A Novel RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride & Glimepiride in Tablet Dosage Form

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ABSTRACT
A new simple, fast accurate and reproducible reverse phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Metformin Hydrochloride and Glimepiride from tablet dosage form. The method was developed using Waters HPLC system on C18 column (Spherisorb ODS 2: 250mm x 4.6 µm) using a mixture of 25mM Phosphoric Acid pH 3.0 (with KOH) and Acetonitrile (40:60 v/v) as mobile phase in an isocratic elution mode at a flow rate of 1.00 ml/min at 40ºC with a load of 20µl. The detection was carried out using UV-Visible detector set at 240 nm. The retention time of Metformin Hydrochloride and Glimepiride were found to be 3.20 min and 6.7 min respectively. The method was validated with respect to linearity, robustness, precision and accuracy. The method had been successfully applied in other pharmaceutical formulations of the same composition.

Keywords: Metformin Hydrochloride, Glimepiride, High Performance Liquid Chromatography.

INTRODUCTION
Metformin Hydrochloride is 1, 1-Dimethylbiguanide hydrochloride 1 and is used in the treatment of diabetes. It is completely different from the hypoglycemic sulfonamides 2 both in its structure and its mode of action 2. It possibly interferes with mitochondrial respiratory chains and promote peripheral glucose utilization by enhancing anaerobic glycolysis or it enhances binding of insulin to its receptors and potentiates its action 3. Other explanation is that it suppresses hepatic gluconeogenesis and inhibits intestinal absorption of glucose. It causes little or no hypoglycemia in non diabetic patients 4. Glimepiride i.e. 1-[(4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrrole-1-carboxamido)-ethyl] phenyl] sulphonyl]-3- trans-(4-methylcyclohexyl) urea is a hypoglycemic agent belonging to the second generation.

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sulfonylureas. It appears to lower blood glucose by stimulating insulin release from beta cells in the pancreatic islets possibly due to increased intracellular cAMP.

EXPERIMENTAL
Reagents and Chemicals
Acetonitrile was used of HPLC grade (Merck, India) and Milli Q water was used for the preparation of the mobile phase. All other reagents used were of HPLC or AR grade.

Drugs used
CDL Reference Standards of Metformin HCl (100%) and Glimepiride (99.54%) were used. Tablet formulation GEMER 1 containing Metformin HCl (extended release) and Glimepiride was purchased from market used as sample under test.

Instrumentation
An Isocratic Waters HPLC with a 515 pump, 2487 dual λ UV-Visible detector and C18 column (Spherisorb ODS 2: 250mm x 4.6 µm) were used for the analysis. The HPLC system was well equipped with Empower 2 software for data processing.

Chromatographic Condition
The mixture of 25m (M) Phosphoric Acid pH 3.0 (with KOH) and Acetonitrile (40:60 v/v) was used as mobile phase in an isocratic elution mode. The mobile phase was filtered through 0.45 micron membrane filtered and then ultrasonicated for 10 minutes. The flow rate of 1.00 ml/min was set for elution and detection was carried out using UV-Visible detector set at 240 nm. All determinations

Vasudeban et al. developed a reverse phase HPLC method for the estimation of metformin hydrochloride in multicomponent dosage form. Cheng et al. reported a reverse phase method of estimation of metformin hydrochloride in formulation. The objective of the present investigation was to establish and validate the fast and sensitive high performance liquid chromatography (HPLC) method for simultaneous determination of metformin hydrochloride and glimepiride in dosage form.
were performed at a constant column temperature of 40°C with a load of 20µl. **Stock and working standard solutions**

A standard stock solution of Glimepiride RS was prepared by dissolving 10 mg Glimepiride in 100 ml Acetonitrile. A mixed standard solution was prepared by weighing 50 mg Metformin HCl and then 1 ml Glimepiride stock solution was added to it and final volume was made up to 100 ml with mobile phase. The final concentration of the solution was Metformin HCl 500 mcg/ml Metformin HCl 1 mcg/ml Glimepiride.

**Tablet Assay**

Twenty tablets were weighed and powdered. An accurately weighed quantity of tablet powder equivalent to about 500 mg Metformin HCl and 1 mg Glimepiride was transferred to 100 ml volumetric flask and 20 ml Acetonitrile was added to it. The solution was then sonicated for 10 minutes. A further 30 ml mobile phase was then added and the solution was again sonicated for another 15 minutes with intermittent shaking. The volume was made up to the 100 ml mark with mobile phase. Solution was mixed and filtered through 0.22 mcm membrane filter. 1 ml of this solution was further diluted to 10 ml with mobile phase to obtain the final concentration of Metformin HCl 500mcg/ml and Glimepiride 1 mcg/ml (Table-1)

**METHOD VALIDATION**

**Linearity**

The linearity of the method is the ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The linearity study was made from a series of mixed standard solutions of Metformin HCl and Glimepiride. Since in the available tablet dosage forms contain Metformin HCl and Glimepiride in the ratio 500:1, it was not possible to prepare mixed standard solutions by directly weighing & dissolving both the components together. Rather a stock solution of Glimepiride was prepared by dissolving 10.1 mg Glimepiride in 100 ml Mobile phase (101mcg/ml). Then suitable volumes of Glimepiride stock solution was mixed with different weighed amount of Metformin HCl in each case to obtain a series of mixed standard solutions having concentration range 125 mcg/ml to 750 mcg/ml for Metformin HCl and 0.25mcg/ml to 1.5 mcg/ml Glimepiride. Each solution was injected in replicate and chromatogram was recorded. The average peak areas were plotted against concentration to obtain calibration curves.

**Robustness**

To evaluate the robustness of the developed method, small deliberate variations in the optimized method parameters were done. The method was also tried in different HPLC system with different C18 column.

**System Repeatability**

To check the degree of repeatability of the method six injections of mixed standard were carried out and % RSD was calculated for the peak areas for both the components.
Accuracy (Recovery Study)
To study the accuracy of the developed method, recovery study was carried out by spiking standard of Metformin HCl and Glimepiride to the pre-analyzed sample at three different levels 50%, 100% and 150% std. addition.

RESULTS AND DISCUSSION
Different composition of mobile phases containing 25m (M) Phosphoric Acid (pH 3.0 adjusted with KOH) and Acetonitrile were tried but the mobile phase containing in 25mM Phosphoric Acid (pH 3.0 adjusted with KOH) and Acetonitrile in the ratio 40:60 (v/v) was found as optimal for obtaining well defined and resolve peaks at a flow rate of 1.0 ml/min at 40 degree. The optimum wavelength for detection was used at 240 nm, at which best detector response for both the substances was obtained. The mean retention time and standard deviation for Metformin HCl and Glimepiride were found to be 3.194± 0.0263 min and 6.923± 0.0015 min. Since the ratio of Metformin HCl: Glimepiride is 500:1 in all tablet dosage formulations available in the market, so it was really very difficult task to resolve such a small peak of Glimepiride from huge peak of Metformin HCl. A concentrated mixed standard solution of Metformin HCl (258.7 mcg/ml) and Glimepiride (252.85 mcg/ml) was injected in duplicate on the HPLC system to study the Resolution value and clear cut separation in the base line was observed.

Table 1- Assay of Metformin HCl and Glimepiride

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount present (mg)</th>
<th>Amount Found (mg)</th>
<th>% Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>500 mg</td>
<td>496.833 mg</td>
<td>99.37%</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>1 mg</td>
<td>0.9425 mg</td>
<td>94.25%</td>
</tr>
</tbody>
</table>

The proposed method was evaluated by the assay of commercially available tablets containing Metformin HCl (as sustained release) 500 mg and Glimepiride 1 mg. The linearity study shows the method is linear in the concentration range 125 mcg/ml to750 mcg/ml for Metformin HCl and 0.25mcg/ml to1.5 mcg/ml Glimepiride. Table 2 summarizes Beer’s law limits and system suitability parameters for the method are listed in Table 3.
Table -2 Validation Parameters for Metformin HCl & Glimepiride

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metformin HCl</th>
<th>Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Wavelength</td>
<td>240 nm</td>
<td>240 nm</td>
</tr>
<tr>
<td>Beer’s Law Limit (mcg/ml)</td>
<td>125-750</td>
<td>0.25-1.5</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9982</td>
<td>0.9960</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>7.55X10^6</td>
<td>2.76X10^6</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>5.10X10^4</td>
<td>4.56X10^4</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.52</td>
<td>0.73</td>
</tr>
<tr>
<td>Detection Limit (mcg/ml)</td>
<td>0.005</td>
<td>0.008</td>
</tr>
<tr>
<td>Quantitation Limit (mcg/ml)</td>
<td>0.012</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Table -3 System Suitability parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metformin HCl</th>
<th>Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration Range (mcg/ml)</td>
<td>125-750</td>
<td>0.25-1.5</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>3605</td>
<td>10938</td>
</tr>
<tr>
<td>Selectivity</td>
<td>-</td>
<td>2.5</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.94</td>
<td>1.19</td>
</tr>
<tr>
<td>Capacity Factor (k’)</td>
<td>2.4</td>
<td>5.99</td>
</tr>
<tr>
<td>Resolution</td>
<td>-</td>
<td>14.94</td>
</tr>
</tbody>
</table>

The accuracy of the method was established from the recovery study i.e. by external addition of standard in pre-analyzed tablets as described earlier. The result of recovery analysis is given below in Table 4.
Table – 4 % Percentage recovery for Metformin HCl and Glimepiride

<table>
<thead>
<tr>
<th>Recovery solutions</th>
<th>% Recovery for Metformin HCl</th>
<th>% of Recovery for Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Standard Addition</td>
<td>105.6%</td>
<td>94.41%</td>
</tr>
<tr>
<td>100% Standard Addition</td>
<td>100.37%</td>
<td>99.55%</td>
</tr>
<tr>
<td>150% Standard Addition</td>
<td>94.2%</td>
<td>102.7%</td>
</tr>
</tbody>
</table>

CONCLUSION
The developed RP-HPLC method was proved to be simple, fast and reliable. The method was validated for its performance parameters e.g. Linearity, Repeatability, Accuracy etc. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in simultaneous determination of Metformin.
HCl and Glimepiride in tablet dosage form as well as in other formulations.

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