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

Teneligliptin ve Metformin Eş Zamanlı Tahmininde RP-HPLC Yöntemini Gösteren Stabilitenin Gelişimi ve Doğrulanması

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ABSTRACT

Objectives: The main objective of the present work is to develop a simple, precise, specific and stability method indicating reverse phase high performance liquid chromatography method for simultaneous estimation of teneligliptin and metformin in bulk and tablet dosage form.

Materials and Methods: The analysis was performed with a Kromasil C18 column (250×4.6 mm, 5 µm) at 30°C using buffer: acetonitrile: methanol (65:25:10, v/v/v) as mobile phase. The detection was carried out with a flow rate of 1.0 mL/min at 254 nm.

Results: The retention time of teneligliptin and metformin was 2.842 min and 2.017 min, respectively. The linearity range was 5-30 µg/mL for teneligliptin and 125-750 µg/mL for metformin. The forced degradation studies were performed as per the guidelines of the The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions.

Conclusion: This method was successfully validated for all the parameters and could detect the correct amounts of active drug substance in formulations that are available in the market. This developed method in the present study could be successfully employed for the simultaneous estimation of teneligliptin and metformin in bulk and tablet dosage form.

Key words: Teneligliptin, metformin, RP-HPLC, validation, stability studies

ÖZ

Amaç: Bu çalışmanın temel amacı, teneligliptin ve metformini bulk ve tablet dozaj formunda eş zamanlı belirlemek için kolay, kesin ve kararlı bir ters faz yüksek performanslı sıvı kromatografisi yöntemi geliştirmektir.

Gereç ve Yöntemler: Analiz, hareketli faz olarak tampon: asetonitril: metanol (65:25:10, h/h/h) kullanılarak 30°C’dede Kromasil C18 kolonu (250×4,6 mm, 5 µm) kullanılarak gerçekleştirilmiştir. Saptama 1,0 mL/dak akış hızında 254 nm’de gerçekleştirilmiştir.

Bulgular: Teneligliptin ve metformin alıkonma süresi sırasıyla 2,842 dk ve 2,017 dk olarak bulunmuştur. Doğrusallık aralığı, teneligliptin için 5-30 µg/mL ve metformin için 125-750 µg/mL’dir. Zorunlu bozunma çalışmaları asit, alkali, oksidatif, termal, fotostabilite ve nötr koşullar altında Beşeri İlaçlar için Teknik Gereksinimlerin Uyumlaştırılması Uluslararası Konseyi’nin kilavuzlarına göre gerçekleştirilmiştir.

Sonuç: Bu yöntemdeki tüm parametreler başarıyla doğrulanmıştır ve yöntem piyasadaki formülasyonlardaki etkin maddelerin doğru miktarlarını belirleyebilir bulunmaktadır. Bu çalışmada geliştirilen yöntem, teneligliptin ve metforminin hammadde ve tablet dozaj formunda eş zamanlı tahminin için başarıyla kullanılabilir.

Anahtar kelimeler: Teneligliptin, metformin, RP-HPLC, validasyon, stabilite çalışmaları
INTRODUCTION
Teneligliptin (TEN) (Figure 1) is chemically \((2S, 4S)-4\{(5-
 methyl-2-phenylpyrazol-3-yl)piperazin-1-yl\}pyrrolidin-2-yl\)-(1,3-thiazo-
ilin-3-yl) methanone. It is highly effective in lowering blood glucose levels. This drug inhibits the enzyme dipeptidyl peptidase-4, which degrades incretin, a hormone adjusting blood glucose control. It is effectively used to treat type-2 diabetes mellitus.\(^1,2\)

Metformin (MET) (Figure 2) is chemically 1-carbamimidamido-N,N-dimethylmethanimidamide. It belongs to the biguanide class of antidiabetic drugs. It is the first line drug of choice for the treatment of type-2 diabetes. It activates adenosine monophosphate activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and metabolism of glucose and fats.\(^3,4\)

A literature survey reveals a good number of analytical methods for the estimation of TEN and MET individually or in combination with other drugs using ultraviolet (UV) spectrophotometry,\(^6-8\) high performance liquid chromatography (HPLC),\(^9,19\) HPTLC,\(^20\) and LC-MS/MS.\(^21\) Moreover, methods were reported for the estimation of the selected drugs in their combinations using UV spectrophotometry,\(^22,23\) and HPLC.\(^24-28\) To the best knowledge of the authors, no stability indicating RP-HPLC method has been reported so far for the simultaneous estimation of TEN and MET. Hence, we tried to develop a simple stability indicating HPLC method for the estimation of the selected drugs. The developed method has been validated as per the guidelines of the ICH.\(^29\) To establish the stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photostability, and neutral conditions.\(^30\)

MATERIALS AND METHODS

Materials and reagents
Reference standards of TEN and MET were provided as gift samples by Spectrum Labs, (Hyderabad, India). Commercially available tablet formulation Tendia M tablets for the assay studies were purchased from a local pharmacy. HPLC grade methanol, HPLC grade acetonitrile, analytical grade orthophosphoric acid, and HPLC grade water were purchased from Merck Specialities (Mumbai, India). Ethic committee approval was not required for our study.

Instrumentation
The development and validation of the method were performed on a Waters HPLC 2695 system equipped with quaternary pumps, an autosampler, and a photodiode array detector. Empower 2 software was applied for data collection and processing.

Methodology

Preparation of standard stock solutions
Standard stock solutions of 200 \(\mu\)g/mL for TEN and 5000 \(\mu\)g/mL for MET were prepared by accurately weighing and transferring 2 mg of TEN and 50 mg of MET into 10 mL volumetric flasks. About three fourths of the volume of diluent was added, followed by sonication for 10 min. Finally, the flasks were made up to the mark with diluent to obtain the mentioned concentrations. Next, 1 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 \(\mu\)g/mL for TEN and 500 \(\mu\)g/mL for MET.

Preparation of sample solution
Twenty tablets were weighed and average weight was calculated. Then they were powdered using a mortar and pestle and the powder equivalent to 20 mg of TEN and 500 mg of MET was accurately weighed and transferred into a 100 mL volumetric flask. Next 50 mL of diluent was added and the mixture sonicated for 25 min. Further the volume was made up with diluent to obtain a concentration of 200 \(\mu\)g/mL for TEN and 5000 \(\mu\)g/mL for MET. Filters of 0.45 micron size were employed for filtration in the mentioned procedure. Next, 1 mL of the above solution was pipetted out and transferred into a 10 mL volumetric flask and diluted up to the mark with diluents to obtain a concentration of 20 \(\mu\)g/mL for TEN and 500 \(\mu\)g/mL for MET.

Preparation of buffer
One milliliter of orthophosphoric acid was diluted to 100 mL with HPLC grade water to obtain 0.1% orthophosphoric acid buffer.
Mobile phase
Buffer, acetonitrile, and methanol were taken in the ratio of 65:25:10 (v/v/v) and used as mobile phase.

Method validation
Method validation was done as per the guidelines of the ICH.29,30

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

Specificity
Specificity 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 performed to check the interferences in the optimized method. To assess the method’s specificity, blank and placebo were injected into the 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 6 different concentrations in 6 replicates. The calibration curve plots for TEN and MET were obtained by plotting the peak areas on the y-axis and concentrations on the 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 to 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 98%-102%.

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

Method precision
Sample solutions were injected under optimized conditions 6 times on 6 different days and their peak areas were recorded. RSD % for the peak areas of the 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 the 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, and temperature. However, one should not find remarkable changes in the results and the obtained results should be within the ranges in the ICH guidelines.

Effect of variation in flow
A sample was analyzed at 0.9 mL/min and 11 mL/min flow rate instead of 1.0 mL/min; the remaining conditions were kept unchanged.

Effect of variation in temperature
Temperature of 25°C and 35°C was maintained instead of 30°C. Samples were injected in triplicate and chromatograms were recorded.

Limit of detection and limit of quantitation (LOD and LOQ)
LOD is the smallest concentration that can be detected but not necessarily be quantified as an exact value. It is calculated using the formula
LOD=3.3 σ/S, where σ=SD; s=slope
LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ=10 σ/S, where σ=SD; s=slope

Forced degradation studies
TEN and MET standard samples were subjected to degradation under different stress conditions like acidic, alkali, oxidative, thermal, photostability, and neutral conditions.

For acidic and alkali degradation samples were refluxed with 2 N HCl and 2 N 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, a sample was placed in an oven at 105°C for 6 h; for photostability degradation, the drug was exposed to UV light by keeping the sample in a UV chamber for 7 days or 200 W h/m2 in a photostability chamber; for neutral degradation, the drugs were refluxed in water for 6 h at 60°C. All the samples were diluted to obtain a final concentration of 20 µg/mL of TEN and 500 µg/mL of MET. Ten microliters 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 short-term stability and autosampler stability. Short-term stability was tested by keeping the samples at room temperature (25°C) for 24 h. Autosampler stability was determined by storing the samples for 24 h in the autosampler. Each sample was injected 6 times into the HPLC and the results obtained were compared with the nominal values of QC samples.
RESULTS

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

**Forced degradation study**

The standard solutions were subjected to different stress conditions as mentioned in the procedure. Under acidic conditions, the drugs showed degradation of about 3.66% for TEN and 3.14% for MET and we noted about 3 degradation peaks (Figure 6). Under alkali conditions, the drugs showed degradation of about 2.75% for TEN and 2.67% for MET and 2 degradation peaks were noted (Figure 7). Under oxidative conditions, the drugs showed degradation of about 1.01% for TEN and 1.62% for MET and 1 degradation peak was noted (Figure 8). Under the remaining conditions, i.e. thermal, photostability, and neutral conditions, the degradation was less than 1% for both drugs and no degradation peak was noted (Figures 9-11).

**Table 1. Optimized chromatographic conditions**

| Parameter       | Condition                                      |
|-----------------|-----------------------------------------------|
| Column          | Kromasil C18 (250×4.6 mm, 5 µm)               |
| Mobile phase    | Buffer: acetonitrile: methanol (65:25:10, v/v/v) |
| Diluent         | Acetonitrile: water (50:50, v/v)              |
| Column temperature | 30°C                                        |
| Wavelength      | 254 nm                                        |
| Flow rate       | 1 mL/min                                      |
| Run time        | 6 minute                                      |
| Injection volume | 10 µL                                        |

**Table 2. Summary of system suitability parameters**

| Parameter          | TEN    | MET    | Acceptance criteria |
|--------------------|--------|--------|---------------------|
| Tailing factor     | 1.30   | 1.06   | ≤2                  |
| Retention time     | 2.842  | 2.017  | ≥2                  |
| Theoretical plates | 4463   | 6783   | ≥2000               |
| RSD % of area      | 0.72   | 1.08   | ≤2                  |

**Table 3. Linearity values of TEN and MET**

| Parameter                  | TEN            | MET            |
|----------------------------|----------------|----------------|
| Linearity range (µg/mL)    | 5-30           | 125-750        |
| Regression coefficient ± SD| 0.999±0.0003   | 0.999±0.0005   |
| Slope ± SD                 | 8891±4.358     | 4665±8.386     |
| Intercept ± SD             | 1773±58.66     | 35.915±2654.363|

TEN: Teneligliptin, MET: Metformin, RSD: Standard deviation relative
The results of the forced degradation studies are tabulated in Table 9.

### Table 4. Recovery values of TEN and MET

| Drug | Level | Analyte amount (mg) | Recovery amount (mg) | Mean % recovery | RSD % |
|------|-------|---------------------|----------------------|----------------|-------|
| TEN  | 50%   | 10                  | 9.94                 | 99.43          | 0.20  |
|      | 100%  | 20                  | 19.98                | 99.91          | 0.60  |
|      | 150%  | 30                  | 29.97                | 99.92          | 0.67  |
| MET  | 50%   | 250                 | 249.50               | 99.80          | 0.90  |
|      | 100%  | 500                 | 503.07               | 100.61         | 0.40  |
|      | 150%  | 750                 | 752.82               | 100.37         | 0.50  |

TEN: Teneligliptin, MET: Metformin, RSD: Standard deviation relative

### Table 5. Ruggedness values of TEN and MET

| Drug | Analyst-1 (Peak area)* | Analyst-2 (Peak area)* | SD | RSD % |
|------|------------------------|------------------------|----|-------|
| TEN  | 172.792                | 173.167                | 1248| 0.72  |
| MET  | 2,363.854              | 2,320.575              | 12.026| 0.50  |

TEN: Teneligliptin, MET: Metformin, RSD: Standard deviation relative, SD: Standard deviation

*Average of six readings

### Table 6. Robustness values of TEN and MET

| Condition                  | TEN | MET |
|----------------------------|-----|-----|
| Retention time             |     |     |
| Area                       | 2.837| 2.827|
| Tailing factor             | 268.209| 269.076|
| Retention time             |     |     |
| Area                       | 2.027| 2.012|
| Tailing factor             | 3,521,349| 3,540,846|

At 35°C

| TEN | MET |
|-----|-----|
| 267.89 | 2,828|
| 1.13 | 2.028|
| 3,520,932| 3,519,867|

| TEN | MET |
|-----|-----|
| 269,218 | 269,218|
| 1.09 | 2.029|
| 3,519,867| 3,519,867|

TEN: Teneligliptin, MET: Metformin

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

| Drug  | Label claim | Amount found | % Assay |
|-------|-------------|--------------|---------|
| TEN   | 20 mg       | 19.98 mg     | 99.90   |
| MET   | 500 mg      | 498.85 mg    | 99.77   |

TEN: Teneligliptin, MET: Metformin

### Table 8. LOD and LOQ values of TEN and MET

| Drug  | LOD (µg/mL) | LOQ (µg/mL) |
|-------|-------------|-------------|
| TEN   | 0.02        | 0.07        |
| MET   | 1.88        | 5.69        |

TEN: Teneligliptin, MET: Metformin, LOD: Limit of detection, LOQ: Limit of quantitation

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**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 photostability conditions

**Figure 11.** Chromatogram showing degraded peaks under neutral conditions
Table 9. Forced degradation data* (± SD) of the method

| Stress condition | Amount of TEN degraded (%) | Amount of TEN recovered (%) | Amount of MET degraded (%) | Amount of MET recovered (%) |
|------------------|-----------------------------|-----------------------------|---------------------------|---------------------------|
| Acidic           | 3.67±0.89                   | 96.33±3.12                  | 3.15±0.62                 | 96.85±2.19                |
| Alkali           | 2.76±0.58                   | 97.24±2.98                  | 2.68±0.85                 | 97.32±2.81                |
| Oxidative        | 1.02±0.69                   | 98.96±1.98                  | 1.63±0.98                 | 98.37±2.06                |
| Thermal          | 0.75±0.08                   | 99.25±1.87                  | 0.62±0.10                 | 99.38±0.91                |
| Photostability   | 0.63±0.06                   | 99.37±1.39                  | 0.59±0.09                 | 99.41±1.92                |
| Neutral          | 0.53±0.08                   | 99.47±2.01                  | 0.05±0.08                 | 99.95±1.79                |

TEN: Teneligliptin, MET: Metformin, SD: Standard deviation,
*Average of three determinations (each condition)

Stability studies
The drug solutions were found to be stable for 24 h at 25°C for short-term stability and 24 h for autosampler stability.

DISCUSSION
For method optimization different ratios of acetonitrile and buffer were tried but peak resolution was not achieved. Hence, methanol was used in the mobile phase. Different ratios of orthophosphoric acid buffer, acetonitrile, and 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 were fixed as the mobile phase. A Kromasil C18 (250×4.6 mm, 5 μm) column, 1 mL/min flow rate, 10 μL injection volume, 30°C column oven temperature, and 254 nm wavelength 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 and MET. All the validation results were as per the limits of the ICH and hence showed the method to be reliable and economical 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%, showing the stability of the selected drugs. The RSD % values were less than 2.

CONCLUSION
The method developed possesses all the qualities to be a reliable, rapid, sensitive, specific, and economical method according to the above discussed results and data. The study showed the 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 TEN and MET in bulk and pharmaceutical dosage form.

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