Method Development, Validation and Stress Degradation Study of Teneligliptin by RP-HPLC

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ABSTRACT

To develop and validate simple, rapid, linear, accurate, precise and economical reverse phase-high-performance liquid chromatography (RP-HPLC) method for of stress degradation study of Teneligliptin. The separation and quantization were achieved on Inertsil C18(250 mm × 4.6ID,5 um). The mobile phase selected was Methanol: Water (90:10) at a flow rate of 0.8 ml/min and detection of analytes was carried out at 248nm at pH 3. The method exhibited good linearity over the range of 10–50 µg/mL. The drug is freely soluble in organic solvents Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done by capillary tube method. The drug which when subjected to thermal, photolytic, oxidative, and acidic stress degraded into many degradation products. In most of the cases, the degradation rate was seen to be directly proportional to the amount of stress applied. The thermal stress was increased by increasing the incubation temperature, the faster the degradation took place. The values of LOD were found to be 0.956 ug/ml for TNG and the calculated LOQ values were found to be 0.171 ug/ml.

Keywords: RP-HPLC, Validation, Methanol, Teneligliptin, Force degradation

INTRODUCTION

Validation [1,2,3]

Validation is an integral part of quality assurance which helps to maintain current good manufacturing practices which results in safety, quality, purity and efficacy of product. According to WHO "Validation is establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce products which meet its predetermined specifications and quality characteristics."

The documented act of demonstrating that any procedure, process and activity will consistently lead to the expected results. It also includes the qualification of systems and equipment.

Manufacturers should plan validation in a manner that will ensure regulatory compliance and ensuring that product quality, safety and consistency are not compromised.
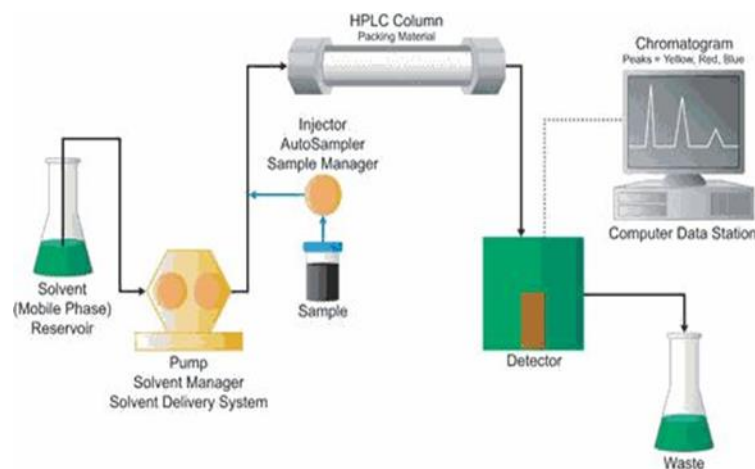
Chromatography[4,5,6,7]

One of the most widespread definitions of IUPAC*:* Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other (the mobile phase) moves in a definite direction*.*

Classification: [4,5,8]

In all type of chromatography, separation of the component of mixture results either by adsorption or partition for the column material. Binding of a compound to the surface of solid phase takes place in adsorption while in case of partition, a compound gets distributed into two liquid phases. The usual type of chromatography involves movement of liquid phase over the stationary phase carrying with it, a solute which has varying degree of affinity towards stationary phase.

High Performance Liquid Chromatography (HPLC)

HPLC is the most widely used from all of the analytical separation techniques. The reasons for the popularity of the method is its sensitivity, ready adaptability to accurate quantitative determinations, suitability for separating non-volatile species or thermally fragile ones and above all, its widespread applicability to substances that are of prime interest to industry. Examples of such materials includes: amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoides, pesticides, antibiotics, steroids, metal- organic species, and a variety of inorganic substances.

Figure 1: High Performance Liquid Chromatography (HPLC)

Application of HPLC

Quantitative analysis- HPLC is used for the identification of components of liquid mixtures. Identification is based on retention time and comparisons with standards. Quantitative analysis- It is used for the quantitative determination of components of high molecular weight compounds.

Stability Indicating Method[9,10,11,12]

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the re-test or expiration dating periods. Stability testing of an active substance or finished product provides evidence on how the quality of a drug substance or drug product varies with time. It is influenced by a variety of environmental factors such as temperature, humidity and light. Knowledge from stability studies enables understanding of the long-term effects of the environment on drugs. If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure (independent procedure). As appropriate, this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation. It is generally recommended that about 20-30 % of analyte degradation at least in one medium should be achieved. Neither ICH nor FDA guidelines specify how forced degradation studies are actually performed. Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g. diode array, mass spectrometry).
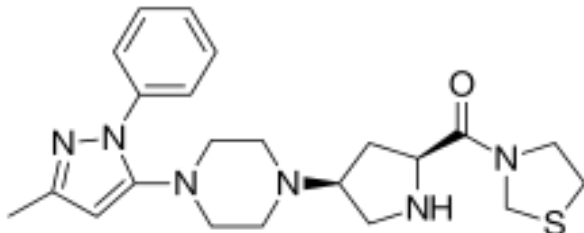
Regulatory Basis of Stability-Indicating Assays and Forced Degradation Studies [13, 14]

The ICH guidelines have been incorporated as law in the Europe, Japan and in the United States, but in reality, besides these, other countries are also using them. A stability indicating profile that provides assurance on detection of changes in identity, purity and potency of the product has been

put on the manufacturer. The expiry date should be based on real-time/real-temperature data. However, it is suggested that studies be conducted on the drug substance and drug product under accelerated and stress conditions.

MATERIALS AND METHOD

Table 1: Active drug

Name Of Drug	**Tenegliptin:** (TNG) is a novel drug, which is used for the treatment of type 2 diabetes mellitus. It is an anti- diabetic drug that belongs to dipeptidyl peptidase-4 inhibitors or "gliptins".
Structure	
IUPAC Name	{(2S,4S)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]-2-pyrrolidinyl}(1,3-thiazolidin-3-yl)methanone
Molecular Formula	$C_{22}H_{30}N_6OS$
Molecular mass	426.58 g/mol
Category	Anti Diabetic
Solubility	Water, Methanol, DMSO.
Melting point	209 ° - 211°C
Dose	In adults, teneligliptin is orally administered at a dosage of 20 mg once daily, which can be increased up to 40 mg per day.
Mechanism of action	Glucagon increases blood glucose levels, and DPP-4 inhibitors reduce glucagon and blood glucose levels. The mechanism of DPP-4 inhibitors is to increase incretin levels which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels.
Uses	Teneligliptin reduces blood glucose levels in patients with type II diabetes mellitus.

Table 2: Solvents and Chemical:

Sr. No.	Solvents and Chemicals	Name of Company
1.	Methanol HPLC Grade	E. Merck Ltd.,Mumbai, India
2.	Water HPLC Grade	E. Merck Ltd.,Mumbai, India
3.	O – phosphoric acid AR Grade	E. Merck Ltd.,Mumbai, India

Table 3: Marketed Formulation:

Sr. No.	Marketed Formulation	Manufacturer
1	ZITEN (Teniligliptin 20 mg tablet)	Glenmark Pharmaceuticals Ltd. Mumbai, Maharashtra.

Table 4: Equipment:

Sr. No	Equipment	Make/ Model
1	HPLC	Shimadzu LC2010
2	Detector system	UV detector
3	Analytical column	4.6 × 250 C-18 (inertsil)

| 4 | Software | LC Solution |
5	Precision balance	Mettler Tolledo
6	pH meter	Labinda
7	Grade 'A' certified Glassware	Borosil
8	Ultrasonicator	FAST CLEAN Ultrasonic Cleaner
9	Electronic balance	Electrolex New

RESULTS AND DISCUSSION

Development of Chromatographic Validation Method for Estimation of Drug:

The standard solution of teneligliptin was used for HPLC method development to determine teneligliptin in presence of degraded products. Degraded samples were prepared by systematic forced degradation study. These samples were used for method development trials to optimize the method as a stability indicating method.

MeOH : WATER (90:10)
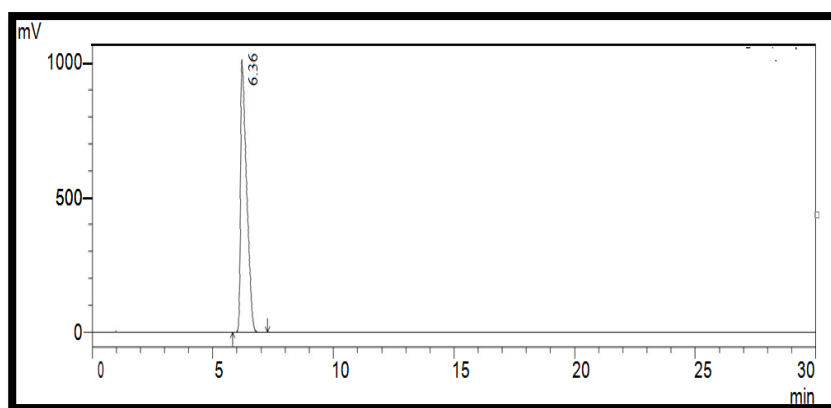
Figure 2: Chromatogram of Standard Drug TNG (10 µg/ml)
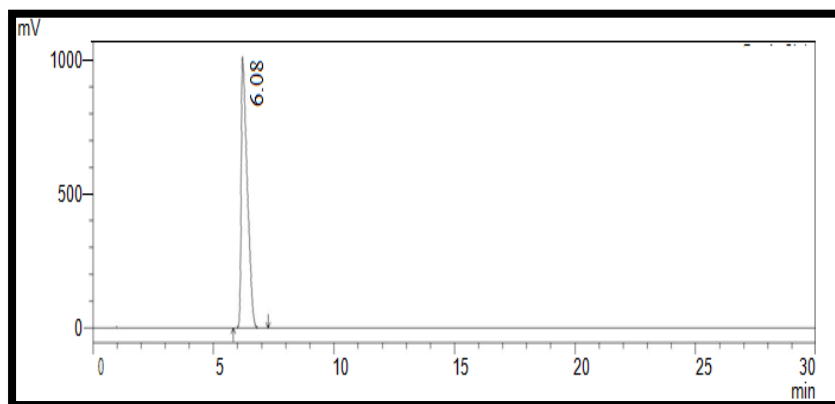
Figure 3: Chromatogram of Tablet TNG (10 µg/ml)

Selection of Mobile Phase :
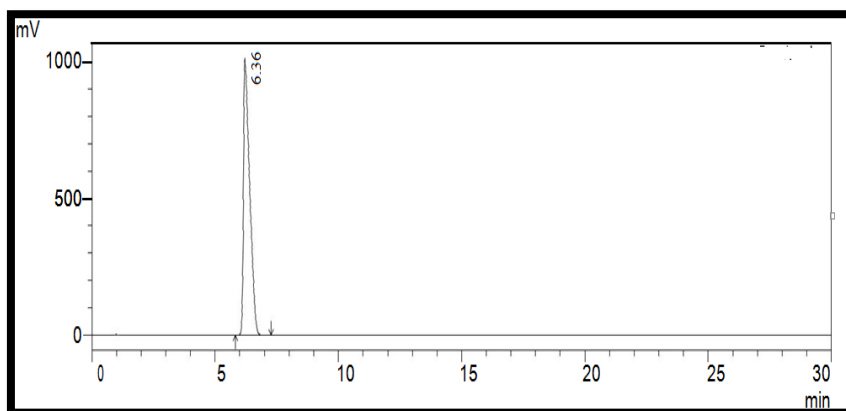
Stationary phase   : Inertsil C18(250 mm × 4.6ID,5 um)

Mobile phase   : Methanol :Water (90:10)

pH   : 03

Detection wavelength   : 248 nm

Flow rate   : 0.8 ml/min

Temperature   : Room temperature

Run time   : 30 min

Retention time   : 6.36 min

The retention time of TNG was found to be 6.36 min as shown in figure no : 15

Figure 4: Chromatogram of Standard Drug TNG (10 µg/ml)

 Optimization of Chromatographic Condition

The following chromatographic condition were established by trial and error and were kept constant throughout the experimentation

Table 5. Chromatographic Condition

Column	Inertsil (250× 4.6 mm) column
Partical Size Packaging	5 µm
Stationary Phase	C_{18} Inertsil
Mobile Phase	Methanol (90%) : water (10%)
Detection Wavelength	248 nm
Flow Rate	0.8 mL/min
Temperature	Ambient
Sample Volume	10 µl

The method was validated with respect to parameter like linearity, accuracy, precision, ruggedness, LOD, LOQ and repeatability.

Validation of Chromatographic Method

The optimized method obtained from TNG was validated according to the ICH guidelines using following parameters.

➢   **Linearity and Range:**

The linearity and range of the method was performed. From the obtained data, calibration graph were plotted using peak areas of standard drug vs concentration for establishing linearity and range

of the method shown in figure16. The teneligliptin was found to be linear in the concentration range 10 -50 ul/ml.depicted in table10.
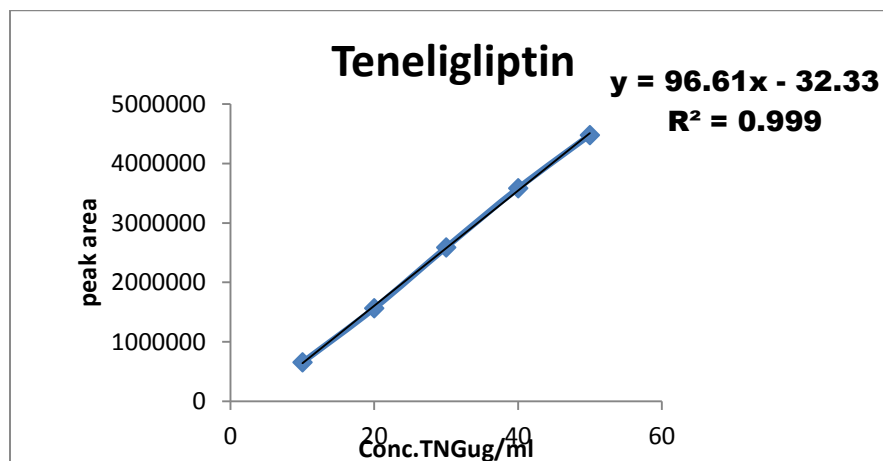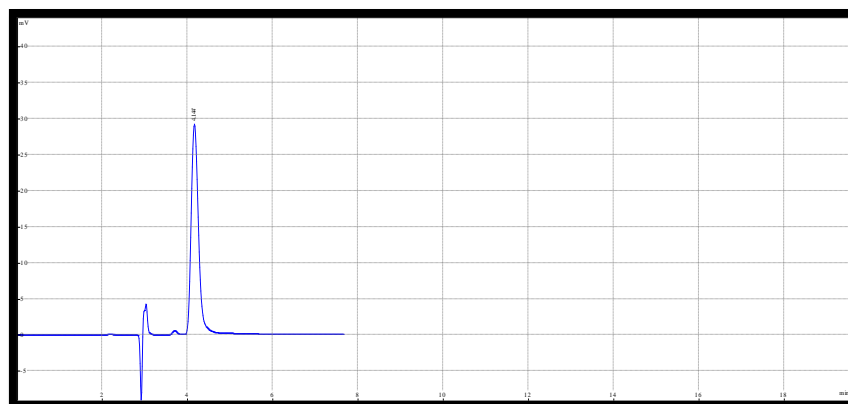

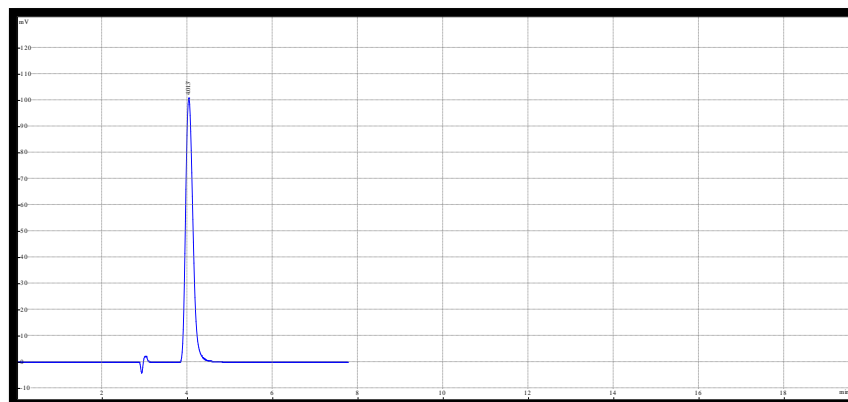**Figure 5: Calibration Curve of TNG (10-50ul/ml) at 247 nm**

Table 6: Data for calibration curve of TNG by optimized method

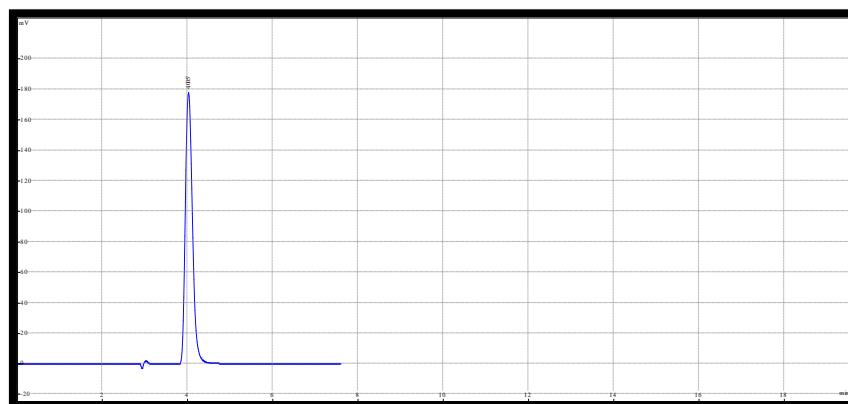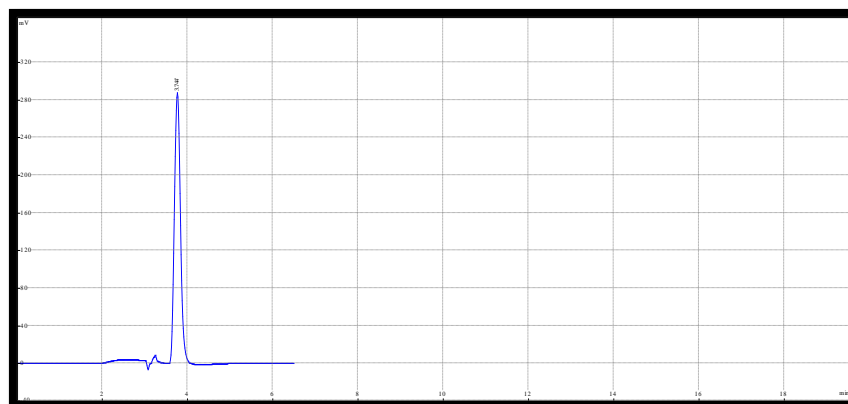| Sr.No	Conc ug/ml	Peak area (±SD)*			%R.S.D.
1	10	62.27	±	0.363	1.165
2	20	263.01	±	1.088	0.765
3	30	276.29	±	1.862	0.865
4	40	362.87	±	2.181	0.822
5	50	462.12	±	3.441	0.845

(n=5)number of determination

Figure 5: Chromatogram of Standard TNG (10ul/ml)

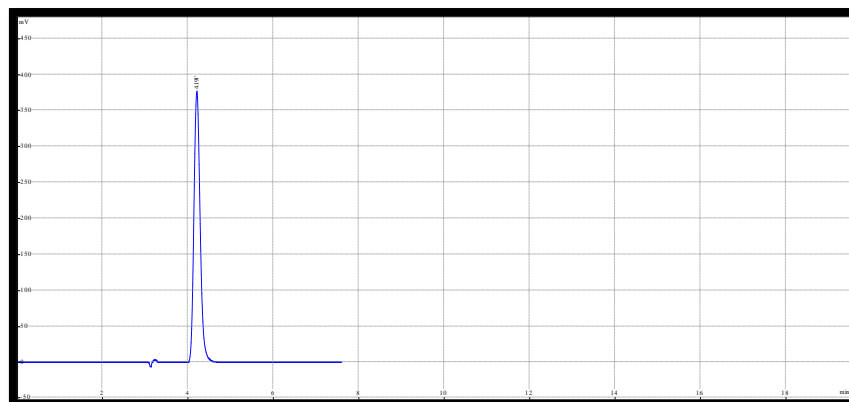**Figure 6: Chromatogram of Standard TNG (20ul/ml)**

Figure 7: Chromatogram of Standard TNG (30ul/ml)

Figure 8: Chromatogram of Standard TNG (40ul/ml)

Figure 9: Chromatogram of Standard TNG (50ul/ml)

Accuracy:

Accurately weighed quantities of preanalysed tablet powder equivalent to 10 mg teneligliptine was taken in 10.0 ml volumetric flask and then known amount of teneligliptine was added at different concentration levels so as to produce solutions containing 80%, 100% and 120% of the label claim. The contents in the flasks were shaken with HPLC grade methanol volumes were adjusted upto the mark. The solutions were filtered through a 0.45µm- membrane filter. The content of drug was calculated.

Table 6: The Result of Accuracy Studies

Initial amt of formulation(ug/ml)	Standard added (ug/ml)	Area	Mean	SD	%R.S.D.*
20	10	861256	8618791	1136.5937	0.13%
	10	861020			
	10	863096			
20	30	2215203	2216458	2323.3878	0.10%
	30	2219139			
	30	2215032			
20	50	3615645	3617905	3165.7862	0.08%
	50	3616546			
	50	3621523			

(n=3)number of determination

Precision:

The precision of the method was checked by carrying out repeatability, intraday and interday precision as described in section. Result of precision studies expressed in % RSD according to ICH guidelines acceptable limit (< 2) which indicates good repeatability and low variability in inter-day.

For intraday and interday study solution at single concentrations (10µg/ml) were prepared using stock solution. The absorbance of the resulting solutions were recorded at 248 nm and the obtained data were used to calculate S.D. and %R.S.D.

Table 7: Result of Repeatability of Measurement

Repeatability				
Sr.No	Conc (ug/ml)	Peak Area	Mean ±SD*	%R.S.D.
1		1073458		
2		1092374		
3	10	1093783	1089793	0.74%
4		1092862		
5		1092742		
6		1093536		

(n=6)number of determination

Table 8: Result of Interday Precision

Intraday Precision				
Sr.No	Conc(ug/ml)	Peak area	Mean	%R.S.D.*
1	10	1019685	1010970	0.82%
2		1006372		
3		1012107		
1	10	1014045		
2		996825		
3		1016784		

(n=3)number of determination

Table 9: Result of Intraday Precision

Intraday Precision				
Sr.No	Conc(ug/ml)	Peak area	Mean	%R.S.D*
1	10	1018852	1017934	1.25%
2		1012553		
3		1013253		
1	10	1002058		
2		1020685		
3		1040198		

(n=3) number of determination

➢   **LOD and LOQ :**

Limit of detection (LOD) and limit of quantification (LOQ) of the development method were determined by dilution progressively low concentration of standard solution. It is calculated by using slope and standard deviation from linearity and precision respectively:

Limit of detection (LOD):

**LOD** = $3.3 \times$ SD / Slope

LOQ = $10 \times$ SD / Slope

Where, SD – Standard deviation

The LOD and LOQ were calculated as per the equation given in section. The values of LOD were found to be 0.956 ug/ml for TNG and the calculated LOQ values were found to be 0.171 ug/ml. The low values of LOD and LOQ indicates the sensitivity of the method.

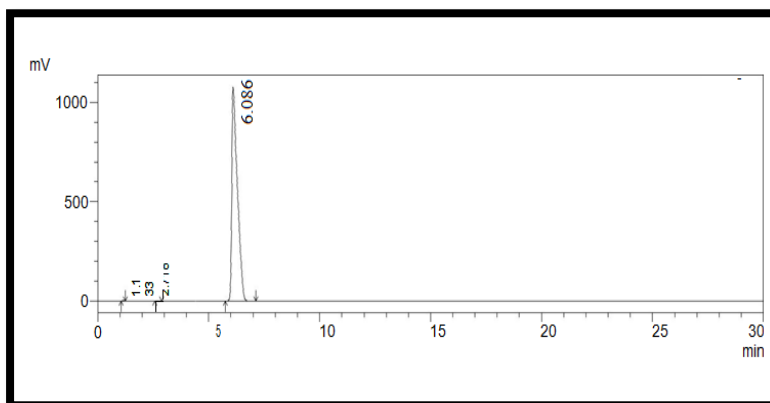❖ **Degradation Study:**

➢ **Acidic Hydrolytic Degradation:**

To 1 ml of s tock solution teneligliptin, 1 ml of 0.1N HCl was added into separate 10ml std flask and refluxed for 1 hour at 60 ^{0}C .The resultant solutions was diluted to obtain 100μg/ml solution of teneligliptin respectively with mobile phase and 10 μl solution was injected into the system and the chromatogram was recorded to assess the stability of sample.
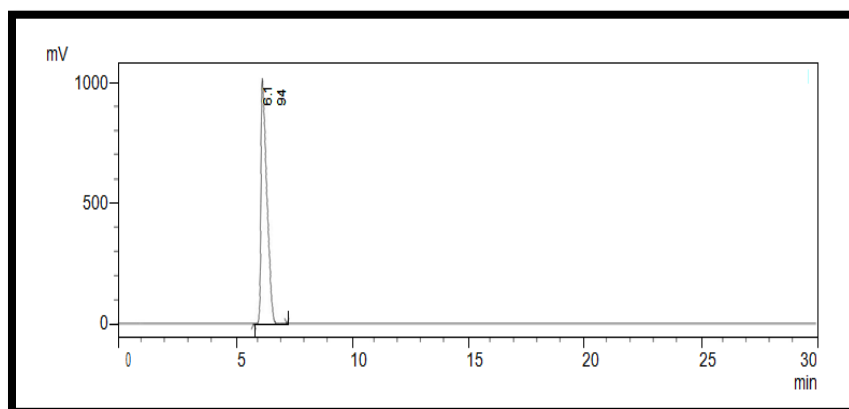
Figure 10: Chromatogram for Acidic Hydrolytic Degradation of TNG on HPLC

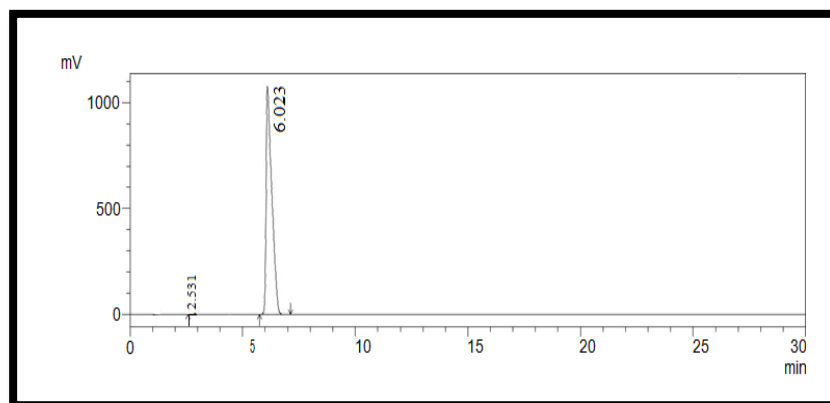➢ **Basic Hydrolytic Degradation:**

To 1 ml of s tock solution of teneligliptin, 1 ml of 0.1M NaOH was added into separate 10ml std flask and refluxed for 1 hour at 60 ^{0}C . The resultant solutions was diluted to obtain 100μg/ml solution of teneligliptin respectively with mobile phase and 10 μl solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Figure 11: Chromatogram for Basic Hydrolytic Degradation of TNG on HPLC

➢ **Oxidative Degradation:**

To 1 ml of s tock solution Teneligliptin, 1 ml of 30% H2O2 was added into separate 10ml std flask and refluxed for 12 hour at 60 ^{0}C. The resultant solutions was diluted to obtain 100μg/ml solution of teneligliptin respectively with mobile phase and 10μl solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Figure 12: Chromatogram for Oxidative Hydrolytic Degradation of TNG on HPLC

➤ **Thermal Degradation:**

To 1 ml of s tock solution teneligliptin was added into separate 10ml std flask and refluxed for 1 hour at 80 ^{0}C . The resultant solution was diluted to obtain 100μg/ml solution of teneligliptin respectively with mobile phase and 10μl solution was injected into the system and the chromatogram was recorded to assess the stability of sample
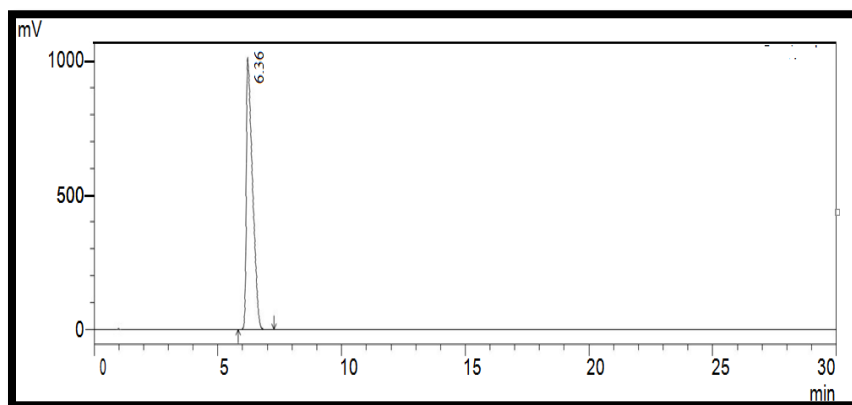
Figure 13: Chromatogram for Basic Thermal Degradation of TNG on HPLC

➤ **Photolytic Degradation:**

1mg of teneligliptine was placed in petri plate and exposed to sunlight for 1 hour and later the volume was made up to mark with methanol then aliquot portion of above solution was diluted with mobile phase as a methanol(90%) : water (10%) to get final concentration of about 100μg/mL. and 10μL of sample solutions were injected and analyzed against control samples (lacking of degradation treatment). teneligliptine is found to be degraded in Photolytic condition.
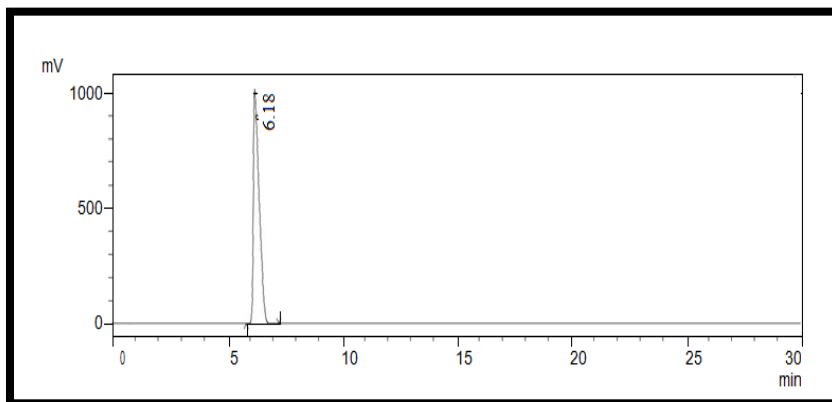
Accuracy was reported as % recovery which was calculated from the expression as equation given below,

$$\% \ Recovery = \frac{B - A}{C}$$

Where,

B = Total amount of drug estimated

A = Amount of drug found on pre-analyzed basis

Figure 14: Chromatogram for Photolytic Hydrolytic Degradation of TNG on HPLC

Table 10: Result of Recovery Studies

Sr. No	% Composition	Area of Standard	Area of Sample	%Recovery
1	80% recovery	2216028	2213693	99.89
2	100% recovery	3614446	2969700	82.16
3	120% recovery	3620740	3616075	99.87

CONCLUSION

A validity stability indicating method was achieved HPLC. The validation of the system carried out effectively indicating method to be linear, precise, accurate, specific and robust. The stability studies on the drug were carried out successfully. The drug which when subjected to thermal, photolytic, oxidative, and acidic stress degraded into many degradation products. In most of the cases, the degradation rate was seen to be directly proportional to the amount of stress applied. The thermal stress was increased by increasing the incubation temperature, the faster the degradation took place. The more the concentration of H_2O_2 faster the drug degraded. Displayed a uniform rate of degradation when acidic stress applied. It showed a very high degradation rate when photolysis using UV radiations. The areas of degraded peaks were found to be lesser than area of standard drug concentration indicating that TNG undergo degradation under all condition.

ABBREVARTIONS

HPLC-High Performance Liquid Chromatography

IUPAC – International union of pure and applied chemistry

ICH -International Council for Harmonization

RSD -Relative Standard Deviation

SD -Standard Deviation

Qty –Quantity

°C -Degree Celsius

Fig. –Figure

Qty -Quantity

% -Percentage

TNG-Teneligliptin

LOD - Limit of detection

LOQ - limit of quantification

ml – Milli liter

H_2O_2 –Hydrogen peroxide

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