



ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENELIGLIPTIN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, rapid, precise and accurate Gradient reversed-phase HPLC method was developed and validated for the determination of Teneligliptin in commercial tablets. Separation was achieved on a C8, 150x4.6mm i.d., 5 μ m stainless steel analytical column i.e. using a mobile phase A consisting of 1ml OPA in 1000ml water and Acetonitrile: Methanol (80:20)v/v as mobile phase B with pH 5.3 at a flow rate of 1.0ml/min and UV detection at 213nm. The retention time was found to be 9.8min. The linearity of the proposed method was investigated in the range of 48.6-148.9 μ g/ml ($r^2 = 0.999$). The percent amount of drug estimated by all developed methods was nearly 100%, found to be in good agreement with label claim of marketed tablet formulation. The recovery study was carried out at three different levels and results were found to be satisfactory. The validation parameters like accuracy, precision, ruggedness, linearity and range were studied for all the developed methods and were found to be within limits. It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

KEYWORDS: Tenelegliptin, RP-HPLC, Validation.

INTRODUCTION

Structure

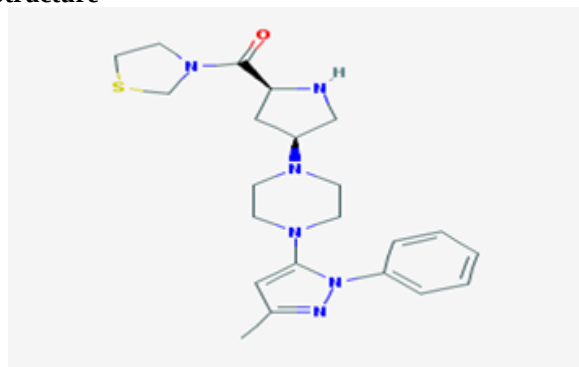


Fig.1 structure of teneligliptin

Teneligliptin Hydrobromide hydrate^[1] is chemically described as [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl]-(1,3thiazolidin-3-yl)methanone. Teneligliptin act by inhibiting the activity of DPP-4 enzyme^[2] there by increasing the incretin levels (GLP-1 & GLP-2) which inhibit glucagon release which in turn increases insulin secretion decreases gastric emptying and decreases blood glucose levels. It is given orally for the treatment of type-2 diabetes mellitus. Teneligliptin is soluble in DMSO, Methanol, Acetonitrile and Water. Recent studies have revealed that

this drug is unique in its nature and exhibits multiple pharmacological effects. It includes vasoprotective, neuroprotective effects. The literature review revealed a UV-spectroscopic^[3] and HPTLC^[4,5] Methods, and a Stability indicating RP-HPLC method^[6,7,8,9] for estimation of Teneligliptin Hydrobromide in pure and tablet dosage form, liquid chromatography–tandem mass spectrometry method for estimation of Teneligliptin HBr in rat blood plasma.^[10] No official or draft monograph of Teneligliptin Hydrobromide Hydrate was published in any of the pharmacopoeia for compendia applications. The proposed work represents the new simple, economical, rapid RP-HPLC method for the quantification of new drug substances in teneligliptin in Tablet dosage form. The developed methods were validated according to ICH guidelines.

EXPERIMENTAL

MATERIALS AND METHODS

Chemicals and reagents

Teneligliptin API (purity, 94.6%), were kindly supplied by Process Research Department of Teneligliptin hydrobromide hydrate was obtained as gift sample from Micro Labs Ltd, Bangalore, India. Methanol, Acetonitrile and Water (HPLC grade), ortho phosphoric acid were purchased from Merck Mumbai Ltd., India. All other chemicals and reagents employed were of

analytical grade and were purchased from S.D Fine chemicals, India. Commercial Pharmaceutical preparations which were claimed to contain 20 mg Teneligliptin tablets of was used in analysis.

Instrumentation

Agilent HPLC 1100 series with photo-diode array detector with empower software.

Chromatographic Conditions

Mobile phase composition of 1ml of OPA dissolved in 1000ml water as a Mobile phase A and Acetonitrile: water in the ratio of (80:20) V/V Mobile phase B to the column at a flow rate of 1.0ml/min whereas run time set was 20min. The separation was performed on Inertsil ODS-3V, 150x4.6mm i.d., 5µm stainless steel analytical column with gradient elution mode. The column was maintained at 40°C and the volume of each injection was 20µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The eluents were monitored at 213 nm and the retention time was found to be 9.8min.

Preparation of the standard solution

Weigh accurately about 77.5mg of Teneligliptin (Equivalent to 50mg) working standard was transferred into a 50mL volumetric flask. 35mL of diluent was added, sonicated to dissolve and upto volume with

diluent. 5ml of standard stock solution was pipette into a 50 mL volumetric flask and the volume was made upto mark with diluents to obtain a concentration of 100ppm. Filter about 2ml through 0.45µm pall pharma lab nylon 66 membrane filter or 0.45µm Durapore PVDF hydrophilic membrane filter.

Preparation of the Sample solution

Weigh not less than 20 tablets and determine the average weight and crush. Weigh and transfer the powdered the tablet powder equivalent to 50mg of Teneligliptin in 50mL volumetric flask, added about 35mL of the diluent. The contents of flask were sonicated for 10minutes with intermediate shaking (by maintaining the sonicator temperature at about 25°C). Finally, volume was completed with diluent and transfer 5ml of teneligliptin in 50ml and mixed well so as to obtain about 100 ppm of the concentration of the Teneligliptin in the test solution. The solution was centrifuged in a tight enclosure for about 5 min at 3500 rpm and 20µl of clear supernatant solution was injected directly on to the column.

Table-1 Optimized Chromatographic Conditions and system suitability parameters for proposed HPLC method of Teneligliptin

Column	C8, 150x4.6mm i.d., 5µm stainless steel analytical column
Mobile phase A	1ml OPA in 1000ml water
Mobile phase B	Acetonitrile : Methanol(80:20)
Mode of separation	Gradient
Diluent	Mobile phase A
Flow Rate	1.0 ml/min
Column Temperature	40°C
Sample temperature	10°C
Injection Volume	20µl
Wave Length	213nm
Run time	20min
Retention time	9.8min

Time in minutes	%Mobile Phase A	%Mobile Phase B
0.0	90	10
5.0	90	10
12.0	60	40
14.0	40	60
14.5	90	10
20	90	10

Table-2 Assay of Teneligliptin

S.No.	Sample Peak Area	Standard Peak Area	Labelled Amount (Mg/Tab)	Amount Found (Mg)	% Assay (95-105%)
1	2605233	2603932	20mg/ Tablet	19.87mg	99.4%

RESULTS AND DISCUSSION

After several systematic trails the present study was aimed at developing a sensitive, precise and accurate RP-HPLC method for the analysis of Teneligliptin in pharmaceutical dosage forms. The present method contains mobile phase A as 1ml of OPA in 1000ml water, Mobile phase B as Acetonitrile : Methanol(80:20) and Diluents as Mobile phase A which was found to be the most suitable as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. Under the above conditions the retention time obtained for Teneligliptin was 9.8min. A model Chromatogram is shown in **Fig.1**.

VALIDATION OF THE PROPOSED METHOD

The developed method of analysis was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness.

SYSTEM SUITABILITY TESTS

System suitability test parameters were checked by repetitively injecting the drug solution at the Concentration level 100µg/ml for Teneligliptin to check the reproducibility of the system. System suitability parameters like number of theoretical plates (N), Tailing factor, resolution and relative standard deviation of peak height or peak area or repetitive injections were studied. The %RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The results were shown in **Table-1**.

LINEARITY

Linearity for the developed method was checked by The standard solutions containing Teneligliptin in the concentration range of 49.66-148.99 µg/ml were prepared and injected in duplicate into the chromatographic system and chromatograms were recorded. A linear relationship between peak area vs. concentration was observed in the range of study. The slope, y-intercept and correlation coefficient were found to be 16795, -18643.055 and 0.9999 respectively. The chromatograms were developed and the results were given in **Table-3 and figure 2**.

PRECISION

Precision of the test method was determined by Six replicates (n=6) solutions were prepared and each

1. System precision and system suitability

Table3: System Suitability Data for Teneligliptin

S.No	System Suitability Parameter	Observed Value	Acceptance Criteria
1	The % RSD of peak areas of Teneligliptin	0.1	NMT 2.0
2	The Tailing factor for Teneligliptin peak in standard solution	1.54	NMT 2.0
3	USP plate count	60993	NLT 3000

solution was injected in duplicate under the same conditions and mean value of peak area response for each solution were considered. The results were given in **Table 4**.

ACCURACY

The accuracy of the proposed method was evaluated by the recovery studies which were carried out by extracting the tablets in the diluent at different spiking levels of 50%, 100%, 150%. Three samples were prepared at each concentration and calculated the exact percentage recovery at each level for Teneligliptin. The results were analyzed and the results are found to be within the limits. The chromatograms were developed and the results were given in **Table-5 and figure 3**.

ROBUSTNESS

Robustness carried out by varying 3 parameters from the optimized chromatographic conditions such as making small changes in Flow rate (± 0.2 ml/min), Absorption maxima ($\pm 5\%$) and column temperature ($\pm 5^\circ\text{C}$). %RSD was not more than 2%. No significant changes were observed the results were shown in the **table 6**.

STABILITY OF ANALYTICAL SOLUTIONS

The stability of the standard and test solution was checked by analyzing these solutions at frequencies of initial, 24 h, 48 h and 72 h at room temperature, against freshly prepared standards. The result demonstrated that the standard solution, as well as the sample solution is stable for at least 72 h.

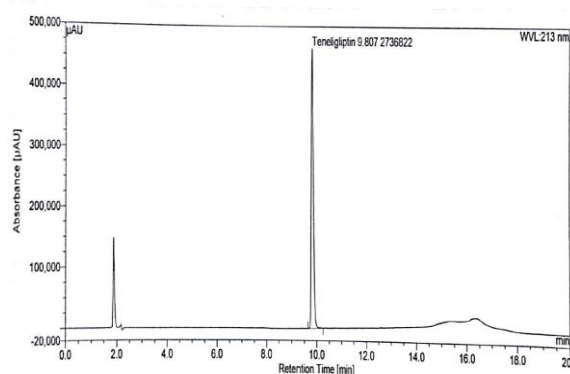


Fig. 7 Standard Chromatogram for System Suitability

2. Linearity

Table: 4 Linearity of Teneligliptin

S.No	Spike level Ss Conc(ppm)	Standard Concentration(ug) ccccccccccccccoconcentration)	Area
1	50%	49.66	825753
2	80%	79.46	1321204
3	100%	99.33	1651506
4	120%	119.19	1981807
5	150%	148.99	2477259
Correlation Coefficient			0.999
Slope (m)			16795
Intercept (b)			-18643.055

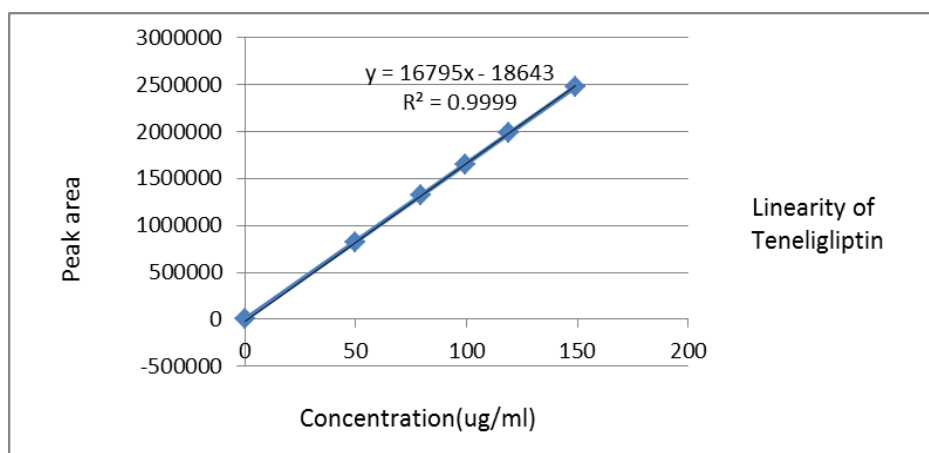


Fig.2 Linearity/Calibration Graph of Teneligliptin

Table: 4 Results of precision studies

Spiked (µg/ml)Concentration (100ppm)	Intra-day	Inter-day
%RSD	0.8	0.76

Table: 5 Recovery Results for Teneligliptin

S.no	Spike level (%)	Spiked (mg)	Peak Area	Mg recovered	Recovery (%)	Mean Recovery (%)
1	50	25.27	1293437	24.88	98.5	99.5
	50	25.21	1304119	25.09	99.5	
	50	25.23	1305245	25.11	99.5	
2	100	50.43	2602470	50.07	99.3	99.6
	100	50.46	2610885	50.23	99.5	
	100	50.41	2622955	50.46	100.1	
3	150	75.68	3908578	75.19	99.4	99.4
	150	75.65	3920057	75.42	99.7	
	150	75.72	3900857	75.05	99.1	
Mean					99.4	
SD					1914.4	
RSD					0.4	

Table: 6 Robustness Studies for Teneligliptin

S.no	Parameter	optimized	used	%RSD	Tailing factor
1	Flow rate (±0.1ml/min)	1.0	0.8	0.4	1.41
			1.0	0.8	1.54
			1.2	0.6	1.55
2	Column	40°C	35°C	0.5	1.42

	temperature($\pm 5^{\circ}\text{C}$)		40°C	0.8	1.54
			45°C	0.6	1.57
3	Absorption maxima ($\pm 5\%$)	213nm	208nm	0.1	1.1
			213nm	0.8	1.54
			218nm	0.3	1.34

CONCLUSION

This method presents a simple analytical method on determination of Teneligliptin in finished dosage form by HPLC. The proposed method is a simple Gradient mode short run time method when compared to long run time, isocratic mode reported method.^[1] The proposed HPLC method has the ability to separate Teneligliptin from all its impurities and excipients found in the tablet dosage form and therefore can be applied to the analysis of samples at quality control. The shorter run time analysis is particularly important in quality control to support the In-process samples during the manufacturing, as each step in production is dependent on the result awaited from quality control before proceeding to further step. The proposed method is rapid, direct, specific, accurate, precise, Rugged, Robust and validated for the routine analysis in the finished dosage form. The method also may be extended to the bulk drug substances.

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