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

Dr. Pradnya Lokhande

Assistant Professor, Department of Chemistry,
Bhavan's College, Andheri (W), Mumbai, Maharashtra, India

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ABSTRACT

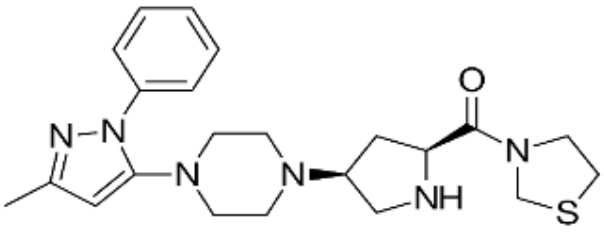
Teneligliptin is drug used against type 2 diabetes mellitus and it is also a member of class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptins". A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Teneligliptin in bulk formulation. The λmax 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 having renal impairment. Mitsubishi Tanabe Pharma Corporation (Osaka, Japan) are doing original synthesis of Teneligliptin and was the first drug of its kind to be synthesized 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]piperazine ($C_{22}H_{30}N_6OS$) and its structure is shown in Figure: 1



Analytical method validation provides that various HPLC analytical techniques shall give repeatable reliable and results; As it is providing information about accuracy, linearity, precision, detection, and quantitation limits and hence it is crucial step in developing new dosage forms. ICH guideline says that, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now mandatory in the process of drug development to provide the validation data for the responsible authorities. The Guidelines for analysis method validation include ICH guidelines. By the literature survey a very few methods reported for determination of Teneligliptin in bulk drug as well as pharmaceutical preparation. This research is tries to develop a new sensitive and rapid HPLC method for the determination of Teneligliptin in Bulk preparation, and this method was also validated according to ICH guidelines.

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 Rheodyne 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 specialities 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, Phosphate buffer 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

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.

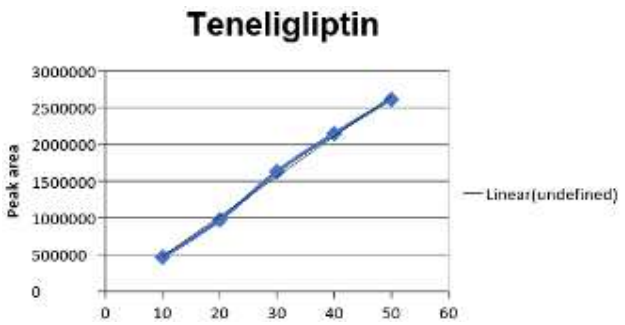
7. System suitability

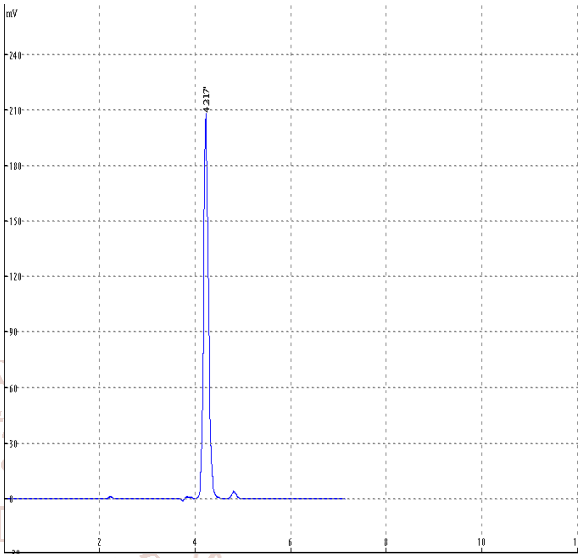
It was ensuring that from the system suitability parameters, the method can generate results of acceptable accuracy and precision**.** System suitability was carried out with three injections of solution of 30 µl/ml of Teneligliptin in to the chromatographic system. Number of theoretical plates (N) obtained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

RESULT AND DISCUSSION

Linearity*:*

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 = 54647 x - 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

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.

			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	% SD	% SRD
1	10	465631	465321	354.8224908	0.0762533	0.076253273
	10	465398				
	10	464934				
2	30	1632648	1629730	2651.272713	0.1626817	0.162681715
	30	1629073				
	30	1627469				
3	50	2608954	2611474.667	2413.756478	0.0924289	0.092428868
	50	2611705				
	50	2613765				

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.

Interday	Day 1			Day 2			Mean	%RSD
Injection	1	2	3	1	2	3		
Area	1629073	1622953	1634124	1628514	1634567	1624254	1628914	**0.30%**
Intraday		**Morning**			**Evening**			**%RSD**
Injection	**1**	**2**	**3**	**1**	**2**	**3**		
Area	1628102	1629073	1626134	1627963	1634124	1629163	1629093	**0.17%**

LOD and LOQ

The LOD and LOQ were calculated by the equations, LOD $= \frac{3.3 \times std.Deviation}{slope}$ and LOQ $= \frac{10 \times std.Deviation}{slope}$ 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
1	Flow rate (ml/min)	0.7	692412	Mean= 692390	Mean= 692318.444	4.339	mean= 4.34666667	mean= 4.26388889
			692352			4.383		
			692406			4.318		
		0.8	692265		SD= 64.4380265	4.203		SD= 0.07429247
		0.9	692423	Mean= 692300.333		4.218	mean= 4.242	
			692352		%RSD= 0.00930757	4.227		%RSD= 1.742364
			692126			4.281		
2	Wavelength (nm)	236	687862	Mean= 688082	mean = 689642.556	4.213	mean= 4.20933333	mean= 4.206
			688961			4.209		
			687423			4.206		
		238	692265		SD= 2284.74906	4.203		SD= 0.0031798
		239	688413	mean= 688580.667		4.206	mean= 4.20566667	
			688367		%RSD= 0.33129467	4.203		%RSD= 0.07560146
			688962			4.208		

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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