RP- HPLC Method Development and Validation for the Simultaneous Estimation of Pioglitazone HCl and Glimepiride in Bulk drug and Pharmaceutical Dosage form

Madhukar A.1*, N. Kannappan2, Mahendra Kumar CB.3
1Department of Pharmacy, BKB Educational Society group of Institutions, Nomula, Ibrahimpatnam, Hyderabad, INDIA.
2Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, INDIA.
3Department of Pharmacy, St. Mary’s College of Pharmacy, Secunderabad, INDIA.

Received on: 13-01-2014; Revised and Accepted on: 16-02-2014

ABSTRACT

A simple, fast, and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of pioglitazone and glimepiride in bulk drug and pharmaceutical dosage form. The quantification was carried out using X-Bridge ODS (150 × 4.6 mm, 5µ) column and mobile phase comprised of Acetonitrile and Ammonium Acetate (pH 4.3; 20mM) in proportion of 40:60 (v/v). The flow rate was 1.0 ml/min and the effluent was monitored at 235 nm. The retention time of Pioglitazone and Glimepiride were 2.61 and 3.50 min respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. Linearity of pioglitazone and glimepiride were in the range of 1.5-225μg/ml and 0.20-30μg/ml respectively. The percentage recoveries of both the drugs were 98.95-101.22% and 98.46-100.98 for Pioglitazone and Glimepiride respectively from the tablet formulation. The proposed method is suitable for simultaneous determination of Pioglitazone and Glimepiride in pharmaceutical dosage form and bulk drug.

Keywords: Pioglitazone, Glimepride, HPLC, Method Validation.

INTRODUCTION

Pioglitazone (PIO) is the class of thiazolidinedione with hypoglycemic action to treat diabetes. While Pioglitazone does decrease blood sugar levels, studies on the main cardiovascular outcomes have not yielded statistically significant results [1]. It is one of the PPAR-alpha agonist, insulin sensitizer used to reduce insulin resistance. By enhancing insulin action on peripheral tissues [2]. Pioglitazone is Chemically (Fig. 1) 

\[
\text{Pioglitazone} = \text{[\(\pm\)-5-[(\(-2\)-[5-ethyl-2-pyridinyl]ethoxy)[phenyl]-methyl]-2,4]thiazolidinedionemono hydro-chloride.}
\]

![Fig. 1: Chemical Structure of Pioglitazone](image1)

Glimepiride is a medium- to long-acting sulfonylurea derivative. Glimepiride is Chemically it is \([\text{[p-}[2-\text{ethyl-4-methyl-2-oxo - 3 - Pyrroline – 1 - Oxamide} \text{ ethyl}] \text{ phenyl} \text{ sulfonyl} \text{ 3} - \text{ (Trans4-methylcyclohexyl)} \text{ urea. It is widely used in type-2 diabetes (Fig. 2). It is an oral Anti Diabetic with prolonged effect and it maintains a more physiological regulation of insulin secretion during physical exercise, which suggests during physical exercise which suggests that there may be less risk of hypoglycemia [3]}.]

![Fig. 2: Chemical Structure of Glimepiride](image2)

*Corresponding author:
Madhukar A.
HOD, Department of Pharmacy,
BKB Educational Society group of Institutions, Nomula, Ibrahimpatnam, Hyderabad, INDIA. Ph. No.: +91-8019899880.
*E-Mail: dr.amk2014@gmail.com
Triplicate samples of mixture of placebo to which known amount of Glimepiride tablets is determined by applying the method in 2. Oxidation: NaOH and analyzed using HPLC.

Linearity & range: The Linearity of detector response is established by plotting a graph to concentration versus area of Pioglitazone HCl and Glimepiride respectively.

Accuracy: Accuracy for the assay of Pioglitazone HCl and Glimepiride tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Pioglitazone HCl and Glimepiride standard is added at different levels (50%, 100%, and 150%).

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

Specificity: The Specificity indicating study of Pioglitazone HCl and Glimepiride were undergoes Acid, Alkali and Oxidation degradation and determining the correlation coefficient. A series of solution of Pioglitazone HCl and Glimepiride standards solution in the concentration ranging from about 1.5µg/ml to 225µg/ml and 0.20 to 30 levels of Pioglitazone HCl and Glimepiride target concentrations respectively were prepared and injected into the HPLC system.

RESULTS AND DISCUSSION

**Table No. 2**

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 1.5 to 225µg/ml and 0.2 to 30µg/ml of targeted concentrations for Pioglitazone HCl and Glimepiride standards with a correlation coefficient value is greater than 0.999. The correlation coefficients of Pioglitazone HCl and Glimepiride \((R^2) = 0.999 \& 0.999\) respectively, which shows that the method is capable of producing good response in UV-detector. And the results of Linearity parameters are listed in **Table 2 & Fig. 3, 4**.
Fig. 3: Linearity Chromatograms of Pioglitazone Hcl (1.5-225) and Glimepiride (0.2-30)
Pioglitazone HCl and Glimepiride Accuracy limit is the % recovery was found in the range of 98.95-101.22% and 98.46-100.98 respectively. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy. And the results of all Accuracy parameters are listed in Table. 3.

Table No. 3: Summary of results of Accuracy parameter for Pioglitazone HCl and Glimepiride

<table>
<thead>
<tr>
<th>Conc.</th>
<th>inj-1</th>
<th>inj-2</th>
<th>inj-3</th>
<th>Mean</th>
<th>% Recovery</th>
<th>STD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>75pm</td>
<td>1182464</td>
<td>1196743</td>
<td>1192567</td>
<td>1190591</td>
<td>98.95359</td>
<td>7341.65</td>
<td>0.616639</td>
</tr>
<tr>
<td>150ppm</td>
<td>2411762</td>
<td>2410783</td>
<td>2409403</td>
<td>2409403</td>
<td>100.1263</td>
<td>31</td>
<td>0.132088</td>
</tr>
<tr>
<td>225pm</td>
<td>3654063</td>
<td>3658946</td>
<td>3647835</td>
<td>3653615</td>
<td>101.2209</td>
<td>5569.05</td>
<td>0.152426</td>
</tr>
</tbody>
</table>

Glimepiride

<table>
<thead>
<tr>
<th>Conc.</th>
<th>inj-1</th>
<th>inj-2</th>
<th>inj-3</th>
<th>Mean</th>
<th>% Recovery</th>
<th>STD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10ppm</td>
<td>280674</td>
<td>281053</td>
<td>280896</td>
<td>280874.3</td>
<td>98.46085</td>
<td>190.42</td>
<td>0.067798</td>
</tr>
<tr>
<td>20ppm</td>
<td>570275</td>
<td>570605</td>
<td>570026</td>
<td>570302</td>
<td>99.96004</td>
<td>290.44</td>
<td>0.050928</td>
</tr>
<tr>
<td>30ppm</td>
<td>864530</td>
<td>863530</td>
<td>864530</td>
<td>864196.7</td>
<td>100.9817</td>
<td>577.35</td>
<td>0.066808</td>
</tr>
</tbody>
</table>

The recovery results indicating that the Pioglitazone HCl and Glimepiride undergoes Acid, Alkali and Oxidation degradation and drug doesn’t undergoes any significant degradation on Photolysis and Heat condition.

Table No. 4: System suitability parameters

<table>
<thead>
<tr>
<th>Conc. of Pio. &amp; Glm.</th>
<th>Injection</th>
<th>Area of Pio.</th>
<th>RT</th>
<th>Area of Glm</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 &amp; 20ppm</td>
<td>inj-1</td>
<td>2410835</td>
<td>2.616</td>
<td>571934</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>inj-2</td>
<td>2409735</td>
<td>2.617</td>
<td>570703</td>
<td>3.48</td>
</tr>
<tr>
<td></td>
<td>inj-3</td>
<td>2411035</td>
<td>2.617</td>
<td>570892</td>
<td>3.514</td>
</tr>
<tr>
<td></td>
<td>inj-4</td>
<td>2410063</td>
<td>2.65</td>
<td>571389</td>
<td>3.49</td>
</tr>
<tr>
<td></td>
<td>inj-5</td>
<td>2409025</td>
<td>2.617</td>
<td>572003</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>inj-6</td>
<td>2412072</td>
<td>2.716</td>
<td>571876</td>
<td>3.47</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2410460.833</td>
<td>2.6388</td>
<td>571505.333</td>
<td>3.4923</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>107816.9637</td>
<td>0.0400</td>
<td>474923.0113</td>
<td>0.0157</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.044728776</td>
<td>1.5187</td>
<td>0.083100364</td>
<td>0.4515</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td></td>
<td>1.000</td>
<td></td>
<td>1.062</td>
<td></td>
</tr>
<tr>
<td>Plate Count</td>
<td></td>
<td>3796.8</td>
<td></td>
<td>3019.1</td>
<td></td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
<td>y = 16140x + 10699</td>
<td>y = 28675x + 8388</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation co-efficient (R²)</td>
<td>0.9998</td>
<td>0.9995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td>0.0000</td>
<td></td>
<td>4.1693</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Analysis

Fig. 4: Linearity of Pioglitazone HCl and Glimepiride

Fig. 5: Standard Chromatogram of Pioglitazone HCl and Glimepiride

Regression equation

$$y = 16140x + 10699$$

$$R^2 = 0.999$$

$$y = 28675x + 8388$$

$$R^2 = 0.999$$

Resolution

58-63
**CONCLUSION**

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Pioglitazone HCl and Glimepiride is done by reverse phase HPLC. The mobile phase consists of buffer (600 volumes buffer, and 400 volumes of Acetonitrile. The ratio pH was found to be 4.3. Then finally filtered using 0.45 μ nylon membrane filter and degassed in sonicator for 10 minutes). The detection is carried out using UV-Detector set at 235nm. The solutions are chromatographer at the constant flow rate of 0.8ml/min. The Retention time for Pioglitazone HCl and Glimepiride were around 2.61 & 3.50 minutes, Linearity ranges for Pioglitazone HCl and Glimepiride is 1.5-225 & 0.20-30μg/ml respectively. The quantity estimation was carried out on the tablet by RP-HPLC taking a concentration of 100μg/ml. The quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 98.95-101.22% and 98.46-100.98 for Pioglitazone HCl and Glimepiride respectively. The degradation of Pioglitazone HCl and Glimepiride undergoes Acid, Alkali and Oxidation degradation and there was not any significant degradation observed in Photolysis and Heat condition.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer’s law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision.

**REFERENCE:**


**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Source of support:** Nil