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Research Article

# Application of Planar chromatography for the simultaneous determination of Metformin Hydrochloride and Glimepiride in combined dosage form

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**ABSTRACT**

A Simple, sensitive, selective and precise high-performance thin layer chromatographic method for analysis of Metformin Hydrochloride and glimepiride in combined dosage form. The method employed TLC aluminium plates Precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene: methanol: ethyl acetate: formic acid (3:6:3:0.2, *v/v/v/v*). This system was found to give compact spots for Metformin Hydrochloride and glimepiride ($R_f$ value of 0.74 ± 0.01 and 0.08 ± 0.01). Densitometric analysis of Metformin Hydrochloride and glimipiride were carried out in the absorbance mode at 216 nm. The linear regression data for the calibration plots showed good relationship with, $r^2$ = 0.9975 ± 0.01 from 200 -1400 ng for Metformin Hydrochloride and $r^2$ = 0.9999 ± 0.02 from 20-140 ng for glimipiride, respectively. The methods were validated for precision, accuracy, ruggedness and recovery. The limits of detection and quantification were 50 and 100 ng per spot for Metformin Hydrochloride and 5 and 50ng per spot for glimipiride, respectively.

**Keywords:** Glimepiride (GLI); High-performance thin layer chromatography-Densitometry; ICH Guidelines; Metformin Hydrochloride (MET)

## INTRODUCTION

For many patients with Type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control (Indian Pharmacopoeia 2007). In such cases a combination of Metformin Hydrochloride and one of the sulfonylureas (SU) is used. The most commonly prescribed medications are Metformin HCl is a biguanide hypoglycemic agent used in the treatment of non insulin –dependent diabetes mellitus. It is chemically [1,1-dimethyl biguanide hydrochloride] (Indian Pharmacopoeia 2007) Fig. 1. The second generation sulfonylureas like glimepiride is a amino acid derivative which lowers the blood glucose levels and stimulates insulin secretion. Chemically it is formulated as (−)-N-[(trans-4-isopropylcyclohexyl) carbonyl-d-phenylalanine salt (United States Pharmacopoeia) Fig. 2. A combination of 500 mg of Metformin HCl and 1 mg of glimepiride are formulated as a tablet in a combined formulation.

Over the past decade HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents, and biomacromolecules. Several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis. It also facilitates automatic application and scanning in situ.

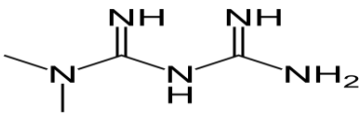

**Figure 1: Chemical structure of Metformin Hydrochloride**

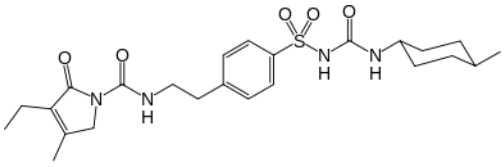

**Figure 2: Chemical structure of glimepiride**

To our knowledge, one article related to the HPTLC determination of Metformin HCl and glimepiride in pharmaceutical dosage form has been reported in literature (or) in pharmacopoeias (Bhat A K et al., 2008 Bonfilio R etal., 2010 Jain D et al., 2008 Sunil R. Dhaneshwar et al., 2010). Therefore, there is a challenge to develop a simultaneous estimation of HPTLC method for Metformin HCl and glimepiride. The present work was involved and validating a simple, accurate, specific, precise and reproducible HPTLC method for the simultaneous determination of Metformin HCl and glimepiride in pharmaceutical formulations. The proposed method was validated as per ICH guidelines and its

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updated international convention (ICH Guidance on Analytical Method 2002; ICH, Q2A Harmonized Tripartite Guideline 1994; ICH, Q2B 1996).

**EXPERIMENTAL MATERIALS**

Metformin Hydrochloride and Glimepiride were supplied by Cipla Pharma Ltd., India. Toluene, Methanol, Ethyl acetate and Formic acid used were of analytical grade (E-Merck Ltd.). All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

**HPTLC Instrumentation**

The sample were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on Precoated silica gel aluminium plate $60F_{254}$, (20 × 10 cm with 250 µm thickness; E. Merck, Germany) using a Camag Linomat IV (Switzerland). The mobile phase consisted of Metformin Hydrochloride and glimepiride ($R_f$ value of 0.74 ± 0.01 and 0.08 ± 0.01; respectively). The plates were prewashed by methanol and activated at 60 $^0$C for 5 min prior to chromatography. Samples were applied at bands 6 mm long, at 5 mm intervals under a stream of nitrogen. The slit dimensions were 5 × 0.45 mm and sensitivity was kept at auto mode. A constant application or spraying rate of 10 s µl$^{-1}$ and scanning speed 20 mm s$^{-1}$ was employed. Linear ascending chromatogram development to distance of 8 cm was performed in 20 × 10 cm twin trough TLC developing chamber (Camag) at room temperature and previously saturated for 30 min with mobile phase. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 216 nm. The source of radiation utilized was deuterium lamp.

**Calibration Curves**

Stock solution of Metformin HCl (100 µg ml$^{-1}$) was prepared in methanol. Different volumes of stock solution 2-14 µl were spotted on the TLC plate to obtain concentrations 200-1400 ng/spot of, MET respectively.

Stock solution of glimipiride (100 µg ml$^{-1}$) was prepared in methanol. Different volumes of stock solution 0.2-1.4 µl were spotted on the TLC plate to obtain concentrations 20-140 ng/spot of, GLI respectively. The data of peak area versus drug concentration was treated by linear least square regression analysis and was selected as working range for the assay and recovery. The data of peak area versus drug concentration was treated by linear least square regression analysis and was selected as working range for the assay and recovery. Densitometric analysis of Metformin Hydrochloride and glimepiride were carried out in the absorbance mode at 216

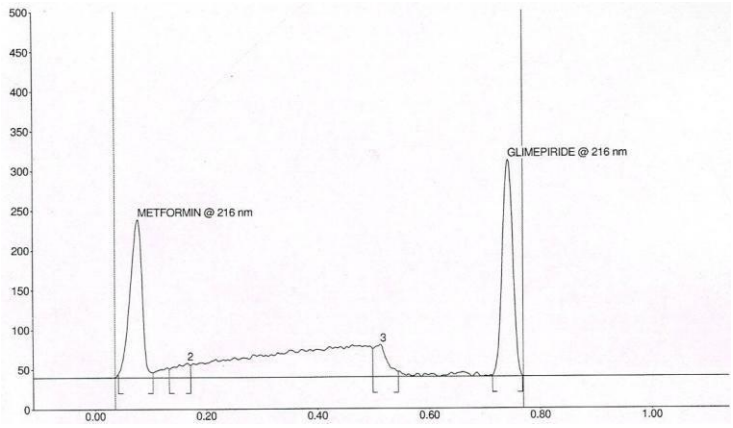


**Figure 3: HPTLC Chromatogram of Metformin Hydrochloride (MET) and Glimepiride (GLI)**

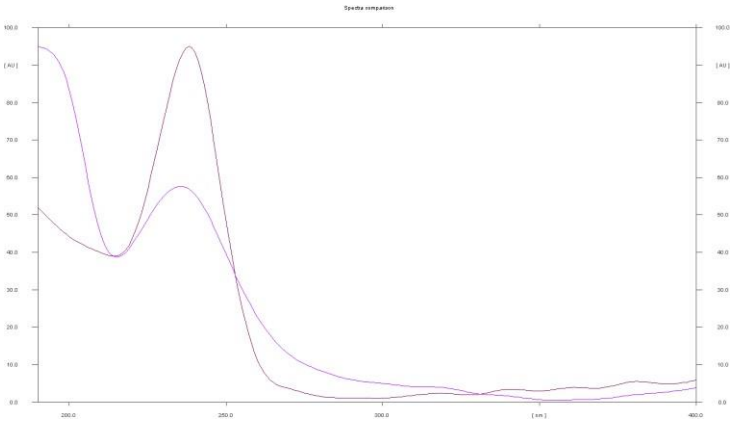

**Figure 4: Typical overlay spectra of standard1 Metformin Hydrochloride and standard 2 glimepiride drug solutions**

nm. The correlation coefficients for all components are close to 1.

**Method Validation**

***Linearity***

The linearity of response for Metformin Hydrochloride was assessed in the range of 200-1400 ng/spot for standard drug

The linearity of response for glimepiride was assessed in the range of 20 -140 ng/spot for standard drug.

***Accuracy and Precision of the Assay***

Accuracy was done in terms of recovery studies and precision. It was measured in terms of repeatability of measurement and application. Recovery studies were carried out by standard addition method. The pre-analysed samples were spiked with extra 80, 100 and 120% of the standards Metformin Hydrochloride and glimepiride and mixtures were analysed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

***Repeatability of Measurement of Peak Area***

Metformin Hydrochloride (500 ng $ml^{-1}$) and glimepiride (100 ng $ml^{-1}$) were spotted on a TLC plate, developed, dried and the spot was scanned seven times without changing the plate position and % co-efficient of variance (% CV) for measurement of peak area was estimated.

***Repeatability of Sample Application***

Metformin Hydrochloride (500 ng $ml^{-1}$) and glimepiride (100 ng $ml^{-1}$) were applied seven times on a TLC plate. The plate was developed and % CV for peak area for different peaks was estimated.

***Ruggedness of the Method***

The Intra-day variation was evaluated in the range of Metformin Hydrochloride 200-1400 ng and glimepride 20-140 ng three times a day. The inter-day variations were similarly evaluated over a period of 3 days.

***Limit of Detection and Limit of Quantification***

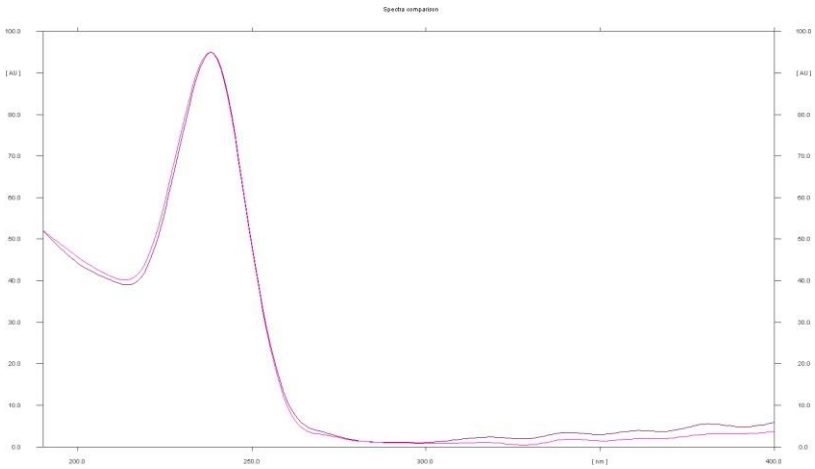

**Figure 5: Peak purity spectra of standard 1 Metformin Hydrochloride Hydrochloride, sample 2 extracted from a marketed formulation , scanned at the peak-start, peak-apex and peak-end positions of the spot (correlation > 0.99)**

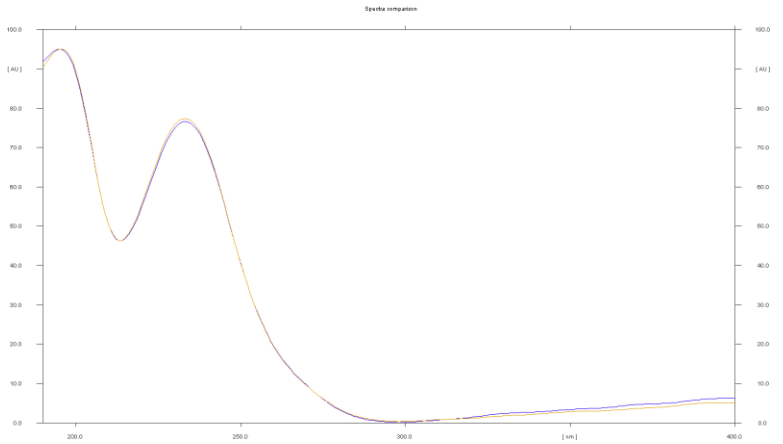

**Figure 6: Peak purity spectra of standard 1 glimepiride, sample 2 extracted from a marketed formulation , scanned at the peak-start, peak-apex and peak-end positions of the spot**

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times. The signal to noise level was determined. LOD was considered as 3:1 and LOQ as 10:1.

***Specificity***

The specificity of the method was ascertained by analysing standard drug and sample. The spots for Metformin Hydrochloride and glimepiride in sample were confirmed by comparing the $R_f$ and spectra of the spots with those of standards. The peak purity of samples was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot.

***Assay of Marketed Formulation***

To determine the contents of Metformin Hydrochloride and glimepiride in conventional tablets (label claim: Metformin Hydrochloride 500 mg and glimepiride 1 mg per tablet), the tablets were powdered and equivalent to 500 mg of Metformin Hydrochloride and 1 mg of Glimepiride were weighed. The extraction solvent employed was methanol. To ensure complete extraction of the drug it was sonicated for 30 min and volume was made up to 100 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and filtered to obtain final concentrations of 5000 ng $ml^{-1}$ (Metformin Hydrochloride) and 100 ng $ml^{-1}$ (glimepiride). One microlitre of the above solution was spotted onto the plate followed by development and scanning. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

**RESULTS AND DISCUSSION**

***HPTLC Method***

Experimental conditions such as mobile phase and detection wave length were optimized to provide precise and repeatability results for the determination of MET and GLI by HPTLC method. The wave length of detection was chosen as 216 nm. And good separation of drug ($R_f$ value of 0.74 ± 0.01 and 0.08 ± 0.01; respectively), were obtained with the mobile phase consisting of toluene: methanol: ethyl acetate: formic acid (3:6:3:0.2, *v/v/v/v*). Typical Chromatograms obtained from the analysis of the drug using the developed method is shown in Fig. 3.

***Calibration Curves***

Calibration graph was found to be linear that is adhe-

**Table 1: Summary of validation parameters**

| Validation parameter | Metformin hydrochloride | Glimepiride |
|---|---|---|
| Specificity | Specific | specific |
| Linear range (ng per spot) | 200-1400 | 20-140 |
| Precision (%RSD) | | |
| Method precision (*n = 6)* | 1.98 | 0.36 |
| Intra-day (*n = 6*) | 1.59 | 0.57 |
| Inter-day (*n = 6*) | 0.99 | 1.42 |
| Different analyst (*n = 6*) | 1.43 | 1.98 |
| Limit of detection (ng per spot) | 50 | 5 |
| Limit of quantification (ng per spot) | 100 | 50 |
| Regression Equation | 3.379 x + 1030 | 3.603 x + 1612 |
| Correlation Coefficient | 0.9975 | 0.9999 |
| Resolution factor ($R_F$) | 0.08 (± 0.02) | 0.74 (± 0.02) |

**Table 2: Results of recovery studies**

| Drug | Initial amount [ng] | Amount added [ng] | Amount recovered ± S.D. [ng] | % Recovered | %RSD |
|---|---|---|---|---|---|
| MET | 800 | 0 | 800.4± 22.48 | 100.05 | 1.12 |
| | 800 | 640 | 1437.55± 10.44 | 99.83 | 0.65 |
| | 800 | 800 | 1598.08± 10.66 | 99.88 | 0.53 |
| | 800 | 960 | 1762.81± 20.39 | 100.16 | 0.84 |
| GLM | 80 | 0 | 80.56± 13.67 | 100.70 | 0.67 |
| | 80 | 64 | 145.59 ± 5.65 | 99.72 | 0.72 |
| | 80 | 80 | 159.71± 5.68 | 99.82 | 0.28 |
| | 80 | 96 | 176.80± 10.72 | 100.46 | 0.44 |

**Table 3: Results of Marketed Formulations**

| Sample | Label claim [mg/tablet] | | Amount Found [%] | | [%] RSD | |
|---|---|---|---|---|---|---|
| | MET | GLM | MET | GLM | MET | GLM |
| T1 | 500 | 1 | 497 | 0.98 | 1.52 | 2.07 |

T1= GLYCOMET-GP1® (USV Pharmaceuticals Ltd., India)

rence of the system to Beer's law was found over the concentration range of 200-1400 ng spot$^{-1}$ MET and 20-140 ng spot$^{-1}$ GLI. The data of Peak area versus concentration were treated by linear least square regression analysis. Table 1, showed a good linear relationship over the low and high concentration range of all drugs.

**Validation of the Method**

***Precision***

For determination of precision of MET and GLI by the proposed method, same homogeneous samples of MET and GLI (real samples) were prepared repeatedly and analyzed. Intermediate precision was evaluated at different times on same day, on different days and even by different analysts. Low values of RSD (less than 2%) obtained in the precision studies indicate that the method is precise and reproducible.

***LOD and LOQ***

The limits of detection and quantification were 50 and 100 ng per spot for Metformin Hydrochloride and 5 and 50 ng per spot for glimipiride.

***Specificity***

Specificity of the method for MET and GLI were proved from the spectral scan Fig. 5 and Fig. 6, and peak purity correlation (r) results for MET and GLI in bulk and in pharmaceutical formulation indicate that there is no merging or co-elution of interfering peaks with MET and GLI, so there is no interference from any excipients present in tablet formulations of MET and GLI.

***Recovery Studies***

The proposed method when used for extraction and subsequent estimation of MET and GLI from pharmaceutical dosage form after spiking with 80, 100 and 120% of additional drug afforded recovery values are given in Table 2.

***Analysis of Marketed Formulation***

The proposed HPTLC method was applied to the simultaneous determination of MET and GLI in GLYCOMET-GP1® tablets. Determination of seven replicate was made. Satisfactory results were obtained which is given in Table 3, for compound in good agreement with label claims.

**CONCLUSION**

The developed HPTLC techniques are precise, specific, simple, accurate, linear, reproducible and repeatable for the estimation of MET and GLI in pharmaceutical dosage form without any interference from the excipients. The HPTLC method offers several advantages over liquid chromatographic methods such as the possibility of simultaneous analysis of sample and standard on the same plate, short system equilibrium time, multiple/repeated scanning of chromatograms, higher mobile phase pH, large sample capacity, short run time, minimum solution consumption and no prior treatment for solvents like filtration and degassing. The methods are sensitive for quantitative detection of the analytes in pharmaceutical dosage form and can thus be used for routine analysis.

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