DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND MITIGLINIDE CALCIUM DIHYDRATE IN COMBINED DOSAGE FORM

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ABSTRACT

A simple, accurate and precise RP-HPLC method was developed for simultaneous estimation of Metformin hydrochloride (MET) and Mitiglinide calcium dihydrate (MTG). C$_{18}$ ODS column (150 x 4.6 mm) was used as stationary phase. The mobile phase used was Acetonitrile:Water (70:30 v/v) at pH 3.0 adjusted by 5% o-phosphoric acid. The mobile phase was delivered at flow rate 1.0 ml/min. UV detection was set at 253 nm. The retention time of MET and MTG was found to be 2.053 min and 2.927 min respectively. Linearity was observed over the concentration range of 20-140 µg/ml and 10-70 µg/ml for MET and MTG respectively. The LOD was found to be 1.477 µg/ml 1.122 µg/ml for MET and MTG respectively. Whereas, LOQ was found to be 5.687 µg/ml for MET and 3.398 µg/ml for MTG. Moreover, the % CV for repeatability, inter and intraday precision was found to be less than 2%, which reveals that the method is precise. The % recovery was found to be 99.34-100.35% for MET and 98.52-101.64% for MTG. However, the change in pH, flow rate and organic phase ratio also did not show any significant variance. Assay of the combined dosage form finalized the applicability of this method for simultaneous estimation of Metformin hydrochloride and Mitiglinide calcium dihydrate in combined dosage forms.

Keywords: RP-HPLC, MET, MTG, LOD, LOQ.

INTRODUCTION

Diabetes Mellitus is generally characterized by deprivation of Insulin secretion or lack of uptake of blood glucose. There are mainly two types of Diabetes, Type I and Type II. Metformin is the most widely used drug for Type II Diabetes. It is the first line Oral hypoglycaemic agent. Various combinations of Metformin act as first line treatment for Diabetes Mellitus. Recently discovered combination of anti diabetic agents Metformin and Mitiglinide is highly effective in Type II Diabetes mellitus. This novel combination is very useful in treating streptozotocin induced Diabetes mellitus.

Metformin [IUPAC Name : N, N-Dimethylimidodicarbonimidic diamide hydrochloride] is a biguanide anti hyperglycemic agent used for treating non-insulin-dependent diabetes
mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain.

Mitiglinide [IUPAC Name : 2-benzyl-4-(octahydro-1H-isooindol-2-yl)-4-oxobutanoic acid] belongs to the Meglitinide class of anti diabetic agents. Mitiglinide is a drug for the treatment of Type II diabetes. Mitiglinide is thought to stimulate insulin secretion by binding to and blocking ATP-sensitive K\(^+\) channels in pancreatic beta-cells. Closure of potassium channels causes depolarization which stimulates calcium influx through voltage-gated calcium channels. High intracellular calcium subsequently triggers the exocytosis of insulin granules.

Literature review revealed that no analytical method has been reported for simultaneous estimation of Metformin hydrochloride and Mitiglinide calcium dihydrate. Aim & objective of this work was to develop and validate of a simple, rapid, precise RP-HPLC method for simultaneous estimation of Metformin hydrochloride and Mitiglinide calcium dihydrate in combined dosage form.

![Chemical Structure of Metformin HCl and Mitiglinide Calcium Dihydrate](image)

**Figure 1**

Chemical Structure of Metformin HCl and Mitiglinide Calcium Dihydrate

**MATERIALS AND METHOD**

**MATERIALS**

**Instruments & Apparatus**

Cyberlab HPLC with C\(_{18}\) ODS column (150×4.6 mm); Labindia Double beam UV-Visible spectrophotometer; Soltec sonica Ultrasonicator; Systronics pH Meter. Borosil
Measuring cylinder 10 ml, 50 ml, 100 ml. Borosil Pipette 1 ml, 2 ml, 5 ml, 10 ml. Borosil Volumetric flask 10 ml, 100 ml. (all apparatus were previously calibrated and made up of glass.)

Reagents & Chemicals

Metformin hydrochloride was procured from Intas Pharmaceuticals & Mitiglinide calcium dihydrate was procured from Cadila Healthcare Ltd, Ahmedabad. HPLC Grade Acetonitrile, Methanol and HPLC Water were procured from Finar Reagents, Ahmedabad.

METHODOLOGY[8-14]

Preparation Metformin hydrochloride stock solution (MET)

Accurately weighed 100 mg of quantity of MET reference standard was transferred into 100 ml volumetric flask and dissolved in 50 ml diluent and sonicated for about 10 min with intermittent shaking and diluted up to the mark with diluent to give a stock solution having strength 1000 µg/ml. 200 µg/ml of MET working standard solution was prepared by diluting 20 ml of stock solution with diluent in 100 ml volumetric flask up to the mark with diluent.

Preparation of Mitiglinide calcium dihydrate stock solution

Accurately weighed 50 mg quantity of MTG reference standard was transferred into 100 ml volumetric flask and dissolved in 50 ml diluent and sonicated for about 10 min with intermittent shaking and diluted up to the mark with diluent to give a stock solution having strength 500 µg/ml. 100 µg/ml of MTG working standard solution was prepared by diluting 20 ml of stock solution with diluent in 100 ml volumetric flask up to the mark with diluent.

Preparation of Solutions for Calibration curve of MET and MTG

MET formin hydrochloride (MET)

Aliquots of stock solution (200 µg/ml) of MET 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with mobile phase to get concentrations 20, 40, 60, 80, 100, 120 and 140 µg/ml. Solutions were injected into the system with stated chromatographic conditions. The graph of area
of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.

Mitiglinide calcium dihydrate (MTG)
Aliquots of working standard solution (100 µg/ml) of MTG 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with mobile phase to get concentrations 10, 20, 30, 40, 50, 60 and 70 µg/ml. Solutions were injected into the system with stated chromatographic conditions. The graph of area of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.

Validation of RP-HPLC method
1) Linearity and Range
The linearity response was determined by analyzing 7 independent levels of calibration curve in the range of 20-140 µg/ml and 10-70 µg/ml for MET and MTG respectively. Plot the calibration curve of Area versus respective concentration and find out correlation coefficient and regression line equation for MET and MTG.

2) Precision
1) Repeatability
It was determined by analyzing MET (40 µg/ml) and MTG (20 µg/ml) seven times in mixture. The areas of seven replicate injections were measured and % C.V. was calculated.

II) Intraday precision
For intraday, MET and MTG in the range of 20, 80, 140 µg/ml and 10, 40, 70 µg/ml were analyzed three times on the same day and % C.V. was calculated.

III) Interday precision
For interday, MET and MTG in the range of 20, 80, 140 µg/ml and 10, 40, 70 µg/ml were analyzed on three different days of a week and % C.V. was calculated.

3) Accuracy
To a fixed amount of pre-analyzed sample of MET 40 µg/ml and MTG 20 µg/ml, increasing amount of MET (32, 40, 48 µg/ml) and MTG working standard solution (16, 20, 24 µg/ml) were injected to system and analyzed as described in and respectively. The
mean % recovery from of peak areas calculated. Combined dosage form was used to perform accuracy.

4) Limit of Detection (L.O.D.)

The L.O.D. was estimated from the set of 5 calibration curves use to determine method linearity. The L.O.D. may be calculated as

$$\text{LOD} = 3.3 \times \left( \frac{\text{S.D}}{\text{Slope}} \right)$$

Where, S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

5) Limit of Quantification (L.O.Q.)

The L.O.Q. was estimated from the set of 5 calibration curves used to determine method linearity. The L.O.Q. may be calculated as

$$\text{LOQ} = 10 \times \left( \frac{\text{S.D}}{\text{Slope}} \right)$$

Where, S.D. = Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

6) Robustness

To evaluate robustness of the method few parameters were deliberately varied. The parameters included variation of flow rate, change in mobile phase ratio. The average value of % CV with respect to peak area and retention time were calculated.

Assay of Combined Dosage Form

Applicability of the proposed method was tested by analyzing the combined dosage form-Tablets. 20 tablets were weighed accurately and powdered. Assay was performed according to the developed method under optimized chromatographic conditions.

RESULT & DISCUSSION

Selection of wavelength

The detection was carried out in the UV region and wavelength selected for detection was 253 nm in mobile Phase. Spectral Data depicted in Figure 2.
Figure 2
Overlain UV spectra of Metformin hydrochloride and Mitiglinide calcium dihydrate in mobile phase

Optimized Chromatographic conditions
Stationary phase: C18 ODS Column (150×4.6mm); Mobile phase: Acetonitrile : Water (70:30 v/v) at pH 3.0 set with o-phosphoric acid; Detection wavelength: 253 nm; Injection volume: 20 μl; Temperature of column: Room temperature; Flow rate: 1.0 ml/min.

Figure 3
Chromatogram of Metformin HCL and Mitiglinide Calcium Dihydrate in Acetonitrile:Water (70:30 v/v)
System Suitability test

System suitability parameters mainly include Resolution, Column efficiency in terms of theoretical plates and tailing factor. These parameters are described below.

**TABLE 1: DATA FOR SYSTEM SUITABILITY TEST FOR MET AND MTG**

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>System Suitability Parameters</th>
<th>Observed Value</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resolution (R₀)</td>
<td>4.438</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>2</td>
<td>Number of theoretical Plates (N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MET</td>
<td>2751</td>
<td>Not Less than 2000</td>
</tr>
<tr>
<td></td>
<td>MTG</td>
<td>2795</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Tailing Factor (Tₐ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MET</td>
<td>1.074</td>
<td>Not greater than 2</td>
</tr>
<tr>
<td></td>
<td>MTG</td>
<td>1.384</td>
<td></td>
</tr>
</tbody>
</table>

Validation of RP-HPLC Method

1) Linearity and Range

The linearity range for Metformin Hydrochloride (MET) was 20-140 µg/ml and for Mitiglinide calcium dihydrate (MTG) it was found to be in the range of 10-70 µg/ml. Calibration curve for MET and MTG are presented in figure 5 & 6. The chromatogram of standard MET and MTG is depicted in below figure 4.

![Figure 4](image)

Chromatogram of Linearity for Metformin Hydrochloride (20-140 µg/ml) and Mitiglinide calcium dihydrate (10-70 µg/ml)

Linearity data for MET and MTG is presented in table below.
TABLE 2: LINEARITY DATA FOR MET AND MTG IN MOBILE PHASE

<table>
<thead>
<tr>
<th>MET µg/ml</th>
<th>MTG µg/ml</th>
<th>Mean±SD MET</th>
<th>Mean±SD MTG</th>
<th>% CV (n=5) MET</th>
<th>% CV (n=5) MTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10</td>
<td>459.803±8.248</td>
<td>258.817±4.2963</td>
<td>1.796</td>
<td>1.660</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>835.918±7.289</td>
<td>448.911±2.902</td>
<td>0.872</td>
<td>0.647</td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>1257.543±3.165</td>
<td>683.589±6.232</td>
<td>0.252</td>
<td>0.912</td>
</tr>
<tr>
<td>80</td>
<td>40</td>
<td>1681.975±10.744</td>
<td>903.932±3.521</td>
<td>0.639</td>
<td>0.390</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>2102.585±3.673</td>
<td>1123.718±2.572</td>
<td>0.175</td>
<td>0.229</td>
</tr>
<tr>
<td>120</td>
<td>60</td>
<td>2490.783±18.283</td>
<td>1329.372±8.943</td>
<td>0.734</td>
<td>0.673</td>
</tr>
<tr>
<td>140</td>
<td>70</td>
<td>2900.662±49.598</td>
<td>1556.762±21.27</td>
<td>1.709</td>
<td>1.366</td>
</tr>
</tbody>
</table>

Calibration curve:

Figure 5
Calibration curve of Metformin Hydrochloride (20-140 µg/ml)
2) Precision
   I. Repeatability:
   The %C.V. was found to be 1.395 and 1.594 for MET and MTG respectively. Chromatogram of standard MET and MTG for repeatability was depicted in Figure 7.

   II. Intraday precision
   The data for intraday precision of MET and MTG were presented by repetition of experiment on the same day at three different timings. The range of %C.V was found to be 0.639-1.801% for MET and 0.117-1.792% for MTG.
III. Interday precision
The data for interday precision of MET and MTG were obtained by repetition of experiment on three different days of a week. The range of %C.V was found to be 1.009-1.717% for MET and 1.652-1.921% for MTG.

3) Accuracy
The data for accuracy of MET and MTG are presented in Table 3. The recovery range for MET and MTG were found to be 99.34-100.35% and 98.52-101.64% from combined dosage form.

<table>
<thead>
<tr>
<th>Amt. of drug added(µg/ml)</th>
<th>Total Amt. of drug(µg/ml)</th>
<th>Amt. recovered mean ±S.D (n=3)</th>
<th>% Recovery (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET 32</td>
<td>MTG 16</td>
<td>MET 72</td>
<td>MTG 36</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>48</td>
<td>24</td>
<td>88</td>
<td>44</td>
</tr>
</tbody>
</table>

4) Limit Of Detection (L.O.D.)
The limit of detection for MET and MTG was found to be 1.877 µg/ml and 1.122 µg/ml.

5) Limit Of Quantification (L.O.Q.)
The limit of quantification for MET and MTG was found to be 5.687 µg/ml and 3.398 µg/ml.

6) Robustness
To evaluate robustness of the method few parameters were deliberately varied. The parameters included variation of flow rate, change in pH of the buffer, change in organic phase ratio. The average value of % CV for determination of MET and MTG, less than 2 % revealed the robustness of the method.

Assay of Combined Dosage Form
The results are shown in Table 4. The data revealed 99.16 % for MET and 98.22 % for MTG. These data confirmed the applicability of this method for combined dosage forms.
TABLE 4: ASSAY OF COMBINED DOSAGE FORM

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Labeled Claim (mg/tab)</th>
<th>Amount Found (mg/tab)</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>MET</td>
<td>250</td>
<td>247.89</td>
<td>99.16</td>
</tr>
<tr>
<td></td>
<td>MTG</td>
<td>125</td>
<td>122.78</td>
<td>98.22</td>
</tr>
</tbody>
</table>

Figure 8
Chromatogram for assay of Metformin hydrochloride and Mitiglinide calcium dihydrate in combined dosage form

All validation parameters are summarized in Table 5.

TABLE 5: SUMMARY OF VALIDATION PARAMETERS

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>MET</th>
<th>MTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity Range</td>
<td>20-140 µg/ml</td>
<td>10-70 µg/ml</td>
</tr>
<tr>
<td>2</td>
<td>Correlation Coefficient</td>
<td>0.9998</td>
<td>0.9997</td>
</tr>
<tr>
<td>3</td>
<td>Precision (% C.V.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>1.395</td>
<td>1.684</td>
</tr>
<tr>
<td></td>
<td>Intraday (n=3)</td>
<td>0.639-1.801</td>
<td>0.117-1.792</td>
</tr>
<tr>
<td></td>
<td>Interday (n=3)</td>
<td>1.009-1.717</td>
<td>1.652-1.921</td>
</tr>
<tr>
<td>4</td>
<td>Mean % Recovery</td>
<td>99.34-100.35</td>
<td>98.52-101.64</td>
</tr>
<tr>
<td>5</td>
<td>Limit of Detection</td>
<td>1.877 µg/ml</td>
<td>1.122 µg/ml</td>
</tr>
<tr>
<td>6</td>
<td>Limit of Quantitation</td>
<td>5.687 µg/ml</td>
<td>3.398 µg/ml</td>
</tr>
<tr>
<td>7</td>
<td>Robustness (% C.V.)</td>
<td>Peak Area</td>
<td>Retention Time</td>
</tr>
<tr>
<td></td>
<td>Change in Mobile Phase</td>
<td>1.870</td>
<td>1.806</td>
</tr>
<tr>
<td></td>
<td>Change in Flow Rate</td>
<td>0.907</td>
<td>1.928</td>
</tr>
<tr>
<td></td>
<td>Change in pH</td>
<td>1.350</td>
<td>1.410</td>
</tr>
</tbody>
</table>
CONCLUSION

A simple RP-HPLC method for simultaneous estimation of Metformin hydrochloride and Mitiglinide calcium dihydrate was developed and validated. The mobile phase used was Acetonitrile : Water (70:30 v/v) at pH 3.0 adjusted by 5% o-phosphoric acid. UV detector was set at 253 nm. The retention time of MET and MTG was found to be 2.053 min and 2.927 min respectively. The proposed method utilizes isocratic elution technique at room temperature. This method reduces the total run time for HPLC, leads to low solvent consumption and makes the analysis more economical. The method is simple, rapid, precise, and accurate for simultaneous estimation of Metformin hydrochloride and Mitiglinide calcium dihydrate in their combined dosage forms. It can be used for the routine quality control of the formulation in the pharmaceutical industries.

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REFERENCES


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