Development and validation of HPTLC method for estimation of Teneligliptin Hydrobromide Hydrate in tablet dosage form

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ABSTRACT
A stability indicating HPTLC method was developed and validated for estimation of Teneligliptin hydrobromide hydrate in tablet dosage form. Chromatographic separation was performed on aluminium plates pre-coated with silica gel 60 F254 aluminium plate using the mobile phase butanol : water : glacial acetic acid (6:2:2 v/v/v) and densitometrically scanned at 245 nm. The Rf value for Teneligliptin hydrobromide hydrate was found to be 0.65. The solvent system was able to separate Teneligliptin hydrobromide hydrate and its degradation products formed under acidic condition. Developed HPTLC method was validated as per ICH guideline Q2(R1). The linear regression analysis data for the calibration plots showed a good linear relationship with $r^2 = 0.998$ in the concentration range of 250-1250ng/band for Teneligliptin hydrobromide hydrate. Percent recovery of drug was found in the range of 98.58 – 99.24% by developed method. Limit of detection and limit of quantitation were found to be 60.50 ng/band and 183.36 ng/band for Teneligliptin hydrobromide hydrate, respectively. Developed method was applied for estimation of Teneligliptin hydrobromide hydrate in tablet dosage form and assay result was found to be 98.34±0.31%.

Keywords: High Performance Thin Layer Chromatography, Teneligliptin hydrobromide hydrate, Stability indicating method

1. INTRODUCTION
Teneligliptin hydrobromide hydrate (THH) is a dipeptidyl peptidase 4 (DPP4) inhibitor and it is highly effective in lowering blood glucose levels. Chemical structure of drug is presented in Figure 1. It inhibits the enzyme dipeptidyl peptidase-4 which degrades incretin, a hormone adjusting blood glucose level. THH is a highly potent, competitive, and long-lasting DPP-4 inhibitor that improves postprandial hyperglycemia and dyslipidemia[1-7]. Few analytical methods have been reported in literature like UV-Visible spectroscopy[11], RP-HPLC[7,9] and LC-MS/MS[10] for the estimation of THH. Literature review revealed that, no stability indicating HPTLC method has been reported for the analysis of THH. Hence attempt was made to develop and validate a stability indicating HPTLC method for its estimation in pharmaceutical dosage forms.

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Figure 1. Molecular structure of Teneligliptin hydrobromide hydrate

2. MATERIALS AND METHODS

2.1. Instrumentation

The HPTLC system (Camag, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag, Muttenz, Switzerland), twin-trough developing chamber (10 x 10cm), UV cabinet with dual wavelength UV lamps, winCATS software, syringe (100 μl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.

2.2. Chemicals and reagents


2.3. Preparation of solutions

2.3.1. Preparation of standard stock solution and working standard solution of THH

Accurately weighed 10mg of standard THH was transferred into 10 ml volumetric flask, dissolved in distilled water and diluted up to mark with distilled water to get standard stock solution having strength of 1000 g/ml of THH.

From the standard stock solution, 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with distilled water to get a concentration of 100 g/ml.

The resulting solution (5 ml) was transferred into 10 ml volumetric flask and diluted with distilled water to obtain a working standard solution having strength of 50 μg/ml.

2.3.2. Preparation of force degradation solutions

Degradation of THH was studied in acidic stress condition. Accurately weighed 100 mg of THH was transferred into 10 ml volumetric flask, dissolved in distilled water and makeup volume up to mark with distilled water, from the above solution transferred 5 ml to 10 ml volumetric flask and diluted up to mark with 2 N HCl. The solution was heated under closed condition for 2 hours at 80°C. The solution was cooled to room temperature. Aliquot of 1 ml was transferred to 10 ml volumetric flask and neutralized using 2 N NaOH and diluted up to mark with distilled water. From the above solution 1 ml was further diluted up to mark with distilled water in 10 ml volumetric flask with.

2.4. Optimized Chromatographic conditions (Table 1)

Chromatographic separation was performed on 10x10 cm aluminium plates pre coated with 250 m layer of silica gel 60 F254 (E. Merk, Darmstadt, Germany). The TLC plate was pre-washed with methanol and activated at 60 °C for 5 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semiautomatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 50nl/s. The TLC plate was developed in twin trough chamber using butanol: water: glacial acetic acid (6:2:2 v/v/v) as mobile phase at temperature, 25 ± 2 °C; chamber saturation time, 30 min; migration distance, 70 mm. The TLC plate was dried, scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 x 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 245 nm.
Table 1. Mobile phase optimization

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Rf value Standard</th>
<th>Degradation in Acidic</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (10 ml)</td>
<td>0.38</td>
<td>-</td>
<td>Standard drug spot observed</td>
</tr>
<tr>
<td>Methanol:Water (9:9:0.1)</td>
<td>0.45</td>
<td>-</td>
<td>Standard drug spot observed</td>
</tr>
<tr>
<td>n-Butanol:Water:Glacial acetic acid (8:1:1)</td>
<td>0.53</td>
<td>0.56</td>
<td>Poor separation of standard drug</td>
</tr>
<tr>
<td>n-Butanol:Water:Glacial acetic acid (7:2:1)</td>
<td>0.6</td>
<td>0.58</td>
<td>Degradation product are well separation</td>
</tr>
<tr>
<td>n-Butanol:Water:Glacial acetic acid (6:2:2)</td>
<td>0.65</td>
<td>0.6</td>
<td>Sharp compact spot of standard and Degredation</td>
</tr>
</tbody>
</table>

2.5. Preparation of calibration curve

Aliquots of 5, 10, 15, 20 and 25 μl of working standard solution (50 μg/ml) were spotted on precoated TLC plate under nitrogen stream using Linomat V semi-automatic sample applicator. The plate was developed in butanol:water:glacial acetic acid (6:2:2 v/v/v) as mobile phase in a twin trough chamber previously saturated with mobile phase for 30 minutes. The plate was dried and scanned at 245 nm in absorbance mode. The calibration curve was constructed by plotting area versus respective concentration (ng/band).

2.6. Validation of HPTLC method

The developed method was validated as per the International Conference on Harmonization (ICH) guidelines with respect to specificity, linearity, precision, limit of detection, limit of quantification and accuracy[7-8].

2.6.1. Specificity

The spots of THH from dosage form were confirmed by comparing its Rf and absorbance/reflectance spectrum with that of standard. The peak purity of THH was determined by comparing the UV spectra of sample scanned at peak start (S), peak apex (M) and peak end (E) positions of the spot.

2.6.2. Linearity and Range

The linearity was expressed in terms of correlation co-efficient of linear regression analysis. The linearity range was determined by analysing five independent levels of calibration curve in the range of 250-1250 ng/band for THH. A volume of 5, 10, 15, 20 and 25 μl of working standard solution (50 μg/ml) were applied on TLC plate and analysed as per the optimized chromatographic conditions described in section 2.4. The calibration curve was prepared by plotting peak area vs. concentration and correlation co-efficient and regression line equation for THH were computed.

2.6.3. Precision

2.6.3.1. Repeatability of sample application

Repeatability of sample application was determined by application of 15 μl of working standard solution (50 μg/ml) seven times on a same TLC plate. The plate was developed, dried and analyzed by the optimized chromatographic conditions described in section 2.4. The areas of seven spots were measured and percent RSD was calculated.

2.6.3.2. Repeatability of area measurement

Working standard solution (15 μl) was spotted on a TLC plate. Plate was developed and analysed by the optimized chromatographic conditions described in section 2.4. The obtained spot of THH was scanned seven times without changing plate position and percent RSD for measurement of peak area was computed.

2.6.3.3. Intermediate precision

Intraday precision of the proposed method was evaluated by analyzing the entire calibration range of THH (250-1250 ng/band), three times on same day. The percent RSD of peak area at each level was calculated.

Interday precision of the proposed method was evaluated by analyzing the entire calibration range of THH (250-1250ng/band) on three different
days. The percent RSD of peak area at each level was calculated.

2.6.3.4. Limit of Detection

The limits of detection of the developed method was calculated from the standard deviation of the intercepts and mean slope of the calibration curves of THH using the equation,

$$\text{LOD} = 3.3 \times \sigma / S$$

Where, $\sigma$ = the standard deviation of the Y-intercepts of the five calibration curves.

$S$ = mean slope of the five calibration curves.

2.6.3.5. Limit of Quantitation

The limits of quantitation of the developed method was calculated from the standard deviation of the intercepts and mean slope of the calibration curves of THH using the equation,

$$\text{LOQ} = 10 \times \sigma / S$$

Where, $\sigma$ = the standard deviation of the Y-intercepts of the five calibration curves.

$S$ = mean slope of the five calibration curves.

2.6.3.6. Accuracy

The accuracy of the method was determined by calculating recovery of THH using the standard addition method. Known amounts of standard THH was added at three levels to preanalyzed sample of THH. The quantity of marketed formulation equivalent to 10 mg of THH was transferred to four individual 10 ml volumetric flasks. Standard THH 8mg, 10 mg, and 12 mg was spiked in second, third and fourth volumetric flasks, respectively. All four flasks were filled to about 80% with distilled water, sonicated for 30 minutes and diluted up to the mark with distilled water. These solutions were filtered through Whatman filter paper- No.41 individually. From each filtrate, 1 ml of was diluted up to 10 ml with distilled water individually. Aliquot of 2.5 ml of each resulting solution was diluted up to 10 ml with distilled water. Each resulting solution (15 µl) was individually spotted on TLC plate. Plate was developed and analyzed as described under optimized chromatography conditions described in section 2.4. From calibration curve, the amount of THH recovered was calculated and % recovery was determined.

2.7. Assay of marketed formulations

Marketed tablet formulation of THH was analyzed using developed method. Twenty tablets of THH having brand name Ziten with 20mg dosage strength, manufactured by Glenmark Pharmaceutical were weighed and finely powdered. Powder equivalent to 10 mg of THH was accurately weighed and transferred into a 10 ml volumetric flask and 5 ml distilled water was added. The flask was sonicated for 30 min and volume was made up to 10 ml with distilled water. The solution was filtered through Whatman filter paper- No.41 and 1 ml solution was further diluted up to 10 ml with distilled water. To 2 ml aliquot of solution was further diluted up to 10 ml with distilled water. A volume 25 µl of resulting solution was applied in triplicate on TLC plate followed by development and scanning as described under chromatographic conditions. The amount of THH present in sample solution was determined by fitting area values of corresponding peak into the equation representing calibration curve of THH.

2.8. Procedure for analysis of forced degradation sample

For the analysis of forced degradation samples, 25 µl of forced degraded solution was applied on TLC plate. The plate was developed in butanol : water : glacial acetic acid (6:2:2 v/v/v) as mobile phase in a Camag twin trough chamber previously saturated with mobile phase for 30 minutes. The plate was removed from the chamber, dried and analysed.

3. RESULTS AND DISCUSSION

3.1. Selection of wavelength for detection

Solution of THH (10 μg/ml) was scanned in the range of 200-400 nm against water as blank in UV visible spectrophotometer. Wavelength selected from spectrum of the drug was 245 nm (Figure 2).
Table 2. Precision results

<table>
<thead>
<tr>
<th>Concentration (ng/band)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (Mean ± SD)(n=3)</td>
<td>% RSD</td>
</tr>
<tr>
<td>250</td>
<td>1163.7 ± 15.07</td>
<td>1.29</td>
</tr>
<tr>
<td>500</td>
<td>2443.6 ± 41.98</td>
<td>1.72</td>
</tr>
<tr>
<td>750</td>
<td>3359.9 ± 61.73</td>
<td>1.54</td>
</tr>
<tr>
<td>1000</td>
<td>4611.7 ± 33.97</td>
<td>0.74</td>
</tr>
<tr>
<td>1250</td>
<td>5953.6 ± 39.058</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table 3. Results for recovery study for THH

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Amount of THH from sample (mg)</th>
<th>Amount of THH added (mg)</th>
<th>Total amount of THH (mg)</th>
<th>Avg. Total Area</th>
<th>Amount of spiked THH recovered (mg) ±SD(n=3)</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenelia_20</td>
<td>0%</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>1725.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>3137.67</td>
<td>7.92 ± 0.05</td>
<td>99.04 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>3470.9</td>
<td>9.85 ± 0.05</td>
<td>98.58 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>3844.47</td>
<td>11.91 ± 0.14</td>
<td>99.24 ± 1.15</td>
</tr>
</tbody>
</table>

3.2. Mobile phase optimization

The mobile phase was optimized to get desired \( R_f \) value range [0.2 - 0.8]. It was achieved by trying different mobile phases containing solvents of different polarities in different ratios like toluene, triethylamine, n-butanol, methanol, formic acid, water, glacial acetic acid. Good separation was found in n-butanol: glacial acetic acid: water (6: 2: 2, v/v/v).

Table 4. Summary of validation parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity Range</td>
<td>250-1250 (ng/band)</td>
</tr>
<tr>
<td>2</td>
<td>Correlation coefficient (RSD)</td>
<td>0.998</td>
</tr>
<tr>
<td>3</td>
<td>Precision (%RSD)</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>Repeatability of Sample Application (n=7)</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Repeatability of Area Measurement (n=7)</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Intraday Precision (n=3)</td>
<td>0.65-1.72</td>
</tr>
<tr>
<td></td>
<td>Interday Precision (n=3)</td>
<td>0.78-1.84</td>
</tr>
<tr>
<td>4</td>
<td>Accuracy (n=3) (% recovery)</td>
<td>98.58-99.24</td>
</tr>
<tr>
<td>5</td>
<td>LOD (ng/band)</td>
<td>60.50</td>
</tr>
<tr>
<td>6</td>
<td>LOQ (ng/band)</td>
<td>183.36</td>
</tr>
</tbody>
</table>

3.3. Calibration curve

The linear regression data for calibration curve (n=5) shows linear relationship over concentration range of 250-1250 ng/spot with respect to peak area (Figure 3 & 4).

3.4. Validation of HPTLC Method

3.4.1. Specificity

The method was found to be specific as excipients did not interfere with analysis. The degradation products were also separated by the method. The \( R_f \) value and UV spectra of THH from sample (Figure 5B & 6B) was comparable with that of standard (Figure 5A & 6A respectively), which confirmed the identity of the sample spot. Peak purity of THH in sample was assessed by comparing spectra acquired at start, middle and end of the band obtained from scanning the spot. The values of \( r (s,m) \) found to be 0.9995 and \( r (m,e) \) found to be 0.9943 (Figure 6A and 6B).

Table 5. Assay of marketed formulations containing THH

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Label claim of Tablet (mg)</th>
<th>Amount of THH found (mg)</th>
<th>% Label claim ± SD(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenelia_20</td>
<td>20</td>
<td>19.66</td>
<td>98.34 ± 0.31</td>
</tr>
</tbody>
</table>

3.4.2. Linearity and Range

The linearity range of THH was found to be 250-1250 ng/band with Correlation co-efficient \( (r^2) \) of 0.998. The 3D chromatogram of standard THH in range 250-1250 ng/band is depicted in Figure 4. The regression line equation for THH is as following,

\[
y = 4.705x - 7.862
\]

Where, \( y = \) Peak area and \( x = \) Concentration in ng/band
750 ng/band of THH by seven replicate determinations.

The percent RSD of repeatability of sample application and area measurement was found to be 1.06 and 0.68 respectively.

All levels of standard solutions were analysed 3 times on the same day. The percent RSD for intra-day precision of THH was found to be in the range of 0.65–1.72%. The result for intra-day precision for THH is depicted in Table 1. Low values of %RSD indicate that method is precise.

All levels of standard solutions were analysed on 3 different days. Percentage RSD for inter-day precision of THH was found to be in the range of 0.78–1.84%. The data for inter-day for THH is depicted in Table 2. The results indicate that method is precise.

### 3.4.4. Limit of Detection and Limit of Quantitation

Based on the formula the LOD and LOQ were calculated for THH. The LOD and LOQ for THH was found to be 60.50 ng/band and 183.36 ng/band respectively.

### 3.4.5. Accuracy

Accuracy was determined in terms of recovery study at three levels i.e. 80%, 100% and 120%. Percent recovery obtained by the proposed method was found to be between 98.58-99.24% indicating that the method is accurate (Table 3).

### 3.5. Summary of Validation Parameters

The results of validation have been summarized in Table 4.

### 3.6. Analysis of marketed formulations

Applicability of the proposed method was assessed by analyzing marketed dosage form of THH (tablet). The amount of THH was calculated from the calibration curve. The assay value for THH varied from 98.34±0.31% of label claim. Assay result of THH is shown in Table 5.
Figure 5. Densitogram of THH in (A) Acidic condition (B) Tablet (C) API

Figure 6. Comparison of Absorbance/Reflectance spectra showing peak purity of (A) standard THH (B) THH in Formulation
3.7. Analysis of forced degraded sample of THH

THH was subjected to acid hydrolysis and analyzed to check stability indicating property of the method.

3.7.1. Acidic Hydrolysis

Degradation products formed when THH was heated with 2N HCl at 80°C for 2hrs. The Rf of THH was 0.66 and the degradation products were observed at Rf 0.26, 0.30, 0.44 (Figure 6).

4. CONCLUSION

The developed stability indicating HPTLC method was found to be simple, specific, precise and accurate. Forced degradation study involved formation of degradation products under acidic stress condition. The developed method successfully separated drug substance from degradation products formed under the stress condition. The method was successfully applied for assay of marketed dosage form of THH and the assay results were found to be in good agreement with the label claim.

5. ACKNOWLEDGMENTS

We are thankful to The Principal, Maliba Pharmacy College for providing all the facilities for this research work. Also we express our gratitude to Glenmark Pharmaceuticals Ltd, for providing the API.

6. REFERENCES

5. European Bioinformatics Database Https://www.ebi.ac.uk/chembldb/compound/inspect/CHEMBL2147777