**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF TENELIGLIPTIN IN PHARMACEUTICAL DOSAGE FORM

SONTAKKE A*, PATIL S, BIYANI K.R. AND KALE R.H.

Anuradha College of Pharmacy, Chikhli-443201, Maharashtra, India

*Corresponding Author: E Mail: Amit Sontakke: amitsontakke24@gmail.com

Received 19th Aug. 2021; Revised 20th Sept. 2021; Accepted 29th Oct. 2021; Available online 1st Dec. 2021

<https://doi.org/10.31032/IJBPAS/2021/10.12.2006>

ABSTRACT

Teneligliptin is a Anti-diabetic agent used in the management of T2DM. It belongs to class DPP-4 (dipeptidyl peptidase-4) inhibitors or "gliptins". A simple, accurate and sensitive method has been developed for the estimation of Teneligliptin in pharmaceutical dosage form. The λ_{max} of Teneligliptin was found to be 248 nm in methanol:water pH3 [70:30(v/v)]. The method shows high sensitivity with linearity 10 to 50 $\mu\text{g/ml}$ (regression equation: $y = 39069x + 38994$; $r^2 = 0.999$). The various parameters according to ICH guidelines are followed for validating and testing of this method. The limit of Detection and limit of Quantitation were found to be 0.1756 $\mu\text{g/ml}$ and 0.5321 $\mu\text{g/ml}$ in Methanol: Water pH: 3 [70:30 (v/v)] respectively. The % purity of tablet formulation was found to be 99.8855%. The results concluded that the procedure is accurate, specific and reproducible (RSD < 2%), and also being simple and cheap and appropriate for the determination of Teneligliptin in bulk and pharmaceutical formulation.

Keywords: Teneligliptin, RP-HPLC, DPP-4 Inhibitors, ICH Guidelines

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia. Diabetes mellitus further classified into 2 types^[1]

Type I - Insulin-dependent diabetes mellitus (IDDM)

Type II – Non-insulin dependent diabetes mellitus (NIDDM)

Type II DM is more common specially in adults where the body doesn't produce enough insulin. Teneligliptin belongs to class DPP-4 inhibitors. Teneligliptin is {(2S, 4S)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5yl)-1-piperazinyl]-2&pyrrolidinyl}(1,3-thiazolidin-3-yl) methanone. The molecular formula and molecular weight of Teneligliptin is $C_{22}H_{30}N_6OS$ and 426.58g/mole resp. The chemical structure of Teneligliptin is shown in fig 1.

Previous literature review of Teneligliptin shows that there were several analytical methods like RP-HPLC^[2-5], HPTLC^[6], RP-UHPLC^[7], UV spectroscopy^[8] and very few methods were reported for RP-HPLC for estimation of Teneligliptin in bulk and dosage form. In the present study, we have developed a simple, accurate and more precised method for the estimation of teneligliptin in tablet dosage form by RP-HPLC and also validated according to ICH guidelines.

MATERIALS AND METHODS:

Instruments- The chromatographic separation was performed on Analytical Technologies HPLC-3000 series compact liquid chromatographic system integrated with a variable wavelength programmable UV detector. A reverse phase C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used. Model - UV-3000-M 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:

Chemicals: All the solvents used are of HPLC grade. It includes Methanol, Ortho phosphoric acid, distilled water, etc.

Teneligliptin sample supplied by RAP Analytical Research and Training centre, Nashik used as Standard drug. TENEPLA (Teneligliptin 20 mg) manufactured by Cipla Pharma is bought from the market.

CHROMATOGRAPHIC CONDITIONS AND INSTRUMENTATION:

C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used for the chromatographic separation at a detection wave length of 248 nm. Methanol:water 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 20 μ l and the flow rate was adjusted to 0.8 ml/min. The chromatographic conditions were mentioned in Table 1.

Preparation of Mobile phase- The mobile phase is consist of Methanol and water in the ratio of 70:30v/v. The pH was adjusted 3 with ortho phosphoric acid.

Preparation of Standard solutions- 10 mg Teneligliptin was accurately weighed and transferred into 10 ml volumetric flasks, dissolved using mobile phase and sonicate for 5 min and the volume was made up with the same solvent to obtain primary stock solution of concentration 1000 μ g/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 upto 10 ml with mobile phase. Then solution was filtered through 0.45 μ m membrane filter. The solution contains 1000 μ g/ml of Teneligliptin. From the above stock solution 0.1ml 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 μ g/ml of Teneligliptin.

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

chromatograph of teneligliptin was shown in figure 2.

RESULT AND DISCUSSION:

Validation of the RP-HPLC method -

Validation of the optimized method was performed according to the ICH Q2 (R-1) guidelines. It includes Linearity, Accuracy, Precision, LOD, LOQ, Robustness.

1. Linearity - For the determination of linearity, 0.1 – 0.5 ml was pipetted out from 1000 μ g/ml stock solution in to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50 μ g/ml of Teneligliptin. Calibration curves were plotted with concentration against observed peak areas. The calibration curves for Teneligliptin were shown in figure 3 and their corresponding linearity parameters given in table 2.

2.Accuracy - To ensure the reliability and accuracy recovery studies were carried out by % recovery method (50%,100%,150%). Sample was inserted in triplicates.

3. Precision: Precision is “the closeness of results obtained from two or more sampling of the same sample under the prescribed conditions,” and it is expressed in the form of RSD. The 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.

4.LOD and LOQ : The LOD was calculated by the equation, $LOD = 3.3 (SD)/S$ and LOQ by $LOQ = 10 (SD)/ S$ where, Standard Deviation taken from accuracy and slope is from linearity . Based on these equations, the calculated LOD and LOQ values for Teneligliptin were found to be 0.1756 and 0.5321 μ g/ml, respectively.

5.Robustness : The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The flow rate was varied by of optimized chromatographic condition. The results of robustness studies are shown in Table 6 and 7. Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

6.Assay of marketed formulation : The % purity obtained from the formulation was given in table 8. And it was found that the Assay results of teneligliptin are within the limits.

System Suitability Parameters: Number of theoretical plate should be greater than 2000 as it indicates efficiency of column.

Value of Asymmetry factor should be less than 2.

These conditions are fulfilled by this method.

Figure 1: Chemical structure of Teneligliptin

Figure 2: UV spectrum of Teneligliptin

Figure 3: Calibration curve of Teneligliptin

Table 1: Chromatographic conditions for Teneligliptin

Parameter	Conditions
Mobile phase	Methanol:water(70:30v/v)
Flow rate	0.8 ml/min
Wavelength	248 nm
Injection volume	20 µl
Detector	UV-3000-M UV visible spectrophotometer

Table 2: Result for Linearity of Teneligliptin

Concentration µg/ml	Area
10	773087
20	1184192
30	1559903
40	1948584
50	2344350

Table 3: Result of Accuracy

Sr. No.	Concentration	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	% RSD
1	10	773087	772896.6667	2079.044572	0.2689939	
	10	770729				
	10	774874				
2	30	1559903	1559405.333	1748.451982	0.112123	0.086382089
	30	1560851				
	30	1557462				
3	50	2344350	2345692.667	2999.548355	0.1278747	
	50	2349129				
	50	2343599				

Table 4: Result of % recovery

Sr. No.	% Composition	Area of standard	Area of sample	% Recovery
1	50% Recovery	1559903	1558330	99.8991604
2	100% Recovery	1948584	1947143	99.92604886
3	150% Recovery	2344350	2341058	99.85957728

Table 5: Results of Interday and Intraday precision of Tenoeligiptin

Interday Day 1			Day 2			Mean	% RSD
1559903	1560851	1557462	1559695	1560420	1561963	1561963	0.10%

Intraday Morning			Evening			Mean	% RSD
1559903	1560851	1557462	1562916	1562418	1561632	1560864	0.13%

Table 6: Result of Robustness Study of Tenoeligiptin (Wave Length)

Sr. No.	Conc (ppm)	Wavelength (nm)	Area	Mean	SD	%RSD
1	20	246	1188274			
2	20	248	1184192	1185351	2549.06	0.2150465
3	20	250	1183588			

Table 7: Result of Robustness Study of Tenoeligiptin(Flow rate)

Sr. No.	Conc. (ppm)	Flowrate (ml/min)	Area	Mean	SD	%RSD
1	20	0.6	1185979			
2	20	0.8	1184192	1183675	2601.86	0.21981239
3	20	1.0	1180853			

Table 8: Result for Assay of Tenoeligiptin

% composition	Area of standard	Area of sample	% Assay
% Assay	1559903	1558117	99.8855

DISCUSSION

The RP-HPLC method of Tenoeligiptin for single tablet doses was carried out successfully. The developed RP-HPLC method was also validated successfully in terms of accuracy, precision, linearity etc. with following conclusions,

- The proposed RP-HPLC method allows precise, accurate and reliable measurement of Tenoeligiptin in single dosage form.
- This RP-HPLC method was found to be simple, rapid, accurate and precise (reproducible) and economical for the concurrent estimation of the drug in

respective multi component tablet dose of Teneligliptin.

REFERENCES

- [1] K. D. Tripathi "Essential of Medical Pharmacology" Jaypee Publishers, 7th edition, Page no 274.
- [2] Rajani Vatepalem, Rajendra Prasad Yejella, Lakshmana Rao Atmakuri "Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Metformin" Turkish Journal of Pharmaceutical Sciences 2020,Page no 141-147.
- [3] Anuruddha Chabukswar, Pramod Sakpal "RP-HPLC and UV-Spectrophotometric Methods Development and Validation for Simultaneous Estimation of Teneligliptin and Metformin in Fixed Dose Combination" Journal of Current Pharma Research 2019, page no. 3146-3154.
- [4] Anvesha Vinit Ganorkar, Rekha S Jibhate, Krishna Radheshyam Gupta "Development of Stability Indicating and Robust RP-HPLC Method for Determination of Teneligliptin" Asian Journal of Applied Chemistry Research 2018, page no 01-12.
- [5] Pradnya Lokhande "Analytical Method Development and Validation of Teneligliptin by using RP-HPLC with ICH Guidelines" International Journal of Trend in Scientific Research and Development 2019, volume 3, page no 2456- 6470.
- [6] Sohan S Chitlange, Diptee G Rawat, Sejal P Gandhi "Estimation of Anti Diabetic Teneligliptin in Bulk and Formulation by Densitometric and Spectrophotometric Method" Analytical Chemistry Letters 2017,page no 556-566.
- [7] K Ganesh Prabhu "Analytical Method Development and Validation for the Estimation of Teneligliptin in Oral Solid Dosage Form by Reversed Phase Chromatographic Technique Using UHPLC" RVS College of Pharmaceutical Sciences 2017.
- [8] Nita Yadav, Anju Goyal "Method development and validation of Teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods" International Journal of Pharmaceutical Chemistry and Analysis 2017, volume 4, page no 54-8.
- [9] <https://www.drugbank.ca/drugs/DB00619>.