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 F254 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 Rf 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 dosages. 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 F254 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 F254 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.
Integrated using WinCATS evaluation software (Version 1.1.3.0).

Calibration plots

HPTLC method

MET was detected at Rf 5.0 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. At 238 nm for all measurements. The slit dimension was kept at 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode.

After chamber saturation, the plates were developed to a distance of 25 ± 2 °C with the mobile phase containing a mixture of ammonium sulphate (0.5%): 2-propanol:methanol (8:1.6:1.6 (v/v/v)).

Twin Trough Chamber was saturated for 20 min at room temperature. Constant flow rate of 150 nl/s by using a nitrogen aspirator. A Camag Plate pretreatment of 7 days). These spots were analyzed (n = 6) by using the above described HPTLC method.

Robustness testing

Track 2, ID: met

Table 1: Linear regression data for the calibration curves.

| 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 |

Table 2: Intra and inter day precision of HPTLC method.

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 (Rf = 0.5 ± 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.
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 Rf 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.

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     |
| Biciphage   | 80                       | 30060                    | 30544.92                         | 99.82      |
| Bigomet     | 80                       | 37400                    | 37171.86                         | 99.39      |
|             | 120                      | 22000                    | 21959.6                          | 99.98      |
|             | 100                      | 20000                    | 19846                            | 99.93      |
|             | 100                      | 34000                    | 33874.2                          | 99.63      |
|             | 120                      | 9000                     | 9003.6                           | 100.04     |
|             | 100                      | 10000                    | 9997                             | 99.97      |
|             | 120                      | 11000                    | 11031.9                          | 100.29     |

Table 5: Recovery studies*.

| Parameter                  | Data              |
|----------------------------|-------------------|
| Linearly 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)           | 99.82             |
| Glycomet                   | 101.0             |
| Biciphage                  | 100.24            |
| Bigomet                    |                   |
| Precision (% RSD)          |                   |
| Repeatability of application Inter – day (n = 6) | 1.82 |
|                           | 1.28              |
| Intra – day (n = 6)        | 1.91              |
| Robustness                 |                   |
| 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/ml 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.

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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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