**Stability Indicating RP-HPLC Method for the Estimation of Metformin Hydrochloride and Repaglinide as API and Estimation in Tablet Dosage Form**

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**ABSTRACT**

A new simple, precise, sensitive, economical RP-HPLC method was developed for the estimation of Metformin hydrochloride and Repaglinide in bulk and tablet dosage form. The λmax for Metformin hydrochloride and Repaglinide was found to be 231 nm and 230 nm respectively. The linearity in the concentration range of 25 µg/ml to 150 µg/ml (r²=0.999) and 0.1 to 0.6 µg/ml (r²=0.999) for Metformin hydrochloride and Repaglinide respectively. The chromatographic values for Metformin hydrochloride and Repaglinide were found to be satisfactory on BDS Hypersil 18, 250 x 4.6 mm, 5 µm, column using mobile phase of ACN: Buffer in the ratio of 58:42 v/v with the flow rate of 1.0 ml/min. The developed method was validated according to ICH guidelines and found to be accurate, precise, rugged and robust and found to be in good accordance with the prescribed values. The newly developed method can be used for routine analysis estimation of Metformin hydrochloride and Repaglinide in bulk and tablet dosage form in pharmaceutical industry.

**Keywords:** Metformin hydrochloride, Repaglinide, Stability studies, method development and validation.

**INTRODUCTION**

Glucose is the obligatory source of energy for the brain and physiological control of blood glucose reflects the need to maintain adequate fuel supplies in the face of intermittent food intake and variable metabolic demands. Increased blood sugar causes increased insulin secretion, whereas reduced blood sugar reduces insulin secretion. Insulin is the main hormone controlling intermediary metabolism. It’s most obvious acute effect is to lower blood glucose¹⁻². Metformin hydrochloride is the drug of choice in Biguanide classification. It is chemically 1, 1-dimethyl biguanide hydrochloride (Figure 1). Metformin lowers blood glucose additionally reduces low density and very low density lipoprotein LDL and VLDL respectively.⁴ Repaglinide is chemically(S)-(+)–2-ethoxy-4-[2-{3-methyl-1-[2-(piperidin-1-yl) phenyl] butylamino}-2-oxoethyl] benzoic acid (Figure 2). It acts by blocking the sulfonyl urea receptor on K<sub>ATP</sub> channels in pancreatic β-cell membranes.⁶ From literature review shows that there is developed method including UV⁷⁻⁸, Fluorometric⁹, HPTLC¹⁰, LC-MS¹¹⁻¹² methods. The review of the literature reveals that the present study is to develop and validate a stability indicating HPLC method for metformin hydrochloride and Repaglinide according to ICH guidelines¹³.

**MATERIALS AND METHODS**

The reagents were used are of Analytical grade. HPLC Model Shimadzu equipped with BDS Hypersil C¹³ (4.6 x 250mm, 5 µm) Column and pump of LC-20 AT VP series with injector of Rheodyne.

**Optimization of Chromatographic Conditions**

The chromatographic conditions were optimized to Acetonitrile: NaH₂PO₄ ratio 58:42, Flow rate: 1.0 ml/min and Wave length: 231 nm.

**Preparation of standard solution of Metformin Hydrochloride and Repaglinide**

250 mg Metformin hydrochloride and 1 mg Repaglinide was taken and made up the volume to 100 ml.

**Preparation of standard solution of Repaglinide**

Accurately weighed 10mg Repaglinide of transferred to…

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Figure 1: Metformin hydrochloride

Figure 2: Repaglinide
Table 1: Standard stock solution of Repaglinide / Metformin Hydrochloride

<table>
<thead>
<tr>
<th>S. No</th>
<th>Solvent</th>
<th>Solubility Metformin Hydrochloride</th>
<th>Solubility Repaglinide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Acetonitrile</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>0.1N NaOH</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>0.1N HCl</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

10ml volumetric flask volume made up to 10ml using mobile phase. Pipetted 0.1ml and made up to the mark using mobile phase.

Solubility
Solubility of drugs was observed by dissolving it in different solvents and it was found that drugs having good solubility in following solvents. The table 1 shows the different solubility of Metformin hydrochloride and Repaglinide.

Standard stock solution of Repaglinide / Metformin Hydrochloride
Accurately 10 mg of Repaglinide / Metformin HCl was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of mobile phase and then made up to the volume with mobile phase. The maximum wavelength was found to be 231 nm. The chromatogram of both the standard and sample in Figure 3.

Assay
Weigh about 20 tablets and powdered. Powder equivalent to 500mg and 2mg of Metformin HCl and Repaglinide were taken into 50 ml volumetric flask. Add about 10 ml of mobile phase and sonicated until the contents were dissolved. Filter the contents by using 0.45µ membrane filter under vacuum. Make up to the mark with mobile phase. Inject 20µl of sample solution into the chromatographic system. Measure the area of Metformin HCl and Repaglinide and calculate the percentage of assay.

Standard Market sample analysis

Linearity
To 250 mg Metformin hydrochloride and 1 mg Repaglinide was taken and made up the volume to 100 ml. Pipette out 1, 2, 3, 4, 5, and 6ml from the stock solution and made up the volume using 100 ml respectively. The results were tabulated in Table 2 and Chart showing the linearity of Repaglinide and Metformin hydrochloride in Figure

Accuracy
From the Standard stock solution pipette out 5 ml and make up to 100 ml (Spiking Standard Solution). From the standard stock solution pipette out 1, 2, 3, 4 and 5ml
respectively and made up the volume using 100ml (Standard Stock Solution). The average % Recovery of Repaglinide and Metformin HCl was found to be 99.80 and 99.70 respectively. The results were tabulated in table 4.

**Precision**
The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. Should be investigated using homogeneous, authentic samples.

**Repeatability**
Established the repeatability of the analytical method by estimating the assay for 5 sample proportion of the same batch under normal operating conditions. Calculated the assay for all 5 sample preparation and reported the %RSD for the sample. The results were tabulated in table 4.

**Ruggedness**
Intermediate precision (ruggedness)
Table 6: Ruggedness for Repaglinide and Metformin HCl

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug</th>
<th>Retention time of Repaglinide (min)</th>
<th>Retention time of Metformin HCl (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Metformin</td>
<td>100+12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>HCl</td>
<td>125+12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>150+12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Table 8: Limit of Detection and Limit of Quantization of Repaglinide and Metformin HCl.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drugs</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Metformin</td>
<td>Theoretical Plates (N)</td>
<td>6169.000</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>LOD, µg/ml</td>
<td>3.73220</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>LOQ, µg/ml</td>
<td>11.64910</td>
</tr>
<tr>
<td>1</td>
<td>Repaglinide</td>
<td>Theoretical Plates (N)</td>
<td>7196.000</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>LOD, µg/ml</td>
<td>0.01291</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>LOQ, µg/ml</td>
<td>0.04030</td>
</tr>
</tbody>
</table>

Intermediate precision study was carried out by repeating the complete experiment with different analysts, on different days in same laboratory as per the following preparation. To 250 mg of Metformin hydrochloride and 1 mg of Repaglinide made up the volume to 100 ml. the sample.

Method Precision
Ability to reproduce data within the predefined precision is called method precision. The standard and sample was prepared and readings were taken.

Robustness
Capacity to remain unaffected by small but deliberate variations in method parameters. Variations may include: stability of analytical solution, variation of pH in a mobile phase, different column (lot/supplier), temperature, and flow rate. From the standard stock solution and sample, the robustness can be measured with different flow rate and wavelength. The method remains unaffected by the small deliberate changes that were introduced. The results were tabulated in table 7.

Specificity
Treating with Acids
To 1 ml from the stock solution into a 10 ml volumetric flask. To that 1 ml of 0.1M hydrochloric acid added. Observed for any change took place in the retention of the peak.

Treating with Base
To 1 ml from stocks solution into a 10 ml volumetric flask. To this 1 ml of 0.1 M sodium hydroxide were added and Observed for any degradedness.

Heating
For the specificity study 1 ml from the stock solution should be taken in a 10 ml flask, make up to the volume with the mobile phase. The solution should be heated at 40°C for a period of 30 min. Observed for any degradation occurs or not. From the specificity performed, various degradation products are formed and there is no change in the detection of the analyte in the presence of other components.

Limit of Detection and Limit of Quantization
The Limit of Detection and Limit of Quantification was done as per ICH guidelines for Metformin Hydrochloride and Repaglinide and tabulated in table 8.

LOD = 3 x STDEV / SLOPE
LOQ = 10 x STDEV / SLOPE

**CONCLUSION**

An HPLC method was developed and validated for various parameters as per ICH guidelines. The system suitability parameters proved that the proposed method is equally suitable for estimation of Metformin HCl and Repaglinide. The chromatogram for Metformin HCl and Repaglinide were found to be satisfactory on RP-18(2), 250 X 4.6mm, 5µm column, using mobile phase combination of ACN: Buffer (58:42 v/v) with flow rate of 1.0 ml/min. The accuracy of the method was determined by recovery with spiked concentration of pure drug at three levels for metformin HCl and Repaglinide. The recovery of drug was well within the acceptance limits of 97-103%. The method was rugged and robust as observed from insignificant variation in the results of analysis on changes in mobile phase composition ratio, pH, flow rate, temperature and analysis being performed by different analysts and on different days respectively. In all above cases the recovery was found to be within the limit. Hence, this study can be extended by studying the degradation kinetics of Repaglinide and Metformin HCl determination by RP-HPLC method and also its estimation in plasma and biological fluids.

**REFERENCES**


