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Simultaneous HPTLC analysis of Gliclazide and Metformin hydrochloride in bulk and tablet dosage form

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Abstract

This paper presents the development and validation of high performance thin layer chromatography (HPTLC) with a densiotometric detection method for the simultaneous analysis of Gliclazide and Metformin hydrochloride. A separation was performed on silica gel 60F254 plates. The mobile phase comprised of toluene, acetonitrile, ethanol, Ammonium sulphate (0.25%) (4 : 4 : 4 : 3, v/v/v/v). The detection of wavelength was found to be 228nm. The Rf values of GLZ and MET were found to be 0.69 and 0.37 respectively. The accuracy and reliability of the method was assessed by evaluation of linearity 200-1000ng per spot of GLZ and MET. Precision intra-day and inter-day RSD values were always less than accuracy (97.37 for GLZ and 94.8 for MET) specificity in accordance with ICH guidelines. The developed HPTLC method was found to be new, accurate and precise.

Keywords: Glicazide, Metformin Hydrochloride, HPTLC, Validation.

Introduction

Gliclazide (GLZ) is chemically N-(hexahydrocyclopenta(c) pyrrol-2-(1H)-ylcarbamoyl)-4-methyl) benzenesulphonamide (Fig. 1). Gliclazide is an oral hypoglycemic (anti-diabetic) and is classified as a sulphonylurea. It is used in type 2 diabetes mellitus.(Non-insulin dependent diabetes mellitus). GLZ was proven to protect human pancreatic beta cells from hyperglycemia induced apoptosis. It was also shown to have an antiatherogenic effect (preventing accumulation of fat in arteries) in type 2 diabetes. GLZ selectively binds to sulphonylurea receptors (SUR-1) on the surface of the pancreatic beta-cells.[1]

Metformin Hydrochloride (MET) is chemically, 1-carbamimidamido-N-N-dimethylmethanimidamide (Fig. 2).[2] It is an oral anti-diabetic drug from the biguanide class. It is the first-line drug for the treatment of type-2 diabetes, particularly in overweight and obese people and those with normal kidney function and evidence suggest it may be the best choice for the people with heart failure. The major action of MET is increasing glucose transport across the cell membrane in skeletal muscle.[3]

The literature survey revealed that many methods are available for estimation of GLZ and MET individually and their combination with other drugs by spectrophotometry[4-6], HPLC[7-8] and HPTLC[9-15] methods. According to the author's best knowledge, till date there is no HPTLC method for simultaneous estimation of GLZ and MET in combined tablet dosage form. Thus a simple and the new HPTLC method has been developed

and validated for their estimation of GLZ and MET in bulk and combined tablet dosage form.

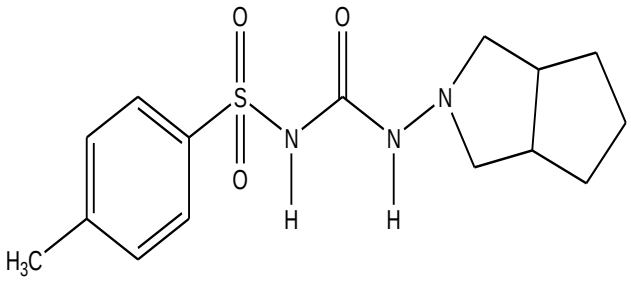

Figure 1: Structure of Glicazide (GLZ)

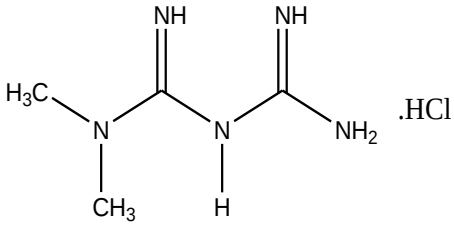

Figure 2: Structure of Metformin hydrochloride (MET)

Materials and Methods

GLZ and MET reference standards were obtained from Wockhardt Pharma Ltd. Fixed dose combination tablets of the two compounds, (GLZ and MET) from manufacturers were brought from retail pharmacy in Aurangabad (Maharashtra, India). Other chemicals were obtained from Merk (India) and were of analytical grade.

A Camag HPTLC system comprising of Camag AS-30-applicator, Hamilton syringe, Camag twin through the chamber, Camag TLC Densitometer CD 60 scanner, and stationary phase pre coated with silica gel 60F254 were used. TLC aluminium plates pre-coated with silica gel 60F254 (20 x 20 cm) were from Merck. Densitometry was carried out with a Camag TLC scanner 3 (Desaga Make) fitted with Desaga ProQuant software. Samples were applied on the TLC plates using the spray on technique of Camag AS-30 under nitrogen gas flow, and developed in a Camag 20 x 20 cm twin trough chambers.

Preparation of standard solution

Weigh accurately 10 mg GLZ and MET individually, dissolve in methanol and make up the volume up to 10 ml in a volumetric flask. These solutions (1 mg/ml) were used as working standard solution of GLY and MET for analysis.

Method development

GLZ and MET working standard solution were prepared by using methanol as solvent. The pre coated TLC plate is prewashed with methanol and activated by keeping at 115ºC for about 15 minutes. Working standard solutions of GLZ and MET (0.5 µl) were applied on TLC plates as spot bands 8 mm with the help of AS-30 applicator. Application positions were at least 15mm from the sides and 15 mm from the bottom of the plate. Mobile phase components were mixed prior to use and develop chamber left to saturate with mobile phase vapor for 20 min before each run. Development of plate was carried out by ascending technique to migration distance 7cm, and then the plates were dried by using the dryer.

Densitometric scanning was done in the absorbance mode at 228 nm using a deuterium lamp. The slit dimensions were set at 5mm× 0.45mm, the scanning speed at 20 mm/s and data resolution at 100m/step. Single wavelength detection was performed because we are dealing with main components analysis.

HPTLC system and the results were evaluated with an aim to achieve an optimum separation between GLZ and MET spots and migration of spots with Rf values between 0.69 and 0.37 in order to ensure separation and reproducibility respectively (Fig. 3 and Fig. 4)

Method Validation

Linearity of calibration curve

A stock standard solution with 1mg/ml each GLZ and MET were prepared in methanol. A volume of 2µl of each solution was applied to the HPTLC plate to deliver 200, 400, 600, 800 and 1000 ng of GLZ and MET per spot. This was done in triplicate and repeated for three days. For each concentration, the applied spot bands were evenly distributed across the plate to minimize possible variation along the silica layer. The linearity was evaluated visually by looking at the calibration curves of GLZ and MET as shown in Fig.5 and Fig. 6.

Precision

Intraday precision of the test method is demonstrated by three applications of the same batch (same concentration) of sample at initial, 24 and 48 hrs. Inter-day precision of the test method is demonstrated by three applications of the same batch (same concentration) of samples on three successive days. The RSD was found to be less than 2 for both intra-day and inter-day precision. From the peak areas, the percentage RSD was determined. The intra-day

and inter-day accuracy and precision of GLZ and MET were shown in Table 1 and 2 respectively.

Accuracy

The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (80%, 100% and 120% concentration) by addition of known amounts of standard to the placebo. Solutions were prepared in triplicate and analyzed. This procedure was repeated for three consecutive days. Calibration curves to estimate the concentration of drug per spot were measured daily on the same plates as the samples. The accuracy was determined and expressed as percentage recovery (Table 3).

Table 1: Evaluation of intra-day and inter-day accuracy and precision of GLZ

GLZ taken (ng/spot)	Intraday accuracy and precision			Interday accuracy and precision		
	GLZ found (ng/spot)	RE %	RSD %	GLZ found (ng/spot)	RE %	RSD %
400	415	1.9	0.24	384	2.4	0.29
600	621	2.1	0.31	591	1.7	0.30
800	817	1.7	0.17	783	1.4	0.28

Table 2: Ealuation Of Intraday And Interday Accuracy And Precision Of MET

MET taken (ng/spot)	Intraday accuracy and precision			Interday accuracy and precision		
	MET found (ng/spot)	RE (%)	RSD (%)	MET found (ng/spot)	RE (%)	RSD (%)
400	404	1.5	0.25	369	2.3	0.21
600	612	2.3	0.19	578	2.4	0.24
800	809	1.4	0.24	785	1.8	0.18

Table 3: Recovery data

Level (%)	Amount added (ng)		Amount found (ng)		% Recovery	
	GLZ	MET	GLZ	MET	GLZ	MET
80	480	480	473.39	472.96	98.62	98.53
100	600	600	589.48	586.88	98.24	97.81
120	720	720	705.67	714.07	98.01	99.17

Analysis of Tablet sample

The method was used for simultaneous quantization of Gliclazide and Metformin Hydrochloride in tablet dosage form. Samples of combined dosage form (Glykind-M) were procured from local pharmacy. For sample preparation, methanol was used as solvent for extraction and dilution. Twenty tablets were ground into fine powder. Portions of powder equivalent to 10 mg of GLZ were accurately weighed into a 10 ml volumetric flask. About 10 ml of methanol was added and the mixture was sonicated for 10 min. The mixture was diluted to volume with methanol, mixed well and filtered through Whatman filter paper no 41 to obtain the sample stock solution.

Further dilute 1 ml of the stock solution with 10 ml of methanol to get the concentration of 0.1 mg/ml GLZ and 1.450 mg /ml MET, used as a test solution for quantitative analysis of Gliclazide from Glykind-M tablets. 2µl of the test solution was applied in the pre-coated silica gel 60F254 plate and developed the plate in mobile phase comprised of Toluene: Acetonitrile: Ethanol: Ammonium sulphate (0.25%) (4:4:4:3 v/v/v). Densiometric evaluation of the separated zones was performed at 228 nm. Chromatogram showing resolution of Gliclazide (Rf =0.67) and Metformin (Rf = 0.39) and from the peak area obtained; the amount of Gliclazide and Metformin hydrochloride in formulation were simultaneously calculated using the respective calibration graph. The assay results of tablet dosage form are shown in Table 4.

Table 4: Assay Results Of Tablet Dosage Form

Formulation	**Actual amount (mg)**		**Amount Found ± SD (mg)**		**% of Drug Found ± SD**	
Tablet (Glykind-M)	**GLZ**	**MET**	**GLZ**	**MET**	**GLZ**	**MET**
	0.20	2.90	0.20	2.88	100 ±1.6	99.31 ± 1.5

Table 5: Optical Characteristic and Validation Parameter of GLZ and MET

Parameters	**GLZ**	**MET**
Linearity range (ng/Spot)	200-1000	200-1000
Correlation co-efficient (r^2)	0.996	0.992
Retention factor	0.67	0.39
Standard Deviation of intercept (Sa)	2362.32	2412.67
Standard Deviation of Slope (Sb)	3.561	3.637
Molar Absorptivity	2213820	1678753
Sandell sensitivity, (µg/ml)	1.46×10^4	9.86×10^4
Limit of detection (LOD, ng/spot)	86.14	106.11
Limit of quantification (LOQ, ng/spot)	261.03	321.54
Intraday Precision (% RSD)	0.24	0.22
Interday Precision (% RSD)	0.29	0.31
Accuracy (%)	98.29	98.5
Assay (%)	100 ±1.6	99.31±1.5

Results and Discussion

During the stage of method development different mobile phases were tried and the mobile phase comprising of Toluene: Acetonitrile: Ethanol: Ammonium sulphate (0.25%) (4:4:4:3 v/v/v/v) was confirmed. A good linear relationship was obtained over the concentration range 200-1000 ng/spot for Gliclazide and Metformin hydrochloride. The linear regression data showed a regression coefficient of 0.994 for Gliclazide (Fig. 5) and 0.992 for Metformin hydrochloride (Fig. 6). The LOD with signal/ noise ratio were found to be 86.140 and 106.11 ng/spot for Gliclazide and Metformin respectively. The LOQ with signal/ noise ratio was found to be 261.03 ng/spot and 321.54 ng /spot for Gliclazide and Metformin respectively. The intraday and inter-day precision showed excellent % RSD less than 2 % (Table 1 and 2). The recovery was 98.62, 98.24 and 98.01% for Gliclazide and 98.53, 97.81 and 99.17% for Metformin hydrochloride at 80%, 100% and 120% levels (Table 3). Assay results show excellent label claim of 100 % for Gliclazide and 99.31% for Metformin HCl. The optical characteristics and validation parameters are summarized in Table 5.

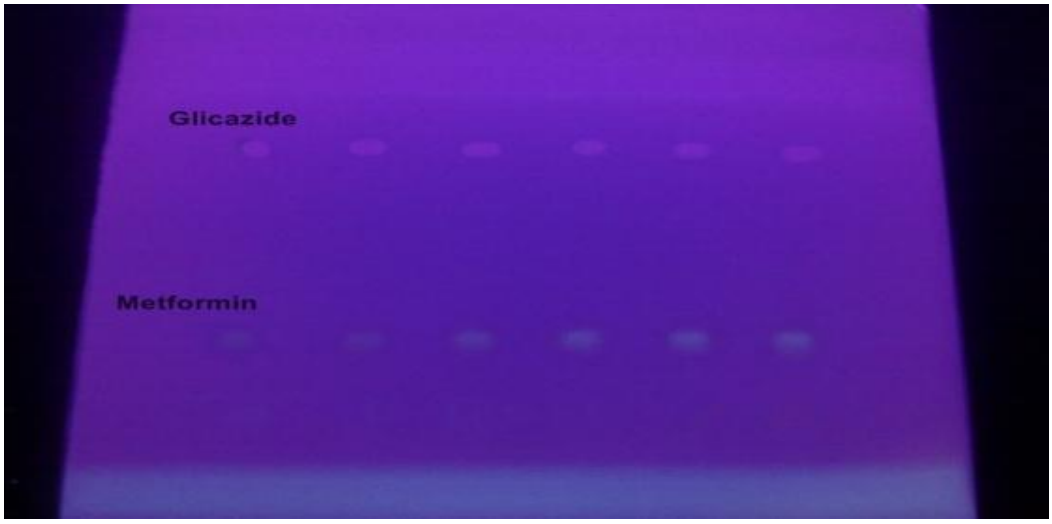

Figure 3: TLC Plate showing separation of (GLZ Rf =0.67) and MET (Rf =0.39)

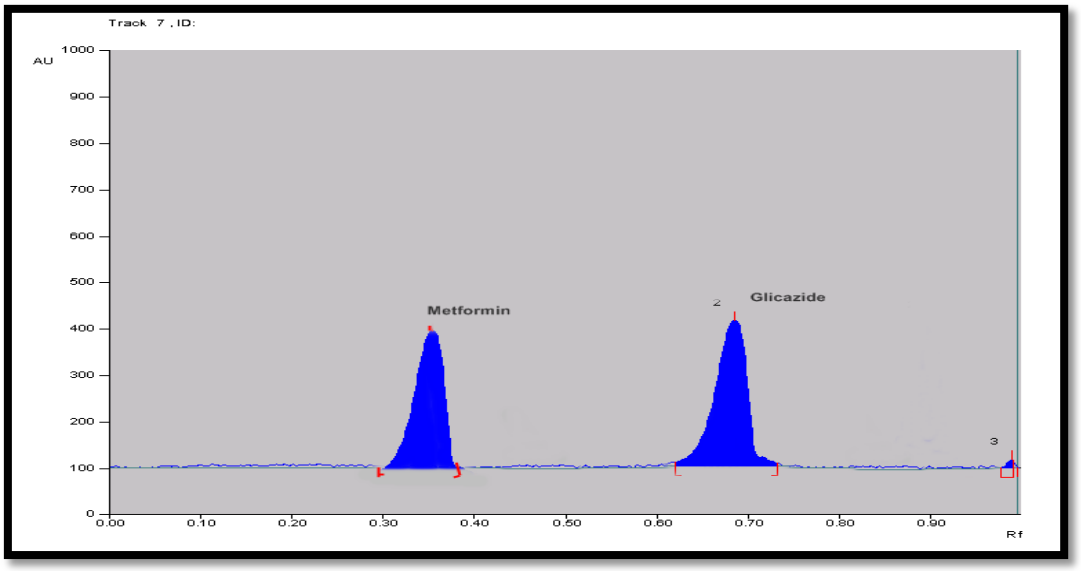

Figure 4: Chromatogram showing resolution of Gliclazide (Rf=0.67) and Metformin hydrochloride (Rf=0.39)

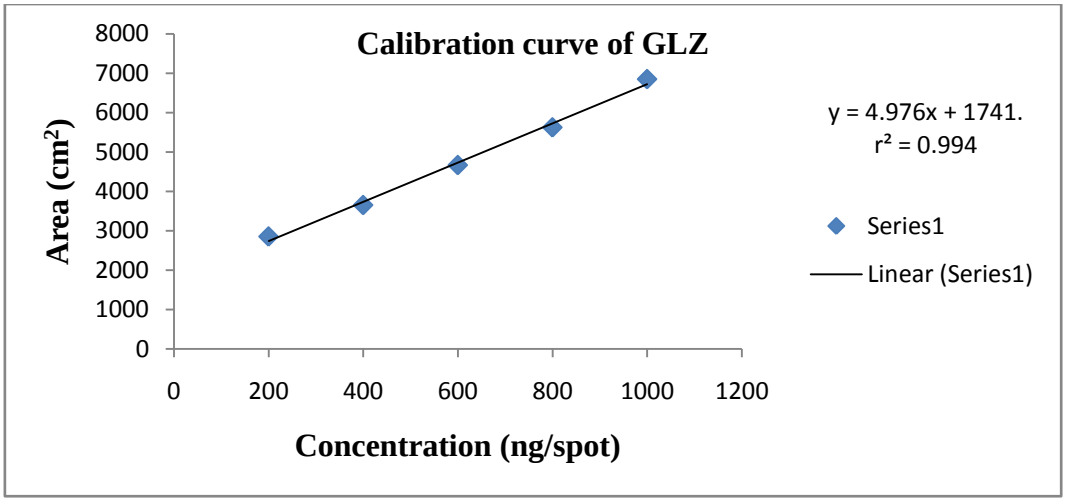

Figure 5: Calibration curve of Gliclazide (GLZ)

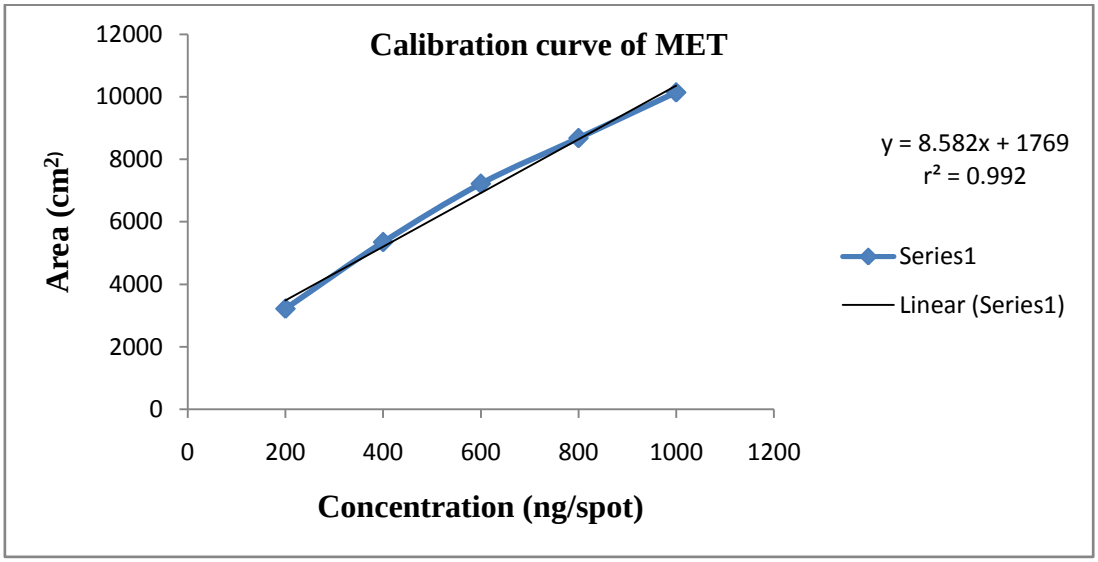

Figure 6: Calibration curve of Metformin HCl (MET)

This developed and validated method for simultaneous analysis of GLZ and MET in pharmaceutical preparations is very rapid, accurate, and precise. The method was successfully applied for determination of GLZ and MET in its pharmaceutical tablet formulations with a mobile phase comprising of Toluene: Acetonitrile: Ethanol: Ammonium sulphate (0.25%) (4:4:4:3 v/v/v/v). The method was successfully validated for linearity, precision and accuracy. Moreover, it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence this method can be conveniently used for routine quality control analysis of GLZ and MET in its pharmaceutical formulations.

Conclusion

The developed TLC method is new, precise and accurate. It is concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and easy sample preparation steps and improved sensitivity made it reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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