Publications
List of Publications


Paper communicated for Publication

Simultaneous determination of two antidiabetic drugs, metformin and glipizide, in pharmaceutical tablet formulations were investigated. Normal phase thin layer chromatography plate (silica gel 60 F254) was used as the stationary phase and water:methanol:0.5% w/v ammonium sulfate solution (6:3:1.5 v/v/v) as the mobile phase to determine two pharmaceutically active ingredients in formulations of Glynase-MF. This system gave a good resolution for metformin ($R_f$ value of 0.22 ± 0.01) and glipizide ($R_f$ value of 0.85 ± 0.01). The $k_{max}$ was at 236 nm. The linear regression data for the calibration plot showed a good relationship with $r = 0.9962$ and 0.9930 for metformin and glipizide, respectively. The method was validated for precision and recovery. The limits of detection and quantification were 991.30 and 3003.95 ng/band for metformin and 9.57 and 29.01 ng/band for glipizide, respectively.

**Keywords** glipizide, HPTLC, metformin hydrochloride, oral antidiabetics, pharmaceutical analysis

**INTRODUCTION**

Metformin hydrochloride (Figure 1a), N,N-dimethylimidodicarbonimidic diamide monohydrochloride, is an antihyperglycemic agent that improves insulin tolerance in patients with type II diabetes, lowering both basal and postprandial plasma glucose.[1] Metformin hydrochloride decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Glipizide (Figure 1b), N-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-5-methylpyrazine-2-carboxamide is an oral antihyperglycemic drug of the sulfonylurea class, which appears to lower
blood glucose by stimulating the release of insulin from the pancreas.\textsuperscript{[2]} These drugs are oral hypoglycemic agents. A combination of 500 mg of metformin and 5 mg of glipizide are available commercially as tablets. This combination is used in the treatment of noninsulin dependent diabetes mellitus (NIDDM).

Various methods have been employed for the individual analysis of metformin both in biological fluids and pharmaceutical formulations, including HPLC,\textsuperscript{[3–14]} LCMS,\textsuperscript{[15]} spectrophotometry,\textsuperscript{[16]} and chemiluminescence.\textsuperscript{[17]} Also, glipizide has been subjected to different methods of analysis including HPLC,\textsuperscript{[18,19]} LCMS,\textsuperscript{[20]} and Stripping voltammetry.\textsuperscript{[21]} Simultaneous determination of metformin hydrochloride with other antidiabetic agents by TLC\textsuperscript{[23–26]} and HPTLC\textsuperscript{[27]} is available in the literature as well.

Fixed dose combinations containing metformin and glipizide are widely available commercially, and LCMS\textsuperscript{[28–31]} and HPLC\textsuperscript{[32]} method for their simultaneous determination has already been reported. However, there is no reported HPTLC method for the simultaneous determination of these two drugs.

Now-a-days, HPTLC is becoming a routine analytical technique due to its advantages. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase. Suspensions and dirty or turbid samples can be directly applied. It facilitates automated application and scanning in situ. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters.

In this paper, we report a new HPTLC method for the simultaneous determination of metformin hydrochloride and glipizide from tablet
dosage form which is simple, sensitive, precise, accurate, and rapid. The method requires less quantity of solvent and is more rapid than HPLC, which saves determination time.

**EXPERIMENTAL**

**Materials**

Ammonium sulfate 99% (AR grade) and methanol (AR grade) were supplied by SD Fine Chemicals (Mumbai, India). Reference standards of metformin hydrochloride and glipizide were procured from Torrent Research Centre (Bhat, Gandhinagar, India) and Cadila Pharma (Dholka, India), respectively. Glynase-MF tablets were purchased from a local market.

**HPTLC Instrumentation**

CAMAG TLC system composed of a linomat 5 autosampler, a TLC scanner 3, and utilizes winCATS 1.2.2 software (CAMAG, Muttenz, Switzerland) was used. Chromatography was performed on precoated silica gel 60 F254 TLC plates (10 × 10 cm) (Merck, Darmstadt, Germany) using water: methanol: 0.5% w/v ammonium sulfate solution 6:3:1.5 (v/v/v) as mobile phase. The band length 5 mm and distance between bands 15 mm was kept constant throughout the study. The numbers of applications on the plates were five for standards and three for real samples. Ascending development was performed on 20 × 20 cm twin through chamber (CAMAG); the mobile phase migration distance in all experiments was 90 mm. Chromatograms were evaluated via peak area after scanning in absorbance mode at 236 nm.

**Preparation of Standard Solution**

Accurately weighed metformin (500 mg) and glipizide (5 mg) were transferred to 100-mL volumetric flask, dissolved in and diluted to the mark with methanol to obtain a standard solution having a concentration of metformin (5000 ng/μL) and glipizide (50 ng/μL). Four μL aliquots of diluted standard solution were automatically applied to the plates.

**Preparation of Sample Solution**

The sample solution was prepared according to dosage form of Glynase-MF tablets. Twenty tablets were weighed and powdered, and an amount of the powder equivalent to 500 mg of metformin and 5 mg of glipizide was taken in a 100-mL volumetric flask, sonicated for 15 min, and
diluted to the mark with methanol. The solution was filtered through Whatman No. 42 filter paper. Four µL of this filtrate was applied to the HPTLC plate and developed, dried, and scanned. Quantity analysis of metformin and glipizide was made on the basis of peak areas received for standard solutions.

**Method Validation**

Validation was done with respect to various parameters required under ICH guideline Q2 (R1).\(^{[33]}\)

**Linearity**

Calibration curve was plotted over a concentration range from 5000 to 25000 ng/band for metformin and 50 to 250 ng/band for glipizide. For the calibration curves, accurately prepared standard solution of metformin and glipizide (1.0, 2.0, 3.0, 4.0, 5.0 µL) were applied to the plate. The plate was developed in a developing chamber previously saturated with the mobile phase for 30 min. Each reading was the average of three determinations.

**Accuracy (% Recovery)**

The accuracy of the method was determined by calculating recoveries of metformin and glipizide by the standard addition method. Three different levels of standards (7500, 10000, and 12500 ng/band for metformin and 75, 100, and 125 ng/band for glipizide) were added to the pre-analyzed tablet sample (10000 ng/band and 100 ng/band), each level was repeated three times, and the percentage recoveries were calculated.

**Method Precision (Repeatability)**

The precision of developed method was checked by repeatedly \(n = 6\) injecting 20000 ng/band sample solution of metformin and 200 ng/band sample solution of glipizide, without changing the position of plate for HPTLC method. Six injections of sample were made by one analyst on the same day.

**Intermediate Precision (Ruggedness)**

To determine intermediate precision (ruggedness) the whole experiment was conducted by a different analyst on different day. The results are reported in terms of relative standard deviation.
Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations $\text{LOD} = \frac{3.3 \times N}{B}$ and $\text{LOQ} = \frac{10 \times N}{B}$ where $N$ is standard deviation of the peak area ($n = 3$) and $B$ is the slope of the corresponding calibration curve.

Selectivity

Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned in a previous section.

RESULTS AND DISCUSSION

Wavelength Optimization

UV-Vis spectra (Figure 2) of metformin and glipizide were measured from 200 to 400 nm and 236 nm was used as the optimum wavelength throughout the experiment for both substances.

Linearity

The calibration curve was linear from 5000 to 25000 ng/band for metformin and from 50 to 250 ng/band for glipizide, respectively. Statistical evaluations of the linear part of calibration dependence of metformin and glipizide are presented in Table 1.

FIGURE 2 Spectra for metformin and glipizide wavelength optimization. (Color figure available online.)
**Accuracy (% Recovery)**

The analyzed samples were spiked with extra concentration levels of 7500, 10000, and 12500 ng/band for metformin and 75, 100, and 125 ng/band for glipizide and the mixtures were reanalyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples. The results are presented in Table 2.

**Precision**

The precision of the developed method was expressed as a percentage of relative standard deviation [RSD (%)] for repeatability (intra-day precision) and intermediate precision (inter-day precision). The RSD (%) values of intra-day were found to be 0.116 and 1.424, while RSD (%) values of inter-day were found to be 0.771 and 1.468 for meformin and glipizide, respectively (Table 3). The data obtained were within 2% RSD.

**Limit of Detection and Limit of Quantification**

The LOD and LOQ values were 991.30 and 3003.95 ng/band for metformin and 9.57 and 29.01 ng/band for glipizide, respectively (Table 3). The data shows that the method is sensitive for the determination of metformin and glipizide.

**TABLE 1** Statistical Evaluation of Linear Part of Calibration Dependence of Metformin and Glipizide

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<th>Parameter</th>
<th>Metformin</th>
<th>Glipizide</th>
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</thead>
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<td>Range (ng/band)</td>
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<td>50–250</td>
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<tr>
<td>Slope</td>
<td>1.12</td>
<td>6.76</td>
</tr>
<tr>
<td>Intercept</td>
<td>+24139.18</td>
<td>+271.75</td>
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<tr>
<td>$r^2$</td>
<td>0.9962</td>
<td>0.9930</td>
</tr>
</tbody>
</table>

**TABLE 2** The Recovery Studies of Metformin and Glipizide

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount Taken (ng/band)</th>
<th>Amount Added (ng/band)</th>
<th>% Recovery ± RSD (%) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>10000</td>
<td>7500</td>
<td>101.5 ± 0.4</td>
</tr>
<tr>
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<td>10000</td>
<td>10000</td>
<td>100.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>10000</td>
<td>12500</td>
<td>99.9 ± 0.2</td>
</tr>
<tr>
<td>Glipizide</td>
<td>100</td>
<td>75</td>
<td>98.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>98.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>125</td>
<td>98.4 ± 0.3</td>
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</table>
Selectivity

Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned in a previous section. The related chromatogram is presented in Figure 3. No interference from additives was obtained.

Stability Indicating Properties

Standard solutions of metformin and glipizide were protected from light and stored for a week at room temperature. Sample solutions were exposed to sunlight for a week at room temperature. Standards and solutions were re-analyzed for the stability study. The results are presented in Figure 4, which shows that glipizide is sensitive to room temperature.

Assay

The proposed validated method was successfully applied to determine metformin and glipizide in their combined dosage form. The label claim was 500 mg of metformin and 5 mg of glipizide per tablet. The % assay of metformin and glipizide found in “Glynase-MF” tablets by the proposed method are 99.9% and 100.2%, with RSD (%) 0.116% and 1.424%, respectively.

The Advantages of HPTLC Method Relative to LC/MS and HPLC Method

The Advantages of HPTLC

The HPTLC method is useful for:

- Simultaneous processing of sample and standard,
- Better analytical precision and accuracy,
- Reduced need for Internal Standard,

| TABLE 3 Data Indicating Various Validation Parameters of HPTLC Method (n = 6) |
|---------------------------------|-----------------|-----------------|
|                                  | Metformin       | Glipizide       |
| Precision (n = 6) Mean ± SD; RSD (%) |                 |                 |
| Intra-day precision             | 99.9 ± 0.115; 0.116 | 100.2 ± 1.426; 1.424 |
| Inter-day precision             | 101.1 ± 0.779; 0.771 | 100.6 ± 1.478; 1.468 |
| LOD (ng/band)                   | 991.30          | 9.57            |
| LOQ (ng/band)                   | 3003.95         | 29.01           |
The ability for several analysts to work simultaneously,
Lower analysis time and less cost per analysis,
Low maintenance cost,
Simple sample preparation,
The ability to handle samples of divergent nature,
No requirements of prior treatment for solvents like filtration and degassing,
Low mobile phase consumption per sample,
No interference from previous analysis,

**FIGURE 3** Chromatogram for standards of metformin and glipizide (a) and Glynase-MF tablet (b). (Color figure available online.)
Fresh stationary and mobile phases for each analysis with no contamination,
- The ability for visual detection with an open system,
- To determine non-UV absorbing compounds detected by postchromatographic derivatization.

The proposed method in this study required less time and less solvent for the analysis; therefore, the proposed method is cost effective as HPLC grade solvents are expensive.

**FIGURE 4** Stability indicating study of standard solution (a) and Glynase-MF tablet (b). (Color figure available online.)
CONCLUSION

A new HPTLC method for simultaneous determination of metformin and glipizide in pharmaceutical tablet formulation has been developed. The method was found to be simple, sensitive, precise, accurate, and specific for quantification of metformin and glipizide in pharmaceutical formulation. The proposed method can be used in detail stability studies of metformin and glipizide.

ACKNOWLEDGMENTS

We are thankful to Torrent Research Center (Bhat, Gandhinagar, India) and Cadila Pharma (Dholka, India) for providing gift samples of metformin hydrochloride and glipizide, respectively. We are also grateful to the K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India, for providing laboratory facilities for research work.

REFERENCES


Decision Letter (LJLC-2011-0186.R1)

From: mmoskovitz@aol.com
To: darshana_pharma@yahoo.co.in
CC:

Subject: Journal of Liquid Chromatography & Related Technologies - Decision on Manuscript ID LJLC-2011-0186.R1

Body: @date to be populated upon sending@

Dear Ms Modi:

Ref: RAPID AND SENSITIVE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE HYDROCHLORIDE IN TABLET FORMULATION BY HPTLC METHOD.

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RAPID AND SENSITIVE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE HYDROCHLORIDE IN TABLET FORMULATION BY HPTLC METHOD.
RAPID AND SENSITIVE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE HYDROCHLORIDE IN TABLET FORMULATION BY HPTLC METHOD.

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ABSTRACT

A rapid, simple, and sensitive HPTLC method for simultaneous estimation of two antidiabetic drugs, metformin hydrochloride and pioglitazone hydrochloride, in pharmaceutical tablet was developed and validated. Separation was achieved on aluminum sheets of silica gel 60F₂₅₄ using butanol: 1, 4-dioxane: glacial acetic acid (5:3:2v/v/v) as mobile phase. This system gave a good resolution for metformin hydrochloride (Rₚ value of 0.36 ± 0.01) and pioglitazone hydrochloride (Rₚ value of 0.73 ± 0.01). Detection and quantification was carried out at 226 nm. Calibration plot was constructed in the range of 2000 to 20000 ng/band for metformin hydrochloride and 60 to 600 ng/band for pioglitazone hydrochloride. The linear regression data for the calibration plot showed a good relationship with r = 0.9960 and 0.9968 for metformin hydrochloride and pioglitazone hydrochloride, respectively. Assays for metformin hydrochloride and pioglitazone hydrochloride were 99.6% (RSD 0.618 %) and 99.7% (RSD 0.511 %) respectively for the brand analyzed. The method was validated for precision and recovery. The limits of detection and quantification were 629.89 and 1908.76 ng/band for metformin hydrochloride and 8.51 and 25.77 ng/band for pioglitazone hydrochloride, respectively.

Keywords: Metformin, Pioglitazone, Oral antidiabetics, Pharmaceutical analysis, HPTLC
INTRODUCTION

Metformin hydrochloride (Figure 1a), N, N- dimethylimidodicarbonimidic diamide monohydrochloride, is an antihyperglycemic agent that improves insulin tolerance in-patient with type II diabetes, lowering both basal and postprandial plasma glucose.[1] Metformin hydrochloride decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Pioglitazone hydrochloride (Figure 1b), (±) - 5 - [4 - [2 - (5 -ethyl - 2 -pyridinyl) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione, Pioglitazone is an oral antidiabetic agent belonging to the class of thiazolidinediones that acts primarily by decreasing insulin resistance. [2] These drugs are oral hypoglycemic agents. A combination of 500 mg of metformin and 15 mg of pioglitazone are available commercially as tablets. This combination is used in the treatment of non-insulin dependent diabetes mellitus (NIDDM).

Pioglitazone hydrochloride is not yet official in any of the pharmacopoeia but metformin is official in IP [3], BP [1] and USPNF [4]. Various methods have been employed for the individual analysis of metformin both in biological fluids and pharmaceutical formulations, including HPLC [5–16], LCMS [17], spectrophotometry [18] and chemiluminescence [19]. Determination of pioglitazone by various analytical methods like spectrophotometric method [20] and HPLC and MECK method [21] in tablet dosage form, HPLC and solid phase extraction method in human serum [22] and in dog serum [23], HPLC and LC MS in human plasma [24,25] and HPTLC [26] have been reported.

Simultaneous determination of metformin and pioglitazone beside other antidiabetic agents by derivative spectrophotometry [27–28], HPLC [28-30] and TLC [31] is
available in the literature as well. The review of the literature revealed that no HPTLC method is reported for simultaneous estimation of metformin and pioglitazone in combined pharmaceutical dosage form. Therefore, it was thought worthwhile to develop a simple, rapid, precise, accurate HPTLC method for simultaneous estimation of metformin and pioglitazone in combined tablet dosage form.

EXPERIMENTAL

Materials

Butanol (AR grade), 1, 4-Dioxane (AR grade) and glacial acetic acid (AR grade) were supplied by SD Fine Chemicals (Mumbai, India). Reference standards of metformin hydrochloride and pioglitazone hydrochloride were procured from Torrent Research Centre (Bhat, Gandhinagar, India). PIOZ’MF–15 tablets were purchased from local market.

HPTLC Instrumentation

CAMAG TLC system composed of linomat 5 autosampler, TLC scanner 3, and winCATS 1.2.2 software (CAMAG, Muttenz, Switzerland) was used. Chromatography was performed on precoated silica gel 60 F<sub>254</sub> TLC plates (10 x 10 cm) (Merck, Darmstadt, Germany) using butanol-1, 4-dioxane-glacial acetic acid 5:3:2 (v/v) as mobile phase. The band length 5mm and distance between bands 15 mm was kept constant throughout the study. Number of applications on the plates were five for standards and three for real samples. Horizontal development to a distance of 90 mm was performed on 20 x 20 cm twin through chamber (CAMAG). Chromatograms were evaluated via peak area after scanning in absorbance mode at 226 nm.
Preparation of Standard Solution

Metformin (250 mg) and pioglitazone (7.5 mg) reference substances were accurately weighed and transferred to a 25-mL volumetric flask. The powder was dissolved in and diluted to volume with methanol to furnish concentrations of 10000 µg/mL metformin and 300 µg/mL pioglitazone. This solution (2 mL) was diluted to 10 mL with methanol to obtain a standard solution having a concentration of metformin (2000 ng/µL) and pioglitazone (60 ng/µL).

Preparation of Sample Solution

Twenty tablets were weighed and ground to a fine powder. A quantity of powder equivalent to 500 mg metformin and 15 mg pioglitazone was weighed and transferred to a 50-mL volumetric flask. The powder was dissolved in methanol, sonicated for 15 min, diluted to volume upto mark to furnish a solution containing 10000 µg/mL metformin and 300 µg/mL pioglitazone. The solution was filtered through Whatman filter paper No. 41. Then 2 mL of the solution was diluted to 10 mL with methanol to furnish a solution containing 2000 ng/µL metformin and 60 ng/µL pioglitazone.

Method Validation

Validation was done with respect to various parameters required under ICH guideline Q2 (R1). [32]

Linearity

Calibration curve was plotted over a concentration range from 2000 to 20000 ng/band for metformin and 60 to 600 ng/band for pioglitazone. For the calibration
curves, accurately prepared standard solution of metformin and pioglitazone (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 µL) were applied to the plate. The plate was developed in a developing chamber previously saturated with the mobile phase for 30 minutes. Each reading was the average of three determinations.

**Accuracy (% Recovery)**

The accuracy of the method was determined by calculating recoveries of metformin and pioglitazone by the standard addition method. The analyzed samples (8000 ng/band and 240 ng/band) were spiked with extra concentration levels of 4000, 8000, and 12000 ng/band for metformin and 120, 240, and 360 ng/band for pioglitazone and the mixtures were reanalyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples.

**Precision**

The precision of developed method was checked by repeatedly (n= 6) injecting 10000 ng/band sample solution of metformin and 300 ng/band sample solution of pioglitazone, without changing the position of plate for HPTLC method. Repeatability of a sample application and measurement of the peak area were determined on the same day by the repeated application (n = 6) of sample solutions, while intermediate precision was evaluated by comparing the assays for three different days.

**Limit of Detection and Limit of Quantification**

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations LOD=3.3 x N/B and LOQ=10 x N/B where N is standard deviation of the peak area (n = 3), taken as measure of the noise and B is the slope of the corresponding calibration curve.

**Selectivity**
Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned before.

**Procedure for Assay**

Twenty tablets were weighed and pulverized, and an amount of the powder equivalent to 500 mg of metformin and 15 mg of pioglitazone was taken in a 50-mL volumetric flask, sonicated for 30 min, and diluted to the mark with methanol. The solution was filtered through Whatman No. 42 filter paper. Then 2 mL of the solution was diluted to 10 mL with methanol. Five µL of this filtrate was applied to the HPTLC plate and developed, dried, and scanned. Quantity analysis of metformin and pioglitazone was made on the basis of peak areas received for standard solutions.

**RESULTS AND DISCUSSION**

**Wavelength Optimization**

UV-Vis spectra (Figure 2) of metformin and pioglitazone were measured from 200 to 400 nm and 226 nm was used as the optimum wavelength throughout the experiment for both substances.

**Method Validation**

**Linearity**

The calibration curve was linear from 2000 to 20000 ng/band for metformin and from 60 to 600 ng/band for pioglitazone, respectively. Statistical evaluations of the linear part of calibration dependence of metformin and pioglitazone are presented in Table 1.

**Accuracy (% Recovery)**
The analyzed samples were spiked with extra concentration levels of 4000, 8000, and 12000 ng/band for metformin and 120, 240, and 360 ng/band for pioglitazone and the mixtures were reanalyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples. The results are presented in Table 2.

**Precision**

The precision of the developed method was expressed as a percentage of relative standard deviation (% RSD) for repeatability (intra-day precision) and intermediate precision (inter-day precision). The % RSD values of intra-day was found to be 0.618 and 0.511, while % RSD values of inter-day was found to be 0.986 and 0.849 for meformin and pioglitazone, respectively (Table 3). The data obtained were within 2% RSD.

**Limit of Detection and Limit of Quantification**

The LOD and LOQ values were 629.89 and 1908.76 ng/band for metformin and 8.51 and 25.77 ng/band for pioglitazone, respectively (Table 3). The data shows that the method is sensitive for the determination of metformin and pioglitazone.

**Selectivity**

Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned before. Related chromatogram is presented in Figure 3. No interference from additives was obtained.

**Assay**

The proposed validated method was successfully applied to determine metformin and pioglitazone in their combined dosage form. The label claim was 500 mg of metformin and 15 mg of pioglitazone per tablet. The % assay of metformin and
pioglitazone found in “PIOZ” MF–15 tablets by the proposed method are 99.6% and
99.7%, with % RSD 0.618 % and 0.511 %, respectively.

CONCLUSION

A new HPTLC method for simultaneous determination of metformin and
pioglitazone in pharmaceutical tablet formulation has been developed. The method was
found to be simple, sensitive, precise, accurate and specific for quantification of
metformin and pioglitazone in pharmaceutical formulation. It does not suffer from
interference from common excipients present in the pharmaceutical preparation and can
be conveniently adopted for quality-control analysis.

ACKNOWLEDGEMENTS

We are thankful to Torrent Research Centre (Bhat, Gandhinagar, India) for
providing gift samples of metformin hydrochloride and pioglitazone hydrochloride. We
are also grateful to K.B. Institute of Pharmaceutical Education and Research
(Gandhinagar, Gujarat, India) for providing laboratory facilities for research work.

REFERENCES

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**Table 1** Statistical evaluation of linear part of calibration dependence of metformin and pioglitazone

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<thead>
<tr>
<th>Parameter</th>
<th>Metformin</th>
<th>Pioglitazone</th>
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<tbody>
<tr>
<td>Range (ng/band)</td>
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<td>Slope</td>
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<tr>
<td>$r^2$</td>
<td>0.9960</td>
<td>0.9968</td>
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Table 2 The recovery studies of metformin and pioglitazone

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount taken (ng/band)</th>
<th>Amount added (ng/band)</th>
<th>% Recovery ± %</th>
<th>RSD (n=3)</th>
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<td>Metformin</td>
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<td>98.1 ± 0.1</td>
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<td>99.3 ± 0.5</td>
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<td>Pioglitazone</td>
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Table 3 Data indicating various validation parameters of HPTLC method (n = 6)

<table>
<thead>
<tr>
<th></th>
<th>Metformin</th>
<th>Pioglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision (n=6)</td>
<td>99.6 ± 0.616; 0.618</td>
<td>99.7 ± 0.510; 0.511</td>
</tr>
<tr>
<td>Intra-day precision</td>
<td>101.0 ± 0.996; 0.986</td>
<td>99.8 ± 0.847; 0.849</td>
</tr>
<tr>
<td>LOD (ng/band)</td>
<td>629.89</td>
<td>8.51</td>
</tr>
<tr>
<td>LOQ(ng/band)</td>
<td>1908.76</td>
<td>25.77</td>
</tr>
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</table>
A Simple and Sensitive HPTLC Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in Tablet Dosage Form

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Abstract

A simple, rapid, and precise high-performance thin-layer chromatographic (HPTLC) method for simultaneous estimation of two antidiabetic drugs, metformin hydrochloride and sitagliptin phosphate, in tablet dosage form has been developed and validated. Chromatography was performed on silica gel 60 F254 plates with butanol: water: glacial acetic acid (6:2:2, v/v/v) as mobile phase. This system gave a good resolution for metformin hydrochloride ($R_s$ value of 0.35 ± 0.01) and sitagliptin phosphate ($R_s$ value of 0.75 ± 0.01). Detection and quantification were carried out at 227 nm. The linear regression data for the calibration plots showed a good relationship with $r = 0.9991$ and 0.9991 for metformin hydrochloride and sitagliptin phosphate, respectively. The method was validated for precision and recovery. The limits of detection and quantification were 13.05 and 39.56 ng/μL for metformin hydrochloride and 2.65 and 8.03 ng/μL for sitagliptin phosphate, respectively. The amounts of the drugs in the marketed formulation were 99.86% and 98.91% for metformin hydrochloride and sitagliptin phosphate, respectively.

1. Introduction

Metformin hydrochloride (MET), N,N-dimethylimidodicarbonimidic diamide monohydrochloride (Figure 1(a)), is an antihyperglycemic agent that improves glucose tolerance in patients with type II diabetes, lowering both basal and postprandial plasma glucose. Metformin hydrochloride decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization [1]. Sitagliptin phosphate (SITA), (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5h-triazolo[3,4-c]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one (Figure 1(b)), is new oral antidiabetic agent that blocks dipeptidyl peptidase-4 (DPP-4) activity. SITA increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels [2].

A literature survey revealed that MET is official in IP [4], BP [3], and USPNF [5], while SITA is not yet official in any of the pharmacopoeias. A detailed literature survey found that LC-MS/MS method has been reported for quantitation of MET and SITA from mouse and human dried blood spots [6]. UV spectrophotometry has also been used for simultaneous determination of MET and SITA in tablet dosage form [7]. Determination of MET and SITA in tablet dosage form by liquid chromatography has also been reported [8]. To our knowledge, this is the first report of HPTLC method for simultaneous estimation of MET and SITA in a tablet dosage form. The HPTLC method is useful for simultaneous processing of sample and standard, reduced need for internal standard, lower analysis time and less cost per analysis, simple sample preparation, no requirements of prior treatment for solvents like filtration and degassing, no interference from previous analysis, fresh stationary and mobile phases for each analysis with no contamination, the ability for visual detection with an open system, and to determine non-UV absorbing compounds detected by postchromatographic derivatization. It reveals that proposed method require less time and less solvent for the analysis. So proposed method is cost effective as HPLC grade solvents are too costly.

2. Experimental

2.1. Materials

Butanol (AR grade) and glacial acetic acid (AR grade) were supplied by SD Fine Chemicals (Mumbai, India). Distilled water was used throughout the study. Reference standard of MET (99.5%) was procured from Torrent Research Centre (Bhat, Gandhinagar, India). Reference standard of SITA (99.8%) was procured from MSD Pharmaceuticals Private Limited (Bhiwand, Maharashtra, India). JANUMET tablets were purchased from local market.

2.2. HPTLC Instrumentation

A CAMAG HPTLC system equipped with Linomat 5 autosampler, TLC scanner 3, and winCATS 1.2.2 software (CAMAG, Bhiwandi, Maharashtra, India). JANUMET tablets were purchased from local market.

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Muttenz, Switzerland) was used. The slit dimension was kept at 5.00 x 0.45 mm, and 20 mm/sec scanning speed was employed. Chromatography was performed on precoated silica gel 60 F254 TLC plates (10 x 10 cm, catalogue number 1.05554.007) (Merck, Darmstadt, Germany) using butanol: water: glacial acetic acid (6:2:2, v/v/v) as mobile phase. The band length 6 mm and distance between bands 15 mm were kept constant throughout the study. Numbers of applications on the plates were five for standards and three for test samples. The application speed was 150 nL/sec. Ascending development to a distance of 85 mm was performed on 20 x 10 cm twin through chamber (CAMAG). Chromatograms were evaluated via peak area after scanning in absorbance mode at 227 nm.

2.3. Preparation of Standard Solution

MET (100 mg) and SITA (10 mg) reference substances were accurately weighed and transferred to a 25 mL volumetric flask. The powder was dissolved in and diluted to volume with methanol to furnish concentrations of 4000 μg/mL MET and 400 μg/mL SITA. This solution (2.5 mL) was diluted to 10 mL with methanol to obtain a standard solution having a concentration of MET (1000 ng/μL) and SITA (100 ng/μL).

2.4. Preparation of Sample Solution

Twenty tablets were weighed and ground to a fine powder. A quantity of powder equivalent to 200 mg MET and 20 mg SITA was weighed and transferred to a 50 mL volumetric flask. The powder was dissolved in methanol, sonicated for 30 min, diluted to volume up to mark to furnish a solution containing 4000 μg/mL MET and 400 μg/mL SITA. The solution was filtered through Whatman filter paper number 41. Then 2.5 mL of the solution was diluted to 10 mL with methanol to furnish a solution containing 1000 ng/μL MET and 100 ng/μL SITA.

2.5. Method Validation

Validation was done with respect to various parameters required under ICH guideline Q2 (R1) [9].

2.5.1. Linearity

Calibration curve was plotted over a concentration range from 500 to 10000 ng/band for MET and 50 to 1000 ng/band for SITA. For the calibration curves, standard solutions of MET and SITA (0.5, 2.0, 4.0, 6.0, 8.0, and 10.0 μL) were applied to the plate. The plate was developed in a developing chamber previously saturated with the mobile phase for 15 minutes. Each reading was the average of three determinations.

2.5.2. Accuracy (Percent of Recovery)

The accuracy of the method was determined by calculating recoveries of MET and SITA by the standard addition method. The analyzed samples (3000 ng/band and 300 ng/band) were spiked with extra concentration levels of 1000, 3000, and 5000 ng/band for MET and 100, 300, and 500 ng/band for SITA, and the mixtures were reanalyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples.

2.5.3. Precision

The precision of developed method was checked by repeatedly (n = 6) injecting 6000 ng/band sample solution of MET and 600 ng/band sample solution of SITA, without changing the position of plate for HPTLC method. Repeatability of a sample application and measurement of the peak area were determined on the same day by the repeated application (n = 6) of sample solutions, while intermediate precision was evaluated by comparing the assays for three different days.

2.5.4. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations LOD = 3.3 × N/β and LOQ = 10 × N/β, where N is standard deviation of the peak area (n = 3), taken as measure of the noise, and β is the slope of the corresponding calibration curve.

2.5.5. Selectivity

Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned before.

2.6. Procedure for Assay

Twenty tablets were weighed and powdered, and an amount of tablet powder equivalent to 200 mg of MET and 20 mg of SITA was taken in a 50 mL volumetric flask, sonicated for 30 min, and diluted to the mark with methanol. The solution was filtered through Whatman paper number 41. Then 2.5 mL of the solution was diluted to 10 mL with methanol. 6 μL of this filtrate was applied to the HPTLC plate and developed, dried, and scanned. Quantity analysis of MET and SITA was made on the basis of peak areas received for standard solutions.

3. Results and Discussion

3.1. Wavelength Optimization

UV-vis spectra (Figure 2) of MET and SITA were measured from 200 to 400 nm and 227 nm was used as the optimum wavelength throughout the experiment for both substances.

![Figure 2: Spectra for MET (10 μg/mL) and SITA (10 μg/mL) wavelength optimization.](image)

3.2. Method Validation

3.2.1. Linearity

The calibration curve was linear from 500 to 10000 ng/band for MET and from 50 to 1000 ng/band for SITA, respectively. Statistical evaluations of the linear part of calibration dependence of MET and SITA are presented in Table 1.

![Table 1: Statistical evaluation of linear part of calibration dependence of MET and SITA.](image)

3.2.2. Accuracy (Percent of Recovery)

The analyzed samples were spiked with extra concentration levels of 1000, 3000, and 5000 ng/band for MET and 100, 300,
and 500 ng/band for SITA, and the mixtures were reanalyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples. The results are presented in Table 2.

Table 2: The recovery studies of MET and SITA.

3.2.3. Precision
The precision of the developed method was expressed as a percentage of relative standard deviation (percent of RSD) for repeatability (intraday precision) and intermediate precision (interday precision). The data obtained were within 2% RSD (Table 3).

Table 3: Data indicating various validation parameters of HPTLC method (n – 6).

3.2.4. Limit of Detection and Limit of Quantification
The LOD and LOQ values were 13.05 and 39.56 ng/μL for MET and 2.65 and 8.03 ng/μL for SITA, respectively (Table 3). The data shows that the method is sensitive for the determination of MET and SITA.

3.2.5. Selectivity
Typical absorption spectra of MET and SITA are shown in Figure 3. Selectivity of the method was tested by comparison of peaks of test compounds with those of standards prepared as mentioned before. Related chromatogram is presented in Figure 3. Thus it can be concluded that the excipients did not interfere with the peaks from standard drug solutions.

Figure 3: Typical absorption spectra obtained from MET and SITA drug solutions.

3.3. Application of the Validated Method to a Pharmaceutical Preparation
The proposed validated method was successfully applied to determine MET and SITA in their combined dosage form. The label claim was 500 mg of MET and 15 mg of SITA per tablet. The percent of assay of MET and SITA found in JANUMET tablets by the proposed method is 99.86% and 98.91%, with percent of RSD 0.88% and 1.23%, respectively (Figure 4).

Figure 4: Chromatogram for standards of MET (6 μg/band) and SITA (0.6 μg/band) (a) and JANUMET tablet (b).

4. Conclusion
A new HPTLC method for simultaneous determination of MET and SITA in pharmaceutical tablet formulation has been developed. The method was found to be simple, sensitive, precise, accurate, and specific for quantification of MET and SITA in pharmaceutical formulation. It does not suffer from interference from common excipients present in the pharmaceutical preparation and can be conveniently adopted for quality-control analysis.

References
# SIMULTANEOUS ESTIMATION OF METFORMIN AND GlicaZIDE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC AND HPTLC METHODS

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<td>Complete List of Authors:</td>
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