STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR METFORMIN AND EMPAGLIFLOZINE IN TABLET DOSAGE FORM

Shaik Mahammad Noorulla¹* and Sadath Ali²

¹Department of Pharmaceutical Analysis and Quality Assurance, Deccan School of Pharmacy, Hyderabad, India.
²Department of Pharmaceutical Chemistry, Glocal University, Saharanpur, India.

ABSTRACT

The purpose of the investigation was to develop a new HPLC method for simultaneous estimation of Metformin and Empagliflozine in pharmaceutical dosage forms. Chromatography was carried out on an ODS 250mm x 4.6 mm, 5μ particle size with a isocratic mobile phase composed of Buffer, Acetonitrile and methanol at a flow rate of 1mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 233 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention times for Metformin and Empagliflozine were 2.211 min and 4.592 min respectively. The percentage recoveries of Metformin and Empagliflozine were 100.39 % and 100.58 % respectively. The relative standard deviation for assay of tablets found to be less than 2%. The method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

KEYWORDS: Metformin, Empagliflozine, Simultaneous estimation, Stability, ICH guidelines.
1. INTRODUCTION
Metformin (MET) is chemically named as N,N-dimethylimidodicarbonimidic diamide (Fig.1). Metformin is a first line oral pharmacotherapy for type 2 diabetes. Activation of the energy-regulating enzyme AMPactivated protein kinase (AMPK), principally in muscle and the liver, is considered a major mode of metformin action.[1-3]

Empagliflozine (EMPA) is chemically named as (3R,4R,5S,6R)-2-[4-chloro-3-[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol (Fig.2).

Empagliflozine is used for type 2 diabetes. Empagliflozine is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), and causes sugar in the blood to be excreted by the kidneys and eliminated in urine. Various UV & HPLC assay methods are also reported in the literature for the estimation of Metformin and Empagliflozine individually and in-combination with other drugs.[4-6] According to literature survey there is no official method for the simultaneous estimation of Metformin and Empagliflozine by RP-HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop new method for simultaneous estimation and validation of Metformin and Empagliflozine in tablet formulation in accordance with the ICH guidelines.

2. MATERIALS AND METHODS
2.1 Materials:
Metformin, Empagliflozine, Synjardy® (500mg Metformin + 5mg of Empagliflozine) tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic
acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, tri ethyl amine, ortho-phosphoric acid all are from Rankem chemicals.

2.2 Instrument
HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Metformin, Empagliflozine solutions.

2.3 Standard Preparation: (500µg/ml Metformin & 5µg/ml Empagliflozine)
Accurately Weighed and transferred 125mg & 5mg of Metformin and Empagliflozine working Standards into a 25ml and 100ml clean dry volumetric flask respectively, add 20ml and 75ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

2.4 Sample Preparation
5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 70ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

Figure 3: A typical Chromatogram of Metformin and Empagliflozine.
2.5 Degradation studies

Oxidation
To 1 ml of stock solution of Metformin and Empagliflozine, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 500 µg/ml & 5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies
To 1 ml of stock solution of Metformin and Empagliflozine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 500 µg/ml & 5 µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.
Alkali Degradation Studies
To 1 ml of stock solution Metformin and Empagliflozine, 1 ml of 2 N sodium hydroxide was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 500µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies
The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 500µg/ml & 5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.
Photo Stability studies
The photochemical stability of the drug was also studied by exposing the 5000μg/ml & 50μg/ml solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 500µg/ml & 5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies
Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60°. For HPLC study, the resultant solution was diluted to 500µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.
3. RESULTS AND DISCUSSION

3.1 Method development

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH: 4.8 both drugs eluted with better separation. Thereafter, buffer: Acetonitrile were taken in isocratic ratio: %buffer / %Acetonitrile : 70/30 and with flow rate of 1mL/min was employed. ODS 250mm x 4.6 mm, 5µ. particle size was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 205nm to 280nm. Both MET and EMPA showed maximum absorption at 233nm of wavelength and 233 nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.211 min and 4.592 min for MET and EMPA, respectively. The chromatogram obtained was shown in the Fig. 2.

<table>
<thead>
<tr>
<th>SYSTEM SUITABILITY PARAMETERS</th>
<th>MET</th>
<th>EMPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of theoretical plates</td>
<td>3246</td>
<td>5814</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.46</td>
<td>1.08</td>
</tr>
<tr>
<td>Resolution</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>2.211 min</td>
<td>4.592 min</td>
</tr>
<tr>
<td>Mean Area</td>
<td>347926</td>
<td>155807</td>
</tr>
<tr>
<td>RSD</td>
<td>0.86</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 2: Results of accuracy of MET and EMPA

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Metformin</th>
<th></th>
<th></th>
<th>Empagliflozin</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount added (µg/ml)</td>
<td>Amount recovered (µg/ml)</td>
<td>% Recovery</td>
<td>Amount added (µg/ml)</td>
<td>Amount recovered (µg/ml)</td>
<td>% Recovery</td>
</tr>
<tr>
<td>50%</td>
<td>250</td>
<td>252.8973</td>
<td>101.16</td>
<td>2.5</td>
<td>2.533139</td>
<td>101.33</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>251.3102</td>
<td>100.52</td>
<td>2.5</td>
<td>2.501912</td>
<td>100.08</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>252.0607</td>
<td>100.82</td>
<td>2.5</td>
<td>2.490416</td>
<td>99.62</td>
</tr>
<tr>
<td>100%</td>
<td>500</td>
<td>497.9804</td>
<td>99.60</td>
<td>5</td>
<td>5.029409</td>
<td>100.59</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>505.6918</td>
<td>101.14</td>
<td>5</td>
<td>5.00816</td>
<td>100.16</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>499.9807</td>
<td>100.00</td>
<td>5</td>
<td>5.111012</td>
<td>102.22</td>
</tr>
<tr>
<td>150%</td>
<td>750</td>
<td>745.3798</td>
<td>99.38</td>
<td>7.5</td>
<td>7.578994</td>
<td>101.05</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>760.3527</td>
<td>101.38</td>
<td>7.5</td>
<td>7.541373</td>
<td>100.55</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>746.5356</td>
<td>99.54</td>
<td>7.5</td>
<td>7.472364</td>
<td>99.63</td>
</tr>
</tbody>
</table>
3.2 Method Validation

3.2.1 System suitability and Specificity

System suitability parameters such as number of theoretical plates, peak tailing, and retention time and resolution factor were determined. The total run time required for the method is only 8 minutes for eluting both MET and EMPA. The results obtained were shown in Table 1.

3.2.2 Linearity

MET showed a linearity of response between 125-750 μg/mL and EMPA showed a linearity of response between 1.25-7.5 μg/mL. These were represented by a linear regression equation as follows: 
y (MET) = 6525.4x + 536.95.3  (r²=0.9992),  
y (EMPA) = 30967x + 196.32(r²=0.9992) and regression line was established by least squares method and correlation coefficient (r²) for MET and EMPA is found to be greater than 0.98. Hence the curves established were linear.

3.2.3 Accuracy

To pre analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % Mean recovery for MET and EMPA are 100.39 and 100.58 respectively and these results are within acceptable limit of 98-102. The % RSD for MET and EMPA are 0.78 and 0.84 respectively and %RSD for MET and EMPA are within limit of ≤2. Hence the proposed method is accurate and the results were summarized in Table 2.

Table 3: Results of Precision for MET and EMPA

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metformin</td>
<td>Empagliflozine</td>
</tr>
<tr>
<td>1</td>
<td>3491527</td>
<td>158326</td>
</tr>
<tr>
<td>2</td>
<td>3482621</td>
<td>159292</td>
</tr>
<tr>
<td>3</td>
<td>3512489</td>
<td>159824</td>
</tr>
<tr>
<td>4</td>
<td>3524749</td>
<td>157206</td>
</tr>
<tr>
<td>5</td>
<td>3559453</td>
<td>159178</td>
</tr>
<tr>
<td>6</td>
<td>3509399</td>
<td>158432</td>
</tr>
<tr>
<td>Mean</td>
<td>3513373</td>
<td>158710</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>27175</td>
<td>925.8</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.77</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Table 4: Results of Robustness for MET

<table>
<thead>
<tr>
<th>Analytical conditions Evaluation parameters</th>
<th>Flow rate (1ml/min)</th>
<th>Column temperature (oc)</th>
<th>Mobile phase composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.1</td>
<td>0.9</td>
<td>35</td>
</tr>
<tr>
<td>Mean RT</td>
<td>2.20</td>
<td>2.58</td>
<td>2.20</td>
</tr>
<tr>
<td>Mean area</td>
<td>3451382</td>
<td>3553238</td>
<td>3557054</td>
</tr>
<tr>
<td>SD</td>
<td>29250</td>
<td>24334</td>
<td>25812</td>
</tr>
<tr>
<td>RSD</td>
<td>0.85</td>
<td>0.68</td>
<td>0.7</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.59</td>
<td>1.62</td>
<td>1.59</td>
</tr>
<tr>
<td>No. of theoretical plates</td>
<td>3339</td>
<td>4386</td>
<td>3344</td>
</tr>
</tbody>
</table>

Table 5: Results of Robustness for EMPA

<table>
<thead>
<tr>
<th>Analytical conditions Evaluation parameters</th>
<th>Flow rate (1ml/min)</th>
<th>Column temperature (oc)</th>
<th>Mobile phase composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.1</td>
<td>0.9</td>
<td>35</td>
</tr>
<tr>
<td>Mean RT</td>
<td>5.13</td>
<td>5.83</td>
<td>5.13</td>
</tr>
<tr>
<td>Mean area</td>
<td>158243</td>
<td>157481</td>
<td>156843</td>
</tr>
<tr>
<td>SD</td>
<td>622</td>
<td>2325</td>
<td>1987</td>
</tr>
<tr>
<td>RSD</td>
<td>0.4</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.07</td>
<td>1.14</td>
<td>1.07</td>
</tr>
<tr>
<td>No. of theoretical plates</td>
<td>7042</td>
<td>8388</td>
<td>7046</td>
</tr>
</tbody>
</table>

3.2.4 Precision

Repeatability

Six replicates injections in same concentration were analyzed in the same day for repeatability and the % RSD for MET and EMPA found to be 0.77 and 0.6 respectively and % RSD for MET and EMPA found to be within acceptable limit of ≤2 and hence method is reproducible and the results are shown in Table 3.

Intermediate Precision

Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for MET and EMPA is found to be 0.86 and 0.7 and it is within acceptable limit of ≤2. Hence the method is reproducible on different days with different analyst and column. This indicates that the method is precise and the results are as shown in Table 3.
3.2.5 Robustness
The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean Rt and RSD is within limit of ≤2. The tailing factor, resolution factor and no. of theoretical plates are found to be acceptable limits for both MET and EMPA. Hence the method is reliable with variations in the analytical conditions and the results of MET are shown in Table 4 and results of EMPA shown in Table 5.

Table 6: Results of HPLC Analysis of Tablet for MET and EMPA

<table>
<thead>
<tr>
<th>No. of sample assayed</th>
<th>Label amount (mg)</th>
<th>Amount found(mg)</th>
<th>% Assay (Mean±SD)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>MET 500</td>
<td>EMPA 5</td>
<td>MET 501.89</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>EMPA 501.89</td>
<td>MET 100.38±0.776</td>
<td>EMPA 100.09±0.583</td>
<td>0.77</td>
</tr>
</tbody>
</table>
| 3.2.6 Stability of sample solution
The sample and standard solutions injected at 0 hr (comparison sample) and after 24 hr (stability sample) by keeping at ambient room temperature 30°C. The RSD for 0 hr. and 24 hr. for sample and standard solutions of MET are 0.77 and 0.86 respectively. The RSD for 0 hr. and 24 hr. for sample and standard solutions of EMPA are 0.58 and 0.7 respectively. RSD results for both MET and EMPA are within limit of ≤2 and hence the sample and standard stock are stable for 24 hr in ambient room temperature and the results are shown in Table 6.

3.2.7 LOD and LOQ
LOD and LOQ for MET were 0.05 and 0.16 μg/mL respectively and for EMPA were 0.01 and 0.03 μg/mL, respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

3.3 Tablet Analysis
The Content of MET and EMPA in the tablets was found by the proposed method. RSD results for both MET and EMPA is within limit of ≤2 and the results were shown in Table 6.

4. CONCLUSION
A new precise accurate and simple HPLC method was developed and validated for simultaneous estimation of Metformin and Empagliflozine tablet dosage form. This method is
fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in QC laboratories and industries.

5. REFERENCES


