Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Metformin

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INTRODUCTION: The main objective of the present work was to develop a simple, precise, specific & stability indicating RP-HPLC method for simultaneous estimation of Teneligliptin and Metformin in bulk and tablet dosage form.

METHODS: The analysis has been performed using Kromasil C18 column (250 x 4.6 mm, 5µ) at 30°C using buffer: acetonitrile: methanol (65: 25: 10, v/v/v) as mobile phase. The detection was carried out at 254 nm with a flow rate of 1.0 ml/min.

RESULTS: The retention time of Teneligliptin and Metformin was found to be 2.842 min & 2.017 min respectively. The linearity range was 5-30 µg/ml for Teneligliptin and 125-750 µg/ml for Metformin respectively. The forced degradation studies were performed as per the guidelines of ICH under acidic, alkaline, oxidative, thermal, photo stability & neutral conditions.

DISCUSSION AND CONCLUSION: The developed method was successfully validated for all the parameters and was found to be within the limits. The developed method could be successfully employed for the simultaneous estimation of Teneligliptin and Metformin in bulk and tablet dosage form.

Keywords: Teneligliptin, Metformin, RP‐HPLC, Validation, Stability studies.

INTRODUCTION

Teneligliptin (TEN) (Fig. 1) is chemically [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl)methanone. It is highly effective in lowering blood glucose levels. This drug inhibits the enzyme dipeptidyl peptidase-4 (DPP4) which degrades incretin, a hormone adjusting blood glucose control. It is effectively used to treat type-II diabetes mellitus1,2.

Metformin (MET) (Fig. 2) is chemically 1-carbamimidamido-N,N-dimethylmethanimidamide. It belongs to biguanide class of anti diabetic drugs. It is the first line drug of choice for the treatment of type-II diabetes. It activates AMP-activated protein kinase (AMPK), a liver enzyme
that plays an important role in insulin signaling, whole body energy balance and metabolism of glucose and fats. Literature survey reveals good number of analytical methods for the estimation of TEN and MET individually or in combination with other drugs using UV spectrophotometry, HPLC, HPTLC and LC-MS/MS. Moreover, few methods were reported for the estimation of the selected drugs in their combinations using UV spectrophotometry, HPLC and LC-MS/MS. As per the knowledge of the authors, no stability indicating RP-HPLC method was reported so far for the simultaneous estimation of the Teneligliptin and Metformin. Hence, we tried to develop a simple stability indicating HPLC method for the estimation of selected drugs. The developed method has been validated as per the guidelines of ICH. To establish stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photo stability and neutral conditions.

EXPERIMENTAL

Materials & reagents

Reference standards of Teneligliptin and Metformin were provided as gift samples by Spectrum Labs, Hyderabad. Commercially available tablet formulation Tendia M tablets for the assay studies were purchased from local pharmacy. HPLC grade methanol, HPLC grade acetonitrile, analytical grade ortho phosphoric acid and HPLC grade water were purchased from Merck specialties, Mumbai, India.

Instrumentation

Development and validation of the method was performed on waters HPLC 2695 system equipped with quaternary pumps, auto sampler & photo diode array detector. Empower 2 software was applied for data collection and processing.
Methodology

Preparation of standard stock solutions

The standard stock solutions of 200 µg/ml for TEN and 5000 µg/ml for MET were prepared by accurately weighing and transferring 2 mg of TEN and 50 mg of MET into 10 ml volumetric flasks. Added about three fourth of the volume with diluent, sonicated for 10 minutes. Finally, flasks were made up to the mark with diluent to obtain the mentioned concentrations. One ml of the above solution was pipetted out and transferred into a 10 ml volumetric flask and diluted up to the mark with diluent to obtain a concentration of 20 µg/ml for TEN and 500 µg/ml for MET.

Preparation of sample solution

Twenty tablets were weighed and average weight was calculated. Then they were powdered using mortar and pestle and the powder equivalent to 20 mg of TEN and 500 mg of MET was accurately weighed and transferred in to a 100 ml volumetric flask. 50 ml of diluent was added and sonicated for 25 min. Further the volume was made up with diluent to obtain a concentration of 200 µg/ml for TEN and 5000 µg/ml for MET. Filters of 0.45 micron size were employed for filtration purpose in the mentioned procedure. One ml of the above solution was pipette out and transferred into a 10 ml volumetric flask and diluted up to the mark with diluents to obtain a concentration of 20 µg/ml for TEN and 500 µg/ml for MET.

Preparation of buffer

One ml of ortho phosphoric acid was diluted to 100 ml with HPLC grade water to obtain 0.1% ortho phosphoric acid buffer.

Mobile phase

Buffer, acetonitrile and methanol were taken in the ratio of 65:25:10, v/v/v and was used as mobile phase.
Method Validation

Method validation was done as per the guidelines of ICH\textsuperscript{30, 31}.

*System suitability*

System performance parameters like retention time, number of theoretical plates, tailing factor, resolution were calculated by injecting standard solutions for six times. The resultant results were compared with the standard limits as per guidelines.

*Specificity*

It is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample. These studies are to check the interferences in the optimized method. To assess the method specificity, blank and placebo were injected into HPLC system under optimized conditions. There should not be any interfering peak in the blank or placebo chromatograms at the retention times of the selected drugs.

*Linearity*

The linearity of the method was obtained by preparation of the calibration standards of six different concentrations in 6 replicates. The calibration curve plots for TEN & MET were obtained by plotting the peaks areas on y-axis and concentrations on x-axis over the concentration ranges of 5-30 µg/ml for TEN and 125-750 µg/ml for MET. The correlation coefficient should be greater than 0.99.

*Accuracy*

The accuracy of the method was assessed by recovery experiments by adding a known quantity of pure standard drug into the sample solution and recovering the same in terms of its peak areas. The sample was spiked with standard at levels of 50%, 100% and 150% of test concentrations.
The resultant spiked sample was assayed in triplicate. The %recovery for each level should be in between 98%-102%.

**Precision**

It is the degree of closeness of agreement between the series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. It is expressed in terms of standard deviation (SD) or relative standard deviation (RSD). Precision may be measure of either degree of repeatability or reproducibility of the analytical method.

**Method precision:** Sample solutions were injected under optimized conditions for six times on six different days and their peak areas were recorded. %RSD for the peak areas of 6 standard injection results should not be greater than 2.

**Intermediate precision:** Six replicates of sample solutions were injected under optimized conditions on the same day and their peak areas were recorded. %RSD for the peak areas of 6 replicate injection results should not be greater than 2.

**Ruggedness**

The ruggedness of the method was determined by carrying out the experiment on different instruments, by different operators and using different columns of similar types.

**Robustness**

The robustness of the method was determined by making small deliberate changes in the method like flow rate, mobile phase ratio & temperature. But, one should not find remarkable change in the results and the obtained results should be within range as per ICH guidelines.

**Effect of variation of flow:** Sample was analyzed at 0.9 ml/min & 1.1 ml/min flow rate instead of 1.0 ml/min, remaining conditions are kept constant.
**Effect of variation of temperature:** Temperature of 25°C and 35°C was maintained instead of 30°C. Samples were injected in triplicate & chromatograms were recorded.

**LOD & LOQ**

LOD is the smallest concentration that can be detected but not necessarily be quantified as an exact value. It is calculated using formula

\[
\text{LOD} = 3.3 \frac{\sigma}{S}; \text{ where, } \sigma = \text{S.D}; S = \text{Slope}
\]

LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with precision & accuracy.

\[
\text{LOQ} = 10 \frac{\sigma}{S}; \text{ where, } \sigma = \text{S.D}; S = \text{Slope}
\]

**Forced Degradation Studies**

TEN & MET standard samples were subjected to degradation under different stress conditions like acidic, alkali, oxidative, thermal, photo stability and neutral conditions. For acidic & alkali degradation samples were refluxed with 2N HCl & 2N NaOH at 60°C for 30 min. For oxidative degradation 20% v/v H₂O₂ was used and the same was refluxed at 60°C for 30 min. For thermal degradation, sample was placed in oven at 105°C for 6 hr; and for photo stability degradation, drug was exposed to UV light by keeping the sample in UV chamber for 7 days or 200 watt hours/m² in photo stability chamber; for neutral degradation, the drugs were refluxed in water for 6 hours at a temperature of 60°C. All the samples were diluted to obtain a final concentration of 20 µg/ml of TEN & 500 µg/ml of MET. Ten micro liters of the samples were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Solution Stability**
The stability of the drug solution was determined for the short term stability, auto sampler stability. Short term stability was carried out by keeping the samples, at room temp (25°C) for 24 hrs. Auto sampler stability was determined by storing the samples for 24 hrs in the auto sampler. Each sample was injected 6 times into HPLC & the results obtained were compared with nominal values of QC samples.

RESULTS

The results of optimized chromatographic conditions were shown in Table 1. System suitability parameters (tailing factor, retention time & theoretical plates) were found to be within acceptance criteria. Summary of system suitability parameters were tabulated in Table 2. We did not find any interfering peaks at the retention times of TEN & MET (Fig. 3), which declares that the method is specific. The quantification was linear in the concentration range of 5-30 µg/ml for TEN with a correlation coefficient 0.999 (Fig. 4); 125-750 µg/ml for MET with a correlation coefficient 0.999 (Fig. 5) respectively. The results of linearity were tabulated in Table 3. The recoveries of TEN and MET were found to be in the range of 99.35-99.94% and 99.80-100.61% respectively. The results were compared with the guidelines & expressed as percentages and the results were given in Table 4. The precision of the method is satisfactory as %RSD is NMT 2%. The ruggedness was performed by different analyst & on different days. The results were given in Table 5. No remarkable changes in the results were notice in robustness studies and hence the method is robust. The results were tabulated in Table 6. The assay results were compared with the labeled claim of TEN & MET marketed formulation and the results were tabulated in Table 7. The LOD & LOQ values were calculated using slope and standard deviation values and the same were tabulated in Table 8.

Forced degradation study
The standard solutions were subjected to different stress conditions as mentioned in the procedure. Under acidic conditions, drugs showed degradation of about 3.66% for TEN & 3.14% for MET and we have noticed about 3 degradation peaks (Fig. 6). Under alkali conditions, drugs showed degradation of about 2.75% for TEN & 2.67% for MET and 2 degradation peaks were noticed (Fig. 7). Under oxidative conditions, drugs showed degradation of about 1.01% for TEN & 1.62% for MET and 1 degradation peak was noticed (Fig. 8). Under remaining conditions, thermal, photo stability and neutral conditions, the degradation was less than 1% for both the drugs and no degradant peak was noticed (Fig. 9-11). Result of forced degradation studies are tabulated in Table 9.

**Stability studies**

The drug solutions were found to be stable for 24 hrs at 25°C for short term stability and 24 hrs for auto sampler stability.

**DISCUSSION**

For method optimization different ratios of acetonitrile & buffer were tried but, resolution of peak was not achieved. Hence, methanol was used in mobile phase. Different ratios of orthophosphoric acid buffer, methanol were tried i.e., 65:15:20, v/v/v, 60:20:20, v/v/v, 65:20:15, v/v/v. Finally, it was found that buffer:acetonitrile:methanol in the ratio of 65:25:10, v/v/v, gave good peaks and hence fixed as mobile phase. Kromasil C18 (250 X 4.6 mm, 5 µm) column, 1 ml/min flow rate, 10 µl injection volume, 30°C column oven temperature, 254 nm wave length were fixed as optimized conditions which were found to be suitable for the separation of peaks. These optimized conditions gave a retention time of 2.842 min and 2.017 min for TEN & MET. All the validation results were as per the limits of ICH and hence, found to be a reliable and economical method for the estimation of drugs. The effectiveness of the method to separate the
degraded peaks from analyte shows its stability indicating nature. The degradation on the lower side i.e., the degradation percent under all conditions is in the range of 0.05% to 3.66% shows the stability of the selected drugs. The %RSD values were found to be less than 2.

CONCLUSION

The developed method posses all the qualities to be a reliable, rapid, sensitive, specific and economical method from the above discussed results and data. The study showed stability indicating nature of the method with the possible short runtime. Hence, the developed method could be conveniently and effectively used for routine simultaneous estimation of Teniligliptin and Metformin in bulk and pharmaceutical dosage form.
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Table 1: Optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Kromasil C18 (250 X 4.6 mm, 5µ)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Buffer:acetonitrile:methanol (65:25:10, v/v/v)</td>
</tr>
<tr>
<td>Diluent</td>
<td>Acetonitrile:water (50:50, v/v)</td>
</tr>
<tr>
<td>Column temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Run time</td>
<td>6 min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Table 2: Summary of system suitability parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TEN</th>
<th>MET</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing factor</td>
<td>1.30</td>
<td>1.06</td>
<td>≤2</td>
</tr>
<tr>
<td>Retention time</td>
<td>2.842</td>
<td>2.017</td>
<td>≥2</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>4463</td>
<td>6783</td>
<td>≥2000</td>
</tr>
<tr>
<td>%RSD of area</td>
<td>0.72</td>
<td>1.08</td>
<td>≤2</td>
</tr>
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</table>

Table 3: Linearity values of TEN and MET

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TEN</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>5-30</td>
<td>125-750</td>
</tr>
<tr>
<td>Regression coefficient±SD</td>
<td>0.999±0.0003</td>
<td>0.999±0.0005</td>
</tr>
<tr>
<td>slope±SD</td>
<td>8891±4.358</td>
<td>4665±8.386</td>
</tr>
<tr>
<td>Intercept±SD</td>
<td>1773±58.66</td>
<td>35915±2654.363</td>
</tr>
</tbody>
</table>
Table 4: Recovery values of TEN and MET

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Analyte</th>
<th>Recovery amount (mg)</th>
<th>Mean % recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEN</td>
<td>50%</td>
<td>10</td>
<td>9.94</td>
<td>99.43</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>20</td>
<td>19.98</td>
<td>99.91</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>30</td>
<td>29.97</td>
<td>99.92</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>250</td>
<td>249.50</td>
<td>99.80</td>
<td>0.90</td>
</tr>
<tr>
<td>MET</td>
<td>100%</td>
<td>500</td>
<td>503.07</td>
<td>100.61</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>750</td>
<td>752.82</td>
<td>100.37</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Table 5: Ruggedness values of TEN and MET

<table>
<thead>
<tr>
<th>Drug</th>
<th>Analysts-1 (Peak area)*</th>
<th>Analyst-2 (Peak area)*</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEN</td>
<td>172792</td>
<td>173167</td>
<td>1248</td>
<td>0.72</td>
</tr>
<tr>
<td>MET</td>
<td>2363854</td>
<td>2320575</td>
<td>12026</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Average of six readings
Table 6: Robustness values of TEN and MET

<table>
<thead>
<tr>
<th>Condition</th>
<th>TEN</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention</td>
<td>Area</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>factor</td>
</tr>
<tr>
<td>Initial conditions</td>
<td>2.837</td>
<td>268209</td>
</tr>
<tr>
<td>More flow (+0.1 ml/min)</td>
<td>2.827</td>
<td>269207</td>
</tr>
<tr>
<td>Less flow (-0.1 ml/min)</td>
<td>2.851</td>
<td>267902</td>
</tr>
<tr>
<td>At 35°C</td>
<td>2.841</td>
<td>267189</td>
</tr>
<tr>
<td>At 25°C</td>
<td>2.840</td>
<td>269218</td>
</tr>
</tbody>
</table>

Table 7: Assay results of marketed formulation (Tendia M tablets)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim</th>
<th>Amount found</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEN</td>
<td>20 mg</td>
<td>19.98 mg</td>
<td>99.90</td>
</tr>
<tr>
<td>MET</td>
<td>500 mg</td>
<td>498.85 mg</td>
<td>99.77</td>
</tr>
</tbody>
</table>

Table 8: LOD & LOQ values of TEN & MET

<table>
<thead>
<tr>
<th>Drug</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEN</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>MET</td>
<td>1.88</td>
<td>5.69</td>
</tr>
<tr>
<td>Stress condition</td>
<td>Amount of TEN degraded (%)</td>
<td>Amount of TEN recovered (%)</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Acidic</td>
<td>3.67±0.89</td>
<td>96.33±3.12</td>
</tr>
<tr>
<td>Alkali</td>
<td>2.76±0.58</td>
<td>97.24±2.98</td>
</tr>
<tr>
<td>Oxidative</td>
<td>1.02±0.69</td>
<td>98.98±1.98</td>
</tr>
<tr>
<td>Thermal</td>
<td>0.75±0.08</td>
<td>99.25±1.87</td>
</tr>
<tr>
<td>Photo stability</td>
<td>0.63±0.06</td>
<td>99.37±1.39</td>
</tr>
<tr>
<td>Neutral</td>
<td>0.53±0.08</td>
<td>99.47±2.01</td>
</tr>
</tbody>
</table>

*Average of three determinations (each condition); SD: Standard deviation
Figure 1: Chemical structure of TEN

Figure 2: Chemical structure of MET

Figure 3: Chromatogram showing resolved peaks of TEN and MET
Figure 4: Linearity plot of TEN

\[ y = 8891 x + 1773, \quad R^2 = 0.999 \]

Figure 5: Linearity plot of MET

\[ y = 4565 x + 35915, \quad R^2 = 0.999 \]
Figure 6: Chromatogram showing degraded peaks under acidic conditions

Figure 7: Chromatogram showing degraded peaks under alkali conditions

Figure 8: Chromatogram showing degraded peaks under oxidative conditions
Figure 9: Chromatogram showing degraded peaks under thermal conditions

Figure 10: Chromatogram showing degraded peaks under photo stability conditions

Figure 11: Chromatogram showing degraded peaks under neutral conditions