Analytical Method Development and Validation of Teneligliptin by using RP-HPLC with ICH Guidelines

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ABSTRACT
Teneligliptin is drug used against type 2 diabetes mellitus and it is also a member of class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptins". A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Teneligliptin in bulk formulation. The $\lambda_{max}$ of the Teneligliptin was found to be 246 nm in Methanol: Phosphate buffer pH:3 [70:30 (v/v)]. The method shows high sensitivity with linearity 10 to 50 µg/ml (regression equation: $y = 54647x - 74133$; $r^2 = 0.9968$). The various parameters according to ICH guidelines are followed for validating and testing of this method. The Detection limit and quantitation limit were found to be 0.109 µg ml$^{-1}$ and 0.3305 µg ml$^{-1}$ in Methanol: Phosphate buffer pH:3 [70:30 (v/v)] respectively. The % purity of tablet formulation was found to be 99.57%. The results demonstrated that the procedure is accurate, specific and reproducible (RSD < 2%), and also being simple, cheap and less time consuming and appropriate for the determination of Teneligliptin in bulk and pharmaceutical formulation.

Keywords: Teneligliptin, dipeptidyl peptidase-4 inhibitors

INTRODUCTION
A new class of anti-diabetic drugs, Dipeptidyl peptidase-4 (DPP-4) inhibitors have recently introduced, that show enthusiastic results in the treatment of glycemic control with a minimal risk of hypoglycemia and weight gain. Teneligliptin, a novel Dipeptidyl peptidase-4 inhibitor, has a unique structure characterized by five consecutive rings, which produce a potent and long-lasting effect. Teneligliptin is now used as treatment in cases of insufficient improvement in glycemic control even after diet control and exercise and also a combination of diet control, exercise, and sulfonylurea- or thiazolidine-class drugs. Teneligliptin is administered orally at a dosage of 20 mg once daily in adults, which can be increased up to 40 mg per day. Because of the excretion of the metabolites of this drug are via renal and hepatic excretion, no special dose adjustment is necessary in patients who have renal impairment. Mitsubishi Tanabe Pharma Corporation (Osaka, Japan) are doing original synthesis of Teneligliptin and was the first drug of its kind to be synthetized in Japan. The drug under the brand name TENERIA® is sold jointly by Mitsubishi Tanabe Pharma Corporation and Daiichi Sankyo Co, Ltd, (Tokyo, Japan).

Teneligliptin is $1-[(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4-[(3S,5S)-5-(1,3-thiazolidine-3-carbonyl)pyrrolidin-3-yl]pipperazine (C$_{22}$H$_{30}$N$_{6}$OS) and its structure is shown in Figure 1.
MATERIALS AND METHOD:

Instruments:
The chromatographic separation was performed on Analytical Technologies HPLC-3000 series compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rhodineyne injector equipped with 20µl fixed loop. A reverse phase C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used. Model - UV 2012 double beam UV visible spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic balance was used for Spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals
Pharmaceutical grade pure Teneligliptin sample was procured from Swaroop Drugs and Pharmaceuticals HPLC grade Methanol and HPLC grade Water were procured from Merck specialties private limited, Mumbai.

Chromatographic conditions
C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used for the chromatographic separation at a detection wave length of 246 nm. Methanol, Phosphatebuffer pH 3 in a ratio of 70:30 v/v was selected as mobile phase for elution and same mixture was used in the preparation of standard and sample solutions. The elution was monitored by injecting the 20µl and the flow rate was adjusted to 0.8 ml/min.

Preparation of Mobile phase
Preparation of Phosphate buffer pH 3: Dissolve 1.36g of Potassium dihydrogen orthophosphate & 2. ml of triethylamine in 800ml of HPLC water, adjust the pH to 3 with orthophosphoric acid and add sufficient HPLC water to produce 1000ml. The mobile phase was sonicated for 15 min and filtered through a 0.45 µm membrane filter paper.

Preparation of Standard solutions
10mg Teneligliptin was accurately weighed and transferred into 10 ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solution of concentration 1000µg/ml of the drug (Working stock solution).

Preparation of Sample Solution
20 tablets of Teneligliptin were initially weighed and powdered and an amount equivalent to 10mg was accurately weighed into a 10ml volumetric flask, mixed with 10ml of mobile phase and sonicated for 5 min after making final volume up to 10 ml with mobile phase. Then solution was filtered through 0.45µm membrane filter. The solution contains 1000µg/ml of Teneligliptin. From the above stock solution 0.1ml aliquot was transferred in to a 10 ml volumetric flask, volume was made up to the mark with mobile phase to obtain a final concentration of 10 µg/ml of metformin.

Optimization of RP-HPLC method
The HPLC method was optimized with an aim to develop a estimation of Teneligliptin. Different mobile phases were tried for the method optimization, but acceptable retention times, theoretical plates and good resolution were observed with Methanol, Phosphate buffer pH 3 (70:30 v/v) using C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] Table:1 and a typical chromatograph of teneligliptin was shown in figure 3.

Parameter | Condition
--- | ---
Column | Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase | 70 : 30 (Methanol: Phosphate buffer pH-3).
Flow Rate | 0.8 ml/min
Wavelength | 246 nm
Injection Volume | 20 µl
Detector | UV-3000-M

Validation of the RP-HPLC method
Validation of the optimized method was performed according to the ICH Q2 (R) guidelines.

1. Linearity
For the determination of linearity, appropriate aliquots were pipetted out from 1000µg/ml (working stock solution). 0.1 – 0.5 ml was pipetted out in to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50µg/ml of metformin. Each solution was injected in triplicate. Calibration curves were plotted with concentration against observed peak areas followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Teneligliptin were shown in figure 2 and their corresponding linearity parameters given in table 2.

2. Accuracy
To ensure the reliability and accuracy of the recovery studies were carried out by % recovery method (standard addition method). A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in tables 3 and 4.

3. Precision
The repeatability of the method was verified by calculating the % RSD of three replicate injections of 100% concentration (30µg/ml of Teneligliptin) on the same day and for intraday precision % RSD was calculated from repeated studies. The results were given in table 5.

4. Limit of Quantitation (LOQ) and Limit of Detection (LOD)
The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 s/s and LOQ = 10 s/s.

5. Robustness
Robustness was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported. In the operational conditions Small changes were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wave length and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate. % RSD was reported in the table 6.

6. Assay of marketed formulation
20 tablets of teneligliptin were weighed and crushed into fine powder. The average weight of the tablet was calculated

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and the amount equivalent to 10 mg of pure teneligliptin was dissolved in 10 ml of solvent. From this stock solution 30 ppm dilution was prepared and injected. The % purity was calculated by comparing the result with result obtained from 30 ppm standard drug and are reported in table 7.

7. System suitability
It was ensuring that from the system suitability parameters, the method can generate results of acceptable accuracy and precision. System suitability was carried out with three injections of solution of 30 µl/ml of Teneligliptin in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table 8.

RESULT AND DISCUSSION
Linarity:
It was clarified from the analytical method linearity as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figure 1) gave linear relationship over the concentration range of 10 - 50 µg/ml for metformin. From the regression analysis, a linear equation was obtained $y = 54647x - 74133$, and the goodness-of-fit ($r^2$) was found to be 0.9968, indicating a linear relationship between the concentration of analyte and area under the peak.

| Conc. (µg/ml) | Peak Area |
|---------------|-----------|
| 10            | 465398    |
| 20            | 974059    |
| 30            | 1629073   |
| 40            | 2146136   |
| 50            | 2611705   |

| Sr. No. | Conc. (µg/ml) | Area (Mean) | Standard Deviation (SD) | Accuracy (%) | Precision (%) |
|---------|---------------|-------------|-------------------------|--------------|---------------|
| 1       | 10            | 465631      | 465321                  | 354.8224908  | 0.0762533     | 0.076253273  |
| 2       | 30            | 1632648      | 1629730                | 2651.272713  | 0.1626817     | 0.162681715  |
| 3       | 50            | 2608954      | 2611474.667             | 2413.756478  | 0.0924289     | 0.092428868  |

Accuracy
The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Teneligliptin and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD, as demonstrated in Table -3.

| Sr. No. | %Composition | Area of Standard | Area of Sample | % Recovery |
|---------|--------------|------------------|---------------|------------|
| 1       | 50% Recovery | 1629073          | 1633181       | 100.252168 |
| 2       | 100% Recovery| 2146136          | 2112757       | 98.44469316|
| 3       | 150% Recovery| 2611705          | 2619697       | 100.306007 |

Precision
Precision is “the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions,” and it is expressed in the form of relative standard deviation. The repeatability, intra-day and inter-day precision results are shown in the table 5. The RSD were calculated for all the results are within limits. Precision was not more than 2.0% RSD, as demonstrated in Table 5.
| Sr.no. | Parameter | Condition | Peak Area | Statistical Analysis | Retention Time | Statistical Analysis |
|-------|-----------|-----------|-----------|----------------------|----------------|----------------------|
|       |           |           | Mean=     | Mean=               | mean=         | mean=               |
| 1     | Flow rate | 0.7       | 692412    | 692890              | 692318.8444  | 4.339               | 4.346               |
|       |           |           |           |                      |               |                     | 4.269               |
| 1     | Flow rate | 0.8       | 692265    | 64.4330265          | 4.218         | mean=               | mean=               |
|       |           |           |           |                      |               | 0.07429247          |                     |
| 1     | Flow rate | 0.9       | 692352    | 692300.333          | %RSD=         | 4.247               | %RSD=               |
|       |           |           |           |                      |               | 1.742364            |                     |
| 2     | Wavelength| 2.03      | 687423    | 688082              | 689642.556   | 4.209               | 4.206               |
|       |           |           |           |                      |               |                     |                     |

LOD and LOQ
The LOD and LOQ were calculated by the equations, LOD = 3.3x Standard Deviation and LOQ = 10x Standard Deviation where, Standard Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for Teneligliptin were 0.109 and 0.3305 µg/ml, respectively.

Robustness
Robustness of the method reflects that the results are unaffected or reliable even if the minute changes in the method parameters. Here, the flow rate and wavelength were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 30µg/mL solution are as shown in Table No. 6.

| Sr.no. | Parameter | Condition | Peak Area | Statistical Analysis | Retention Time | Statistical Analysis |
|-------|-----------|-----------|-----------|----------------------|----------------|----------------------|
|       |           |           | Mean=     | Mean=               | mean=         | mean=               |
| 1     | Flow rate | 0.7       | 692412    | 692890              | 692318.8444  | 4.339               | 4.346               |
|       |           |           |           |                      |               |                     | 4.269               |
| 1     | Flow rate | 0.8       | 692265    | 64.4330265          | 4.218         | mean=               | mean=               |
|       |           |           |           |                      |               | 0.07429247          |                     |
| 1     | Flow rate | 0.9       | 692352    | 692300.333          | %RSD=         | 4.247               | %RSD=               |
|       |           |           |           |                      |               | 1.742364            |                     |
| 2     | Wavelength| 2.03      | 687423    | 688082              | 689642.556   | 4.209               | 4.206               |
|       |           |           |           |                      |               |                     |                     |

Assay of marketed formulation
The % purity obtained from the formulation was given in Table 7. And it was found that the Assay results of teneligliptin are within the limits.

| % Composition | Area of Standard | Area of Sample | % Assay |
|---------------|------------------|----------------|---------|
| % Assay       | 1629073          | 1622184        | 99.5771 |

System Suitability Parameters:
System suitability was performed by injecting three replicate injections of 100% test concentration, number of theoretical plates, asymmetry factor were satisfactory. The chromatographs confirm the presence of Teneligliptin at 4.2 min without any interference.

| Parameter               | Observed Value | Limits |
|-------------------------|----------------|--------|
| No. of Theoretical Plates| 9520           | < 2000 |
| Tailing Factor          | 1.15           | < 1.75 |

CONCLUSION:
The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Teneligliptin from pure and its dosage forms. The mobile phase used for method development is very simple to prepare and economical also. The sample recoveries in the formulation were showing good results with their respective label claims and it was found that there is no interference of formulation excipients in the estimation. And hence, this method can be easily and conveniently adopted for routine analysis of Teneligliptin in pure form and its dosage form.
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REFERENCES
[1] T. N. V. Ganesh Kumar, S. Vidyadhara, Niteen Ashok Narkhede, Y. Sai Silpa and M. Rajya Lakshmi, Method development, validation and stability studies of Teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy, Journal of Analytical Science and Technology, 01-08. (02-08-2017)

[2] Martindale, The complete drug reference. 36th Edition. Vol. I. London (UK): Pharmaceutical Press (An Imprint of RPS Publishing); 2009.

[3] Vishnu C. Shinde, Kiran B. Aher, Girijab. Bhavar, Sachin J. Kakad and Sanjay R. Chaudhari, Development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of Teneligliptin Hydrobromide in pharmaceutical preparation, Der Pharmacia Lettre, Volume 8 (8), 2016, 291-301. (03-08-2017)

[4] Atul T. Hemke, E. A. Rathod, K. R. Gupta, M. J. Umekar, HPLC and UV-Spectrophotometric estimation of Teneligliptin from tablet dosage form, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry, Volume 4(3), 2016, 148-156. (02-08-2017)

[5] Manjusha D. Karad and V. D. Barhate, Spectrophotometric determination of an anti-diabetic drug Teneligliptin bulk and pharmaceutical formulations. World Journal Pharmaceutical Research; Volume. 5(5), 2016, 1625-1632 (02-08-2017)

[6] Beckett AH, Stenlake JB. Instrumental methods in the development and use of medicines. In, Practical pharmaceutical chemistry (Part-2), 4th edition. New Delhi, CBS Publishers and Distributors, 2005

[7] Chunduri R.H.B, Dannana G.S. Development and validation of LC-MS/MS method for quantification of teneligliptin in human plasma and its application to a pharmacokinetic study, World Journal of Pharmacy and Pharmaceutical Sciences, 2016;5(5):833-50.