Development and validation of a Reverse Phase-HPLC method for the determination of Rosiglitazone Maleate in tablet dosage form

S.Gopalakrishnan*, E.Vadivel*, M.Karthika* and B. Jeyashree*

*Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

The Madras Pharmaceuticals, Chennai, Tamil Nadu, India

ABSTRACT

A rapid reverse phase high performance liquid chromatography method has been developed and validated for the determination of Rosiglitazone maleate in tablets. Isocratic chromatography was performed on a Kromasil C18 (250X4.6mm) with buffer:acetonitrile:methanol in the ratio of 65:25:10 as a mobile phase at a flow rate of 1.0 ml min⁻¹ and the detection was monitored out by photodiode array detector at 235 nm. The retention time for Rosiglitazone maleate was found to be 5.720 min. Good linearity was demonstrated in the range of 160-260 µg/ml (r~0.9975). Various chromatographic parameters including precision, accuracy, system suitability, specificity, LOD, LOQ and robustness have been evaluated. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Rosiglitazone maleate.

Keywords: RP-HPLC, Rosiglitazone maleate, Validation, Anti-diabetic, Isocratic.

INTRODUCTION

Rosiglitazone maleate (1) is an anti-diabetic drug in the thiazolidinedione class of drugs(1-3). It works as an insulin sensitizer, by binding to the PPAR receptors in fat cells and making the cells more responsive to insulin(4-7). It is marketed by the pharmaceutical company GlaxoSmithKline as a stand-alone drug (Avandia) and in combination with Metformin (Avandamet) or with Glimepiride(8-10) (Avandaryl).
Objective
A new, sensitive, accurate and precise Reverse Phase-High Pressure Liquid Chromatography method has been developed for the routine determination of Rosiglitazone maleate in tablet formulation in the quality control department.

MATERIALS AND METHODS

Chemicals
Dichromolactose, Macro crystalline cellulose, Sodium starch glycollate, Magnesium stearate were supplied by E.Merck Ltd, Germany. Acetonitrile, Methanol, Potassium dihydrogen orthophosphate were purchased from Sun Pharmaceuticals Ltd. Mumbai. Rosiglitazone maleate, Rosiglitazone Tablet, Placebo granular powder were purchased from Aldrich Chemicals (USA).

Buffer preparation
Potassium dihydrogen ortho phosphate was dissolved in 1000 ml of distilled water and 5 ml of triethylamine was added and the pH was adjusted to 6.0 with orthophosphoric acid.

Preparation of mobile phase
About 65 volumes of 0.01 M potassium dihydrogen ortho phosphate was added and its pH was adjusted to 3.0 with 25 volumes of acetonitrile and 10 volumes of methanol.

Instruments and Chromatographic conditions
The HPLC system (Shimadzu Co, Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVp, A Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 240nm)

Stationary phase : Kromasil C18 250 x 4.6mm
Buffer : 0.01 M Potassium dihydrogen orthophosphate adjusted to pH 3.0 with Orthophosphoric acid.
Mobile phase ratio : 65:25:10 (Buffer:Acetonitrile:Methanol)
Flow rate : 1.0 ml/min
Injection Volume : 10 µl
Detection : 235 nm

Calculation of Rosiglitazone Maleate

<table>
<thead>
<tr>
<th>Spl.area</th>
<th>Std.Wt</th>
<th>100</th>
<th>Std.purity</th>
<th>X Avg.Wt</th>
<th>X Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std.area</td>
<td>100</td>
<td>X Spl.wt</td>
<td>100</td>
<td>X Avg.Wt</td>
<td>X Conversion factor</td>
</tr>
</tbody>
</table>
Validation of parameters

System suitability

System suitability parameters are evaluated by following ICH guidelines injecting five replicates of 200 µg/ml concentration of standard Rosiglitazone maleate solution. Resolution factor, theoretical plate and tailing factor were evaluated by following ICH guidelines. The results are presented in Table.1 and Fig.1.

Linearity

Linearity of the peak area response was determined by making six measurements at six different concentrations point in the range of 160-260 µg/ml of sample Rosiglitazone maleate respectively. The linear regression coefficient was calculated. The results are presented in Table.2 and Fig.2.

Accuracy

Accuracy was assessed by using a minimum of three different concentration (standard Rosiglitazone maleate 80%, 100%, and 120%) and 310 mg of placebo spiked into the standard solution of Rosiglitazone maleate. The mean, standard deviation and RSD were calculated. The results are presented in Table.3.

Specificity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by comparing the blank chromatogram with the chromatogram obtained for the drug spiked with internal standard(placebo) to trace out the interfering peaks.

The specificity of the method was investigated by the analysis of blank preparations spiked with standard Rosiglitazone maleate and the sample Rosiglitazone maleate and internal standard (placebo) is also added. The result is presented in Table.4.

Limit of quantitation (LOQ)

LOQ, the peak area response was determined by analysing five times of 20 µg/ml concentration of standard Rosiglitazone maleate and the sample solution Rosiglitazone maleate of 20 µg/ml. The mean, standard deviation and RSD were calculated. The result is presented in Table.4.

Limit of detection (LOD)

LOD is the peak area response was determined by making twenty one measurements at seven different concentrations of the sample Rosiglitazone maleate in the range of 2 µg/ml – 10 µg/ml. The mean, standard deviation and RSD were calculated. The result presented in Table.4.

Precision

i) Reproducibility

Reproducibility of the method assessed by analysing five times 200 µg/ml of standard solution of Rosiglitazone maleate. The mean, standard deviation and RSD of Reproducibility were calculated. The results are presented in Table.4.
ii) Repeatability
Repeatability of the peak area response was determined by making six measurements at six
different concentration points in the range of 336.1 - 338.5 mg/ml of sample Rosiglitazone
maleate respectively and it is compared with that of the standard Rosiglitazone maleate 26.5
mg/ml. The results are presented in Table.4.

Robustness
Robustness was determined by injecting triplicate injection of standard and three sample
solutions in single and at different concentration with respect to control condition.

Robustness of the method was checked by varying the instrumental condition
Wavelength ±2 nm, temperature 2° C. and the %RSD was calculated. The results are presented
in Table-4

RESULTS AND DISCUSSION

Method development
The present RP-HPLC method for the quantification of Rosiglitazone maleate in bulk and
Pharmaceutical dosage forms, revealed as simple, accurate, precise, robust, specific and stability
indicating. The method has the significant retention time of 5.720 min.

System suitability
System suitability test was employed to establish the parameters such as tailing factor, theoretical
plates and retention time. The tailoring factor is 1.768 and the theoretical plate is 7229.377. The
results are presented in Table-1. The typical chromatogram of Rosiglitazone maleate is shown in
Fig.1

Linearity
Linearity was evaluated by plotting peak area as a function of analyte concentration for
Rosiglitazone maleate. From the linear studies the specified range determined was 160-260
µg/ml. The linear regression coefficient and the correlation coefficient were found to be 0.99975
and 0.099988. It obeys the linear equation Y= 29836.83 X - 96983.4 (n =6). The results are
shown in Table-2 and Fig-2.

Precision
Reproducibility of the method was studied by injecting standard Rosiglitazone maleate for five
times (n=5). The % RSD was found to be 0.0177, for all the solution tested. The percentage RSD
less than 2 indicates good Reproducibility of the method

Repeatability of the method was studied by obtaining data from the precision experiments for six
multiple injections at six different of samples of Rosiglitazone maleate 336.4, 337.2, 337.4,
336.1, 336.6, 338.5 during precision. The method showed %RSD of 0.5769 which is less than 2
% for all the solution tested. This indicates good Repeatability of the method.

Accuracy
The accuracy of a method can be measured in several ways. One way is based on recovery as
determined by spiking analytes into a blank matrix, and we should get 100% recovery. The
method showed % RSD of 0.7513 for all the solution tested which is less than 2. This indicates
good accuracy of the method.
LOD and LOQ
The LOD of Rosiglitazone maleate was found to be 2 µg/ml and the LOQ was 20 µg/ml. Overall summary of validation parameters are presented in Table-4

**Table-1. System suitability parameters of Rosiglitazone maleate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time</td>
<td>16.001</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>7229.377</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.768</td>
</tr>
</tbody>
</table>

**Table-2. Linearity for Rosiglitazone maleate**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Volume of stock solution(ml)</th>
<th>Volume made up to (ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160</td>
<td>4</td>
<td>50</td>
<td>4673534</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>9</td>
<td>100</td>
<td>5279654</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>5</td>
<td>50</td>
<td>5874826</td>
</tr>
<tr>
<td>4</td>
<td>220</td>
<td>11</td>
<td>100</td>
<td>6472821</td>
</tr>
<tr>
<td>5</td>
<td>240</td>
<td>6</td>
<td>50</td>
<td>7031955</td>
</tr>
<tr>
<td>6</td>
<td>260</td>
<td>13</td>
<td>100</td>
<td>7679710</td>
</tr>
</tbody>
</table>

*Linear regression coefficient 0.99757
Correlation coefficient 0.999988
Standard deviation 19254.14*

**Table-3. Accuracy for Rosiglitazone maleate**

<table>
<thead>
<tr>
<th>S.No</th>
<th>% Recovery/ Concentration</th>
<th>Placebo wt in mg</th>
<th>Standard wt in mg</th>
<th>Standard area</th>
<th>Syn.mix. area</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>---</td>
<td>26.5</td>
<td>6091250</td>
<td>--</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>308.2</td>
<td>22.3</td>
<td>---</td>
<td>5132056</td>
<td>100.11</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>307.6</td>
<td>22.1</td>
<td>---</td>
<td>5132934</td>
<td>101.04</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>308.4</td>
<td>22.4</td>
<td>---</td>
<td>5134018</td>
<td>99.71</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>307.5</td>
<td>27.8</td>
<td>---</td>
<td>6471253</td>
<td>101.26</td>
</tr>
</tbody>
</table>
Table 4. Summary of Validation parameters

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limit of detection (LOD) (µg/ml)</td>
<td>2 µg/ml</td>
</tr>
<tr>
<td>2</td>
<td>Limit of quantitation (LOQ) (µg/ml)</td>
<td>20 µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>Reproducibility (% RSD)</td>
<td>0.0177</td>
</tr>
<tr>
<td>4</td>
<td>Repeatability (% RSD)</td>
<td>0.5729</td>
</tr>
<tr>
<td>5</td>
<td>Robustness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1) Change in wavelength (Mean % Assay)</td>
<td>99.68</td>
</tr>
<tr>
<td></td>
<td>(2) Change in Temperature (Mean % Assay)</td>
<td>100.46</td>
</tr>
<tr>
<td>6</td>
<td>specificity</td>
<td>No interference of other peak, so the system is specific.</td>
</tr>
</tbody>
</table>
CONCLUSION

This developed RP-HPLC method for the estimation of Rosiglitazone maleate is accurate, precise, robust, specific, and stability-indicating. The method has been found to be better because of its less retention time, use of an economical and readily available mobile phase, and good resolution of peaks. The run time is relatively short, which will enable rapid quantification of many samples in routine and quality-control analysis of various formulations containing Rosiglitazone maleate. All these factors make this method suitable for quantification of Rosiglitazone maleate in bulk drugs and in pharmaceutical dosage forms without any interference. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating.

REFERENCES

[10]. D.J Graham, JAMA, 2010, 304