

# Estimation of Metformin in Bulk Drug and in Formulation by HPTLC

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## Abstract

A simple and sensitive, HPTLC method has been developed for the quantitative estimation of metformin in its single component tablet formulation. Metformin was chromatographed on silica Gel 60 F<sub>254</sub> TLC plate using ammonium sulfate (0.5%): 2-propanol: methanol in the ratio of 8.0:1.6:1.6 (v/v/v) as mobile phase. Metformin showed R<sub>f</sub> value of 0.50±0.03 was scanned at 238 nm using Camag TLC Scanner 3. The linear regression data for the calibration plot showed a good relationship with r = 0.999. The method was validated for precision and recovery. The limits of detection and quantification were 95 and 200 ng/spot respectively. The developed method was successfully used for the assay of metformin tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

**Keywords:** Thin layer chromatography; Pharmaceutical analysis; Antidiabetic drug; Metformin; Tablet; Bulk drug

## Introduction

Metformin (Metformin HCl, *N,N*-dimethylimidodicarbonimidic diamide hydrochloride, MET, Figure 1) [1] is an oral antidiabetic drug [2]. Metformin hydrochloride is formulated as tablet dosage forms. This drug was approved by the FDA in December 1994 and has been the only clinically available drug that can significantly improve insulin sensitivity in patients that suffer from Diabetes type II (non-insulin dependent). Typically Metformin reduces basal and postprandial hyperglycemia by about 20.5% in more than 90% of the patients. Determination of MET in plasma by various analytical methods like HPLC MS or UV detector [3-11], in formulation [12] multicomponent system, HPLC [13,14]. Several other HPLC methods were also developed for the determination of metformin hydrochloride. But these methods are sophisticated, expensive and time consuming as compared to simple HPTLC method. There is a need for a simple, rapid, cost effective and reproducible method for assay of MET in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise HPTLC method for the analysis of MET in its tablet formulation. The objective of the current work is, therefore, to develop a simple HPTLC method for analysis of metformin hydrochloride in tablet formulations.

## Experimental

### Materials

MET working standard was a generous gift from Ranbaxy, Indore, India. Silica gel 60 F<sub>254</sub> TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used as a stationary phase. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. Glycomet containing 500 mg of MET were purchased from USV limited, Biciphage containing 850 mg MET were purchased from Biochem and Bigomet containing 250 mg of MET were purchased from Genetica (Aristo).

### Instrumentation

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm × 10 cm), Camag winCATS software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on precoated

silica gel 60 F<sub>254</sub> TLC plates (10 cm × 10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

### Preparation of standard solutions

A stock solution of MET was prepared by dissolving 100 mg in 100 ml methanol (1000 µg/ml). Further standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 200-1000 ng/spot.

### Sample preparation

Three brands of tablets Glycomet of (USV limited), Bigomet (Genetica (Aristo)) and Biciphage of (Biochem Pharmaceuticals) were selected. Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to one tablet was dissolved in 50 ml methanol. To ensure complete extraction of the drug it was sonicated for 45 min. This solution was filtered through a Whatman no. 41 paper.

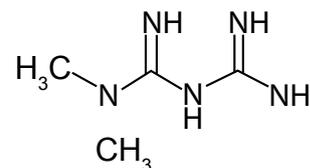


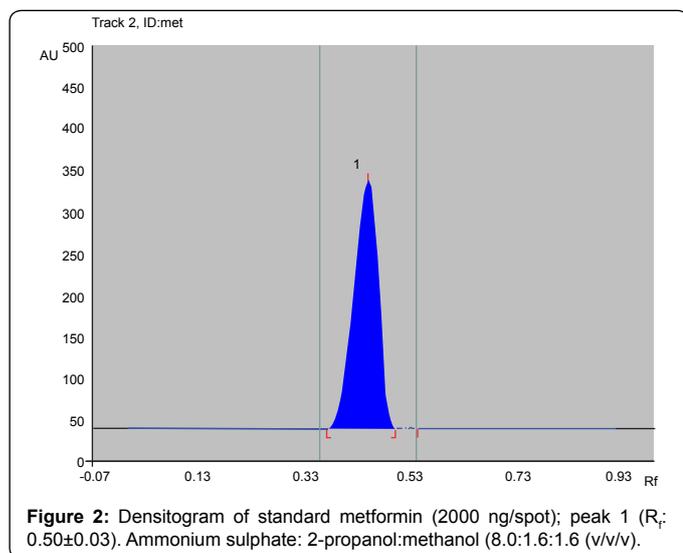
Figure 1: Chemical structure of Metformin.

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**Figure 2:** Densitogram of standard metformin (2000 ng/spot); peak 1 ( $R_f$ :  $0.50 \pm 0.03$ ). Ammonium sulphate: 2-propanol:methanol (8.0:1.6:1.6 (v/v/v)).

| Linearity (ng/spot) | r ± S.D.     | Slope ± S.D. | Intercept ± S.D. |
|---------------------|--------------|--------------|------------------|
| 200-1000            | 0.999 ± 0.09 | 16.73 ± 1.02 | 3.962 ± 0.8      |

<sup>a</sup>n = 6

**Table 1:** Linear regression data for the calibration curves<sup>a</sup>.

| Inter-day precision |          | Intra-day precision |          |
|---------------------|----------|---------------------|----------|
| S.D of areas.       | % R.S.D. | S.D of areas.       | % R.S.D. |
| 1.04                | 1.28     | 1.89                | 1.91     |

<sup>a</sup>n = 6, Average of three concentrations 200, 600, 1000 ng/spot

**Table 2:** Intra and inter day precision of HPTLC method<sup>a</sup>.

| Parameter                            | SD of peak area | % RSD |
|--------------------------------------|-----------------|-------|
| Mobile phase composition             | 0.90            | 1.01  |
| Amount of mobile phase               | 1.60            | 1.89  |
| Temperature                          | 0.60            | 0.90  |
| Relative humidity                    | 1.26            | 1.81  |
| Plate pretreatment                   | 0.79            | 0.08  |
| Time from spotting to chromatography | 1.10            | 0.91  |
| Time from chromatography to scanning | 0.08            | 0.05  |

<sup>a</sup>n = 6, Average of three concentrations 200, 600, 1000 ng/spot

**Table 3:** Robustness testing<sup>a</sup>.

## HPTLC method

In the proposed HPTLC method, the samples were streaked on the precoated TLC plates in the form of a narrow band 6 mm in length, 10 mm from the bottom and margin and 10 mm apart at a constant flow rate of 150 nl/s by using a nitrogen aspirator. A Camag Twin Trough Chamber was saturated for 20 min at room temperature ( $25 \pm 2^\circ\text{C}$ ) with the mobile phase containing a mixture of ammonium sulfate (0.5%): 2-propanol: methanol in the ratio of 8:1.6:1.6 (v/v/v). After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at 238 nm for all measurements. The slit dimension was kept at 5.0 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. MET was detected at  $R_f$  of  $0.5 \pm 0.03$ . The chromatograms were integrated using win CATS evaluation software (Version 1.1.3.0).

## Calibration plots

A stock solution of MET (1000 µg/ml) was prepared in methanol. Calibration curves were prepared over a concentration range of 200–

1000 ng/spot for MET. These solutions were applied to plates, which were then developed and scanned as described above. The data of area under the peak versus drug concentration (ng/spot) were subjected to regression analysis to calculate the regression equations and correlation coefficients.

## Analysis of MET formulations

Twenty tablets of each brands were weighed their average weight calculated, tablet triturated finely powdered and the powder equivalent to containing 500 mg, 850 mg and 250 mg of MET from Glycomet, Biciphage and Bigomet respectively and dissolved in 50 ml of methanol. The solution was sonicated for 45 min and then filtered through Whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined. Each of these solutions (1 µl) were spotted on plates and analyzed for MET in the same way as described earlier.

## Method validation

**Quantitation:** The limit of detection (LOD) was calculated as three times the noise level and limit of quantification (LOQ) was calculated as ten times the noise level.

**Accuracy:** Recovery studies were carried out to check the accuracy of the method. Recovery experiments were performed by adding three different amounts of MET i.e., 80, 100 and 120% of the labeled amount of MET analyzed from the MET formulations and the resultant were reanalyzed (n = 6).

**Precision:** Different amounts of MET covering the low, medium and higher ranges of the calibration curve were spotted on the TLC plate for determining intra-day and inter-day precision (over a period of 7 days). These spots were analyzed (n = 6) by using the above described HPTLC method.

## Results and Discussion

### Chromatography

In this study, the quantitative HPTLC method was developed for the estimation of MET in tablets. The solvent system comprising of ammonium sulfate (0.5%):2-propanol: methanol (8:1.6:1.6 v/v/v) could resolve MET spot with better peak shape. It also offered optimum migration ( $R_f = 0.5 \pm 0.03$ ) (Figure 2) and resolution of MET from other excipients used in various MET formulations. Chamber saturation time was optimized to 30 min in order to get distinct bands of MET. The analytical wavelength, 238 nm, was chosen on the basis of the absorption spectrum recorded in the range 200–800 nm.

### Validation of the method

**Linearity:** Linearity for MET was observed in the range of 200-1000 ng/spot with a correlation coefficient of 0.999 and the linear regression equation was  $y = 3.962x + 16.73$  (Table 1).

**Precision:** The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 1.82 and 1.51 respectively. The results shown in Table 2 revealed intra- and inter-day variation of MET at three different concentration levels 200, 600, 1000 ng/spot. The % RSD for within and day-to-day analysis was found to be <2%.

**Robustness of the method:** The standard deviation of peak area was calculated for each parameter and % R.S.D. was found to be less than 2%. The low values of % R.S.D as shown in Table 3 indicated robustness of the method.

| Formulation | Label Claim (mg) | Drug Content (%) | % R.S.D. |
|-------------|------------------|------------------|----------|
| Glycomet    | 500              | 99.82            | 0.98     |
| Biciphage   | 850              | 101.0            | 1.30     |
| Bigomet     | 250              | 100.24           | 0.36     |

<sup>a</sup>n=6

**Table 4:** Applicability of the HPTLC method for the analysis of the pharmaceutical formulations.

| Formulation | Amount of drug added (%) | Theoretical content (ng) | Amount of MET recovered (ng) mean | % Recovery |
|-------------|--------------------------|--------------------------|-----------------------------------|------------|
| Glycomet    | 80                       | 18000                    | 18018                             | 100.10     |
|             | 100                      | 20000                    | 19846                             | 99.93      |
|             | 120                      | 22000                    | 21995.6                           | 99.98      |
| Biciphage   | 80                       | 30600                    | 30544.92                          | 99.82      |
|             | 100                      | 34000                    | 33874.2                           | 99.63      |
|             | 120                      | 37400                    | 37171.86                          | 99.39      |
| Bigomet     | 80                       | 9000                     | 9003.6                            | 100.04     |
|             | 100                      | 10000                    | 9997                              | 99.97      |
|             | 120                      | 11000                    | 11031.9                           | 100.29     |

<sup>a</sup>n = 6

**Table 5:** Recovery studies<sup>a</sup>.

| Parameter                    | Data           |
|------------------------------|----------------|
| Linearity range              | 200-1000 ng/ml |
| Correlation coefficient      | 0.999 ± 0.09   |
| Limit of detection           | 95 ng/ml       |
| Limit of quantitation        | 200 ng/ml      |
| Recovery (n = 6)             |                |
| Glycomet                     | 99.82          |
| Biciphage                    | 101.0          |
| Bigomet                      | 100.24         |
| Precision (% RSD)            |                |
| Repeatability of application | 1.82           |
| Inter – day (n = 6)          | 1.28           |
| Intra – day (n = 6)          | 1.91           |
| Robustness                   | Robust         |
| Specificity                  | 0.9991         |

**Table 6:** Summary of validation parameters.

**LOD and LOQ:** The signal to noise ratios 3:1 and 10:1 were considered as LOD and LLOQ respectively. The LOD and LLOQ were found to be 95 and 200 ng/spot respectively.

**Specificity:** The peak purity of MET was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.998 and r (middle, end) = 0.9993. Good correlation (r=0.9991) was also obtained between standard and sample spectra of MET.

**Recovery studies:** The proposed method when used for extraction and subsequent estimation of MET from pharmaceutical dosage form after spiking the preanalysed sample with 80, 100 and 120% of label claim of MET afforded recovery of 99-101% as listed in Table 5. The data of summary of validation parameters are listed in Table 6.

**Analysis of MET formulations:** A single spot at R<sub>f</sub> 0.5 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 99-101% of the label claim.

## Conclusion

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

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