Estimation of Metformin Hydrochloride in Bulk and Granules by Ion-Pair Liquid Chromatography

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Abstract

A simple, precise, accurate, cost effective and alternative isocratic high performance liquid chromatography method was developed for the estimation of metformin hydrochloride in bulk and in granule formulation. Chromatographic separation was achieved with Eclipse XDB C18 column (150.00 mm × 4.60 mm) utilizing a mobile phase of sodium chloride, sodium pentane sulfonate along with triethylamine (50.00% W/W). Orthophosphoric acid was added to adjust the mobile phase pH to 3.8. It was filtered through 0.45 μm membrane filter and detection was carried out at 233 nm using PDA detector. Validation was performed as per ICH guidelines to demonstrate system suitability, specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, ruggedness. The linear response (r² = 0.999) was observed in the range of 25-75.00 μg mL⁻¹ with good recoveries (99.79% to 101.75%). Validation acceptance criteria were met in all cases. Thus, the method can be used in pharmaceuticals for routine quality control assay of metformin hydrochloride.

Keywords: Metformin hydrochloride; HPLC; Validation; Ion-pair reagent

Introduction

Metformin hydrochloride (MET), a hypoglycemic agent is chemically 1, 1-dimethylbiguanide hydrochloride FIG. 1 [1]. It is the drug of choice for type 2 diabetes mellitus. It improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization [2,3].


![Chemical structure of metformin hydrochloride.](image)

The objective of the present investigation was to establish and validate the fast and sensitive ion pair HPLC method for MET. Apart from its simplicity and sensitivity, the present method highlights on economic one since mobile phase used is complete devoid of organic solvent FIG. 2.

Ion pair reagent is attracted to the stationary phase because of its hydrophobic alkyl group and charged carried by the reagent (C5- SO3- (Sodium pentane sulfonate)) thereby attached to the stationary phase.

![Mechanism of ion pair chromatography.](image)

**Materials and Methods**

**Chemicals and reagents**

Metformin hydrochloride was obtained as gift sample from Shreeji Pharma International, Vadodara (India). MET granules were purchased from market and used as sample under test. Pentane sulphonic acid sodium salt (Sigma), triethylamine
(Aldrich Chemical Company, Inc.), sodium chloride and ortho phosphoric acid (OPA) used for preparation of mobile phase. The mobile phase was filtered through a 0.45 µm membrane filter (Gottingen, Germany).

**Equipment and chromatographic conditions**

The Agilent HPLC system consisted of a degasser G1379B, a bin pump G1312A, an automatic injector G1329A, a thermostatic column oven G1316A and a multi wavelength detector G1365B, all 1200 Series from Agilent Technologies, which were controlled by an Chem-station software. All chromatographic experiments were performed in the isocratic mode. The mobile phase was consisting of 1.2 gm of NaCl and 0.87 gm of sodium pentane sulfonate in 900 mL of water, 3.00 mL 50% of triethylamine was added to it and it was diluted to 1000 mL with water. Orthophosphoric acid was added to adjust the pH to 3.8. The injection volume was 10.00 µL and detection was at 233 nm (LC Solution). The mobile phase flow rate was set at 1.2 mL min⁻¹ and constant column temperature was 25°C.

**Preparation of solution**

The stock solution was prepared by accurately weighing 50 mg of MET and transferring into 50 mL volumetric flask, 25 mL of water was added as diluent, sonicated for 5 min and volume was made up to the mark. The final concentration of 50 μg mL⁻¹ was achieved by diluting 5 mL of stock solution to 100 mL with diluent.

**Assay of formulation**

Accurately weighed 20 pouches of granules and the average weight were calculated. Weight equivalent to 500 mg was transferred to 1000 mL of volumetric flask; 500 mL of diluent was added, sonicated for 30 min and volume was made up to the mark. The final concentration of 50 μg mL⁻¹ was achieved by diluting 5 mL of above solution to 50 mL with diluent and filtered through 0.45 μm Millipore PVDF filter.

**Validation of the proposed method**

The proposed method was validated following the guidelines of International Conference on Harmonization (ICH) [23]. The validation parameters like; specificity, linearity, accuracy, precision, limits of detection and limit of quantification, robustness, ruggedness and stability were determined.

**Suitability test:** System suitability was determined from six replicate injections of the standard solution before sample analysis.

**Specificity:** To study specificity, drug solution of 50 μg mL⁻¹ was injected into the column, under optimized chromatographic conditions.

**Linearity:** Linearity was studied over the concentration range of 25-75 μg mL⁻¹. Standard calibration curve was plotted with five calibrators over the said concentration with triplicate determinations at each level. The data of peak area vs. drug concentration were treated by linear least square regression analysis.
Accuracy: Accuracy was determined by applying the method in triplicate samples of mixture of placebo to which known amount of standard drug added at three different levels of analyzed sample solution (50%, 100% and 150%). The % recovery and % RSD at each level was calculated.

Precision: The precision studies were performed as intra-day, interday and repeatability. Intra-day was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three consecutive days. Repeatability was performed by analyzing same concentration of drugs for six times.

Limit of detection (LOD) and limit of quantification (LOQ): Sensitivity of the proposed method was estimated in terms of LOD and LOQ. These were determined by using standard deviation of the response and slope.

Robustness: Robustness of the method was performed by deliberately changing the critical chromatographic parameters such as the pH and flow rate of the mobile phase, column temperature and the injection volume.

Ruggedness: Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two different analysts using similar operational and environmental conditions.

Results and Discussion

Optimization of chromatographic conditions
The chromatographic conditions were optimized based on selection of column, optimization of mobile phase, wave length detection, and column temperature and flow rate. The conditions were found to be best suited for analysis as good resolution and sharp peak were observed. The mobile phase found to be the best suited since it devoid of organic solvents. The retention time was obtained at 3.5 min for both standard FIG. 3 and sample FIG. 4.

![Chromatogram for standard solution](image-url)
Validation of proposed method

The proposed method was validated by validating various parameters. The method was found to be system suitable as % RSD was 0.78%, tailing factor was 1.51 and column efficiency (N) was above 3500 plates per column. All these values were within the acceptance range. The specificity of the method was checked for the interference against blank.

![Chromatogram for sample solution](image)

**FIG. 4. Chromatogram of sample solution.**

Separation of the drug indicated no interference of blank and placebo. Standard calibration curve was plotted for linearity over the concentration range of 25-75 µg mL⁻¹. The r² value of 0.999 confirmed good linearity over selected concentration range as shown in FIG. 5. The percentage recovery was found to be in range of 99.79-101.75%. The detailed results of linearity, accuracy and precision are tabulated in (TABLE 1). The values obtained in the respective limits are indicative of accurate and precise method.

![Linearity curve for metformin hydrochloride](image)

**FIG. 5. Linearity curve for metformin hydrochloride.**
TABLE 1. **Optical characteristic of linearity, accuracy and precision for proposed method.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max</td>
<td>233 nm</td>
</tr>
<tr>
<td>Concentration range (µ mL⁻¹)</td>
<td>25-75</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 58213x + 15521</td>
</tr>
</tbody>
</table>

**Accuracy**

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Placebo (mg)</th>
<th>Sample wt. taken (mg)</th>
<th>% Recovery (Mean ± SD)b</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>68.0</td>
<td>250.0</td>
<td>99.79 ± 0.40</td>
<td>0.003</td>
</tr>
<tr>
<td>100</td>
<td>68.0</td>
<td>500.0</td>
<td>101.75 ± 0.01</td>
<td>0.010</td>
</tr>
<tr>
<td>150</td>
<td>68.0</td>
<td>750.0</td>
<td>100.72 ± 0.53</td>
<td>0.014</td>
</tr>
</tbody>
</table>

**Precision**

<table>
<thead>
<tr>
<th>Type of precision</th>
<th>Mean ± SDa</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day Precision</td>
<td>2948508 ± 1039.48</td>
<td>0.03</td>
</tr>
<tr>
<td>Inter-day Precision</td>
<td>2948634 ± 643.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Repeatability</td>
<td>2966157 ± 18571.66</td>
<td>0.63</td>
</tr>
</tbody>
</table>

a: mean of three determinations.
b: Percent recovery according to three replication of same concentration of sample.

The proposed method was found to be sensitive from LOD and LOQ values. The values obtained were found to be 1.5 µg mL⁻¹ and 4.5 µg mL⁻¹ for LOD and LOQ respectively. All the values for peak areas were within the acceptance limits as per ICH guidelines for robustness. Also in case of ruggedness, % RSD of less than 2% indicated the method was rugged. The results of robustness and ruggedness studies are summarized in (TABLE 2). Stock solution of sample and standard were found to be sufficiently stable for the specified period.
TABLE 2. Results of robustness and ruggedness studies.

<table>
<thead>
<tr>
<th>Robustness Parameters</th>
<th>Percent recovery $^{a,b} \pm$ SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal conditions</td>
<td>100.64 ± 0.63</td>
</tr>
<tr>
<td><strong>Variation of the mobile phase flow rate (mL min$^{-1}$)</strong></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>99.58 ± 1.09</td>
</tr>
<tr>
<td>1.4</td>
<td>99.48 ± 1.37</td>
</tr>
<tr>
<td><strong>Variation of column temperature (°C)</strong></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>100.69 ± 1.07</td>
</tr>
<tr>
<td>30</td>
<td>101.27 ± 0.62</td>
</tr>
<tr>
<td><strong>Variation of mobile phase (pH)</strong></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>100.24 ± 0.38</td>
</tr>
<tr>
<td>4.0</td>
<td>100.39 ± 0.70</td>
</tr>
<tr>
<td><strong>Variation of the injection volume (µg mL$^{-1}$)</strong></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>99.63 ± 0.70</td>
</tr>
<tr>
<td>12</td>
<td>100.42 ± 1.54</td>
</tr>
<tr>
<td><strong>Ruggedness parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Analyst</td>
<td>Mean ± SD  % RSD</td>
</tr>
<tr>
<td>I</td>
<td>101.14 ± 0.1285  0.13</td>
</tr>
<tr>
<td>II</td>
<td>101.165 ± 0.1259  0.12</td>
</tr>
</tbody>
</table>

$a$: Percent recovery according to the calibration curve obtained under optimal conditions (mean of six injections ± standard deviation);  
b: each sample contained 50 µg mL$^{-1}$ (100% concentration level)

Conclusion

In the present study, an attempt had made to develop and validate a simple, efficient, and sensitive ion pair HPLC method. The proposed method was found to be accurate, precise, and linear across the analytical range. The method was found to be suitable for the determination of drug content in said formulation. The method may be used to assess the quality of commercially available drug products of metformin hydrochloride. The most significant advantage of proposed method is that mobile phase does not contain organic phase and no pretreatment of sample is required. The obtained results indicated that the drug can be separated completely and there was no interference of excipients present in dosage form. Thus, it will serve as an alternative to routine existing analytical methods in pharmaceutical industry.

Acknowledgment

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REFERENCES

23. International Conference on Harmonization, validation of analytical procedures, Text and Methodology.