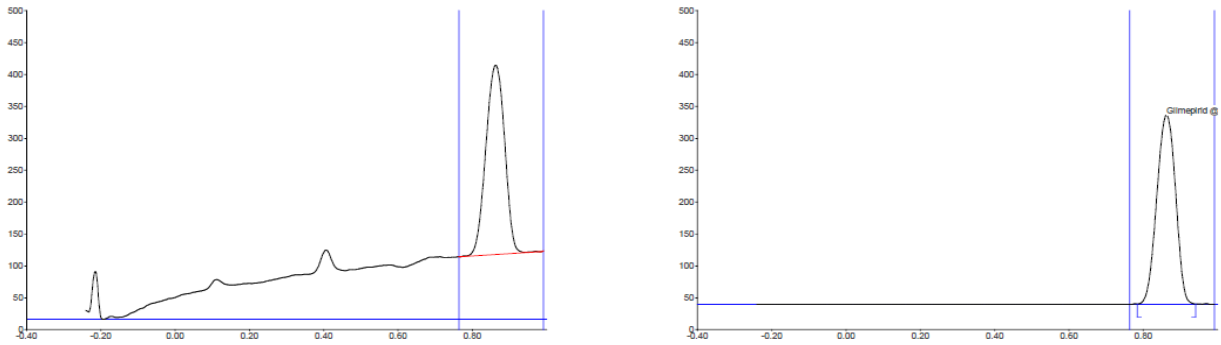
The results of analysis with TLC Scanner method showed that the levels of generic glimepiride tablets and glimepiride tablets under the trade name Metrix® accordance with the provisions of Ministry of Health of the Republic of Indonesia (Table 5) [1]. Densitogram of generic glimepiride and Metrix® samples were shown in Figs. 3 and 4.

Table 4: Evaluation of inter-day precision of glimepiride

Day	AUC	Concentration (ppm)	Average (ppm)	SD	RSD
1	4912.1	205.11	207.50	2.10	1.01
	4983.0	209.05			
	4970.5	208.35			
	8389.2	398.32	400.29	6.65	1.66
	8326.8	394.86			
	8558.0	407.70			
	10495.6	515.37	518.52	7.58	1.46
	10453.2	513.02			
	10707.8	527.16			
2	4912.1	205.11	207.51	2.11	1.02
	4983.5	209.07			
	4970.5	208.35			
	8616.3	410.94	411.52	1.00	0.24
	8616.3	410.94			
	8647.5	412.68			
	10495.6	515.37	520.53	6.03	1.16
	10562.1	519.07			
	10707.8	527.16			
3	4790.2	198.33	195.05	3.48	1.79
	4737.7	195.42			
	4665.4	191.40			
	8525.5	405.90	408.57	3.20	0.78
	8558.0	407.70			
	8637.5	412.12			
	11095.6	548.71	541.23	8.17	1.51
	10983.3	542.47			
	10804.1	532.51			

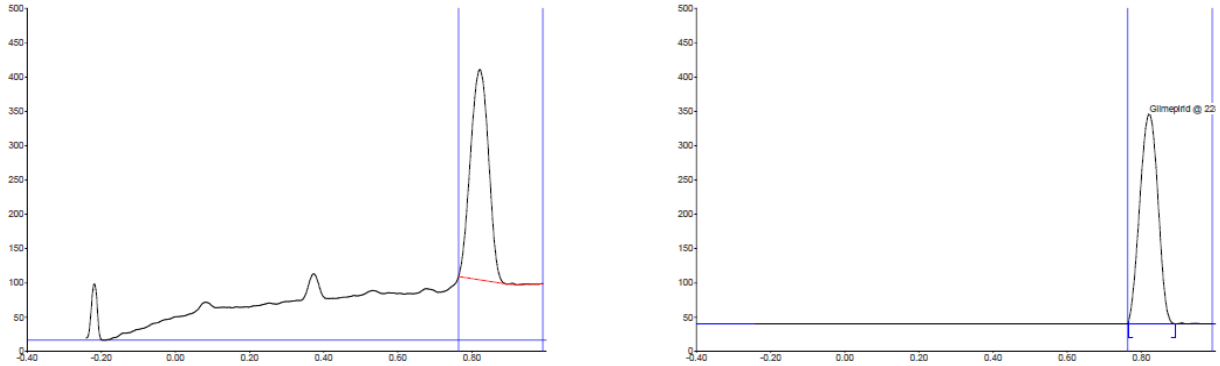
Table 5: The results of measurements of samples glimepiride

Sample	Labeled Content (mg/tablet)	Area Under Curve	Concentration obtained (ppm)	% Glimepiride	Average	SD	RSD
Generic	4	8,714.5	416.3987	104.10	104.68	0.50	0.48
		8,779.2	419.9940	105.00			
		8,775.5	419.7884	104.95			
Metrix	4	8,695.3	415.3318	103.83	104.49	0.60	0.57
		8,753.4	418.5603	104.64			
		8,779.5	420.0107	105.00			



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.60	0.7	0.66	296.6	100.00	0.73	0.5	8714.5	100.00	Glimepirid

Fig. 3: Densitogram samples of glimepiride generic 5 µL volume applications, mobile phase chloroform : methanol (9 : 1), at a wavelength of 228 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	4.0	0.63	306.6	100.00	0.69	0.0	8695.3	100.00	Glimepirid

Fig. 4: Densitogram samples of Metrix 5 µL volume applications, mobile phase chloroform : methanol (9 : 1), at a wavelength of 228 nm

The test results show that the accuracy of glimepiride recoveries were in the range allowed (80-110 %). So this proves that this method gives accurate results (Table 6 and 7).

Table 6: Recovery of glimepiride from generic tablet

% Added	Amount of standard added (mg)	Area Under Curve	Concentration after standard addition (ppm)	Amount before standard addition (mg)	% Recovery	Average	SD	RSD
80	3.3893	14,997.9	765.56	4.24	100.87	99.21	1.46	1.47
		14,830.5	756.25		98.13			
		14,861.0	757.95		98.63			
100	4.2367	16,501.6	849.11	4.24	100.42	101.70	2.04	2.00
		16,778.3	864.49		104.05			
		16,518.0	850.03		100.63			
120	5.0840	18,253.7	946.48	4.24	102.83	101.67	1.01	0.99
		18,087.2	937.22		101.01			
		18,101.6	938.02		101.17			
				Average =		100.86	1.83	

Table 7: Recovery of glimepiride from Metrix tablet

% Added	Amount of standard added (mg)	Area Under Curve	Concentration after standard addition (ppm)	Amount before standard addition (mg)	% Recovery	Average	SD	RSD
80	3.3955	14,817.5	755.53	4.24	97.51	98.05	0.49	0.50
		14,874.7	758.71		98.45			
		14,860.1	757.90		98.21			
100	4.2444	16,618.0	855.58	4.24	101.58	103.52	1.81	1.75
		16,891.6	870.79		105.16			
		16,788.3	865.05		103.81			
120	5.0933	18,101.6	938.02	4.24	100.84	102.00	1.07	1.05
		18,293.9	948.71		102.93			
		18,230.4	945.18		102.24			
				Average =		101.19	2.67	

CONCLUSION

In this work, TLC-densitometry technique was developed and validated for the analysis of glimepiride in pharmaceutical tablets. The proposed method was simple, accurate and highly selective for glimepiride. The satisfactory sensitivity and simplicity make the methods suitable for routine analysis of glimepiride in quality control laboratories.

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