WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 7, 901-918. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF STRESS DEGRADATION STUDIES FOR QUANTIFICATION OF TENELIGLIPTIN BY U V SPECTROSCOPIC METHOD

Kalyani V. Tighare[1]* and Amol V. Sawale[2]

[1]Department of Chemistry Vidyabharti College of Pharmacy, Amravati (MH) India 444-602.

[2]Asst. Professor, Department of Chemistry Vidyabharti College of Pharmacy, Amravati (MH) India 444-602.

Article Received on
28 April 2021,

Revised on 18 May 2021,
Accepted on 08 June 2021

DOI: 10.20959/wjpr20217-20784

***Corresponding Author**
Kalyani V. Tighare
Department of Chemistry Vidyabharti College of Pharmacy, Amravati (MH) India 444-602.

ABSTRACT

To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for of stress degradation studies for quantification of Teneligliptin. 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. It showed absorption maxima was determined in Methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. 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 melting point of the pure drug Teneligliptin was determined by capillary tube method. It was found that in between 209 – 211^{0}C. It showed absorption maxima 248 nm in Methanol. On the basis of absorption spectrum the working concentration was set on 10µg/ml (PPM). The linearity was observed between 2-12 µg/ml (PPM). The values of LOD were found to be 5.88 ug/ml for TNG and the calculated LOQ values were found to be 17.29 ug/ml. The low values of LOD and LOQ indicates the sensitivity of the method**.**

KEYWORDS: Teneligliptin, Validation, force degradation, Methanol, U V Spectrophotomer, Method validation.

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.

The word validation simply means "assessment of validity" or "action of proving effectiveness"

Why validation? [4,5,6]

It would not be feasible to use the equipment without knowing whether it will produce the product we wanted or not. The pharmaceutical industry uses expensive materials, sophisticated facilities & equipment and highly qualified personnel. The proficient use of these resources is necessary for the constant victory of the industry. The cost of product failures, rejects, reworks,

And recalls, complaints are the considerable parts of the total production cost. Detailed study and control of the manufacturing process validation is necessary if failure to be reduced and productivity improved. The pharmaceutical industries are concerned about validation because of the following reasons:

- Assurance of quality
- Cost reduction
- Government regulation
- To provide documentary evidence
- To monitor and control the critical process variables
- For assurance of pre-determined specifications

- For consistency in quality of product
- To fulfill cGMP requirements
- To improve manufacturability and reproducibility

Analytical methods

Analytical methods deals with the methods for determining the chemical composition of samples of matter, and it also may be defined as the science and art of determining the composition of materials in terms of elements or compounds content in them.

Methods of analysis[7,8]

- Quantitative analysis
- Qualitative analysis

Analytical method validation[9,10,11,12]

The validation of analytical method is the process in determining the suitability of a given methodology by laboratory studies; that the method can meet the requirements for intended use. Method validation is not simply a measure of procedure but method validation is a measure of performance of the total analytical system.

According to USP "Validation is the process of providing documented evidence that the method does what it is intended to do." In other words, the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use. Method validation is a regulatory requirement.

Various Guidelines describe typical analytical performance characteristics, how they are determined, and which subset of data is required to demonstrate validity, based on the methods intended use. These analytical performance characteristics are:

- Accuracy
- Precision
- Specificity
- Limit of Detection (LOD)/ Detection Limit (DL)
- Limit of Quantitation (LOQ)/ Quantitation Limit (QL)
- Linearity and range
- Ruggedness
- Robustness

Purpose of validation

- Setting the standards of evaluation procedures for checking compliance and taking remedial action.
- Economic: reduction in cost associated with process sampling and testing.
- As quality of the product cannot always be assured by routine quality control because of testing of statistically insignificant number of samples.
- Retrospective validation is useful for trend comparison of results compliance to CGMP/CGLP.
- Closure interaction with Pharmacopoeial forum to address analytical problems.
- International Conference on Harmonisation particularly in respect of impurities determination and their limits.

Spectroscopy[13,14,12,15]

It may be defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength, approximating monochromatic radiation. The electromagnetic spectrum extends from 100-780 nm and is divided into following regions on the basis of wavelength.

Table 1: Electromagnetic spectrum.

Region	Wavelength
Far (or vacuum) ultraviolet	10-200 nm
Near ultraviolet	200-400 nm
Visible	400-750 nm
Near infrared	0.75- 2.2 µm
Mid infrared	2.5-50 µm
Far infrared	50-1000 µm

Ultraviolet-Visible spectrophotometry[16,17,18.19,20]

The technique of ultraviolet-visible spectrophotometry is one of the most frequently employed in pharmaceutical analysis. It involves the measurement of amount of ultraviolet (190-180 nm) or visible (380-800 nm) radiation absorbed by a substance in solution. Instruments measure the ratio or the functions of the ratio, of the intensity of two beams of light in the ultraviolet-visible region are called ultraviolet-visible spectrophotometer. Absorption of light in both of the ultraviolet and visible region of the electromagnetic spectrum occurs when the energy of light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transition.

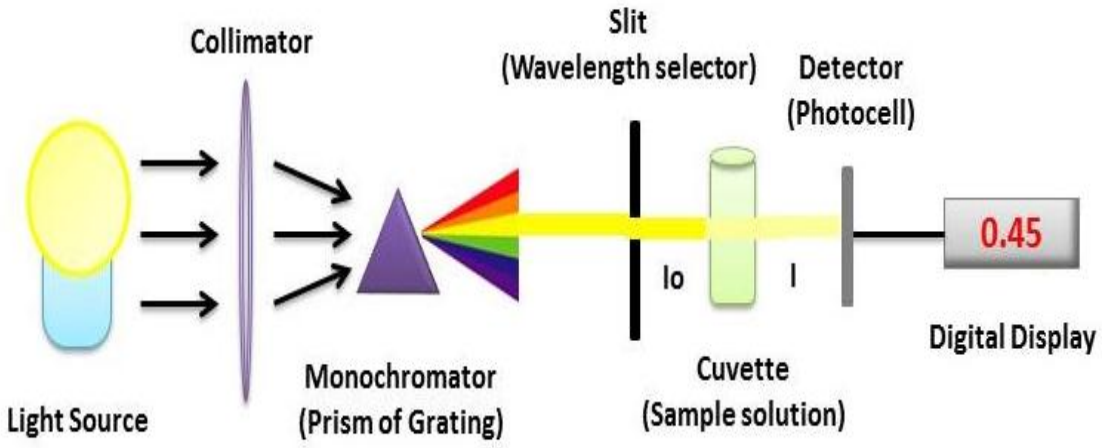


Fig. 1: Basic instrumentation of spectrophotometric.

Beer-Lambert's Law

'When a beam of monochromatic light is passed through a transparent cell containing a solution of an absorbing substance, reduction of intensity of the light may occurs, the rate of reduction in intensity with the thickness of the medium is proportional to the intensity of the light and the concentration of the absorbing substances. Mathematically it is expressed as:

A = a b c

Where A = Absorbance or optical density

a = Absorptivity or extinction coefficient

b = Path length of radiation through sample (cm)

c = Concentration of solute in solution

Regulatory basis of stability-Indicating Assays and Forced degradation studies[21,13]

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.

Development of Stability-Indicating analytical method [Siam][22]

The purpose of the stability study is to investigate how the quality of the drug product changes with time under the influence of environmental factors, to establish a shelf life for the product and to recommend storage conditions.

Forced degradation

Since the conditions that cause instability and result in degradation products of the API cannot be predicted initially, one has to subject the API to a variety of stress conditions. Trial and error are needed to find the proper combination of stress agent concentration and time to effect degradation, preferably in the 5-15 % range. Depending on the API, not every stress agent may affect degradation, but each agent has to be evaluated to determine whether degradation occurs or not.

Typical derivative conditions involve hydrolysis, photolysis, acid/base reactions, and temperature. Achieving 100 % degradation would require too much effort and could be possibly cause secondary degradation. Secondary degradation products are the degradation products of the degradation products, which are not likely to be formed under normal storage conditions.

Depending on the API, not all of the degradation conditions effect degradation, and after a reasonable effort (varying concentrations and time) to produce a degradation product with no success, one can move on to the next condition.

Acidic/Basic hydrolytic degradation

The hydrolytic degradation of a new drug in acidic and alkaline conditions can be studied by refluxing the drug in 0.1 N HCl/NaOH for 8 hrs. If reasonable degradation is seen, testing can be stopped at that point.

However, in case no degradation is seen under these conditions, the drug should be refluxed in acid/alkali of higher strengths and for longer duration. Alternatively, if total degradation is seen after subjecting the drug to initial conditions, acid/alkali strength can be decreased along with decrease in the reaction temperature.

Oxidative degradation

To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range 3-30 % and duration can be from 2 hr to 24 hr.

Photolytic degradation

It should be carried out by exposure to light using either a combination of cool white and ultraviolet fluorescent lamps. In case still no decomposition takes place, the drug can be declared photo stable.

Objective of forced degradation studies

Forced degradation studies are carried out to achieve the following purposes

- To establish degradation pathways of drug substances and drug products.
- To differentiate degradation products that are related to drug products from those that are generated from non-drug product in a formulation.
- To elucidate the structure of degradation products.
- To determine the intrinsic stability of a drug substance in formulation.
- To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product
- To establish stability indicating nature of a developed method.
- To understand the chemical properties of drug molecules.
- To generate more stable formulations.
- To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
- To solve stability-related problems

Development of the analytical method

It is generally experienced that a method can be a Stability-indicating Analytical Method if it is probably based on the approach of forced degradation, with the intention of using the developed method for stability assessment as a final application, after the method has been validated. This approach entails determining the discriminating ability of the selected method upfront before investing time and money in evaluating other analytical parameters prior to assessing the stability-indicating element of the method. So method development experiments are performed on samples arising from the forced degradation studies.

MATERIALS

Table 3: Active drug.

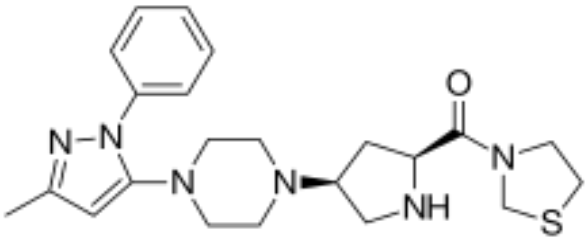
Name of drug	**Tenegliptin:** (tng) is a novel drug, which is used for the treatment of type 2 diabetes mellitus. It is an anti- diabetic drug that belongs to dipeptidyl peptidase-4 inhibitors or "gliptins".
Structure	
Iupac name	{(2s,4s)-4-[4-(3-methyl-1-phenyl-1h-pyrazol-5-yl)-1-piperazinyl]-2-pyrrolidinyl}(1,3-thiazolidin-3-yl)methanone
Molecular formula	$C_{22}h_{30}n_6os$
Molecular mass	426.58 g/mol
Category	Anti diabetic
Solubility	Water, methanol, dmso.
Melting point	209 ^{o} - 211°c
Half life	24.2 hours
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.
Mode of excretion	About 34.4% of teneligliptin is excreted unchanged via kidney and the remaining 65.6% teneligliptin is metabolized and eliminated via renal and hepatic excretion.
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.
Adverse effect	Teneligliptin may cause some adverse effects. Such as, 908ypoglycaemia, constipation, nausea, loss of appetite, diarrhea, abdominal pain and abdominal discomfort.
Uses	Teneligliptin reduces blood glucose levels in patients with type ii diabetes mellitus.

Table 4: Solvents and Chemicals.

Sr. No.	Solvents and Chemicals	Name of Company
1.	Methanol	E. Merck Ltd., Mumbai, India
2.	Water	E. Merck Ltd., Mumbai, India

Table 5: Marketed formulation.

Sr. no.	Marketed formulation	Manufacturer
1.	ZITEN (Teneligliptin 20 mg tablet)	Glenmark Pharmaceuticals Ltd. Mumbai, Maharashtra.

Table 6: Equipments.

Sr. no	Equipment	Make/ Model
1	Precision balance	Mettler Tolledo
2	pH meter	Labinda
3	Grade 'A' certified Glassware	Borosil
4	Ultrasonicator	FAST CLEAN Ultrasonic Cleaner
5	Electronic balance	Electrolex New
6	UV Spectrophotometer	Shimadzu UV 1800, Corporation Japan

RESULT AND DISCUSSION

Development of UV-Spectrophotometric method

Selection of solvent

Drug shows solubility in methanol. The spectra of teneligliptin show the feasibility of using methanol for spectrophotometric analysis for this drug.

Preparation of standard stock solution: (1000 µg/ml)

A 10mg of standard teneligliptin was weighed and transferred to 100ml volumetric flask and dissolved in 50ml methanol. The flask was shaken and volume was made up to the mark with methanol. From this stock solution further 10ml was transferred in 100ml volumetric flask and diluent was added up to mark to give a solution containing 100µg/ml teneligliptin

Determination of absorption maxima

Selection of wavelength: 2, 4, 6, 8, 10, and 12 µg/ml solution of teneligliptin was prepared in diluent and spectrum was recorded between 200-400nm. The overlain spectrum of teneligliptin at different concentration was recorded and peak maxima of drug were found. The peak maximum of Teneligliptin was 248nm. The spectrum of teneligliptin at target concentration was recorded.

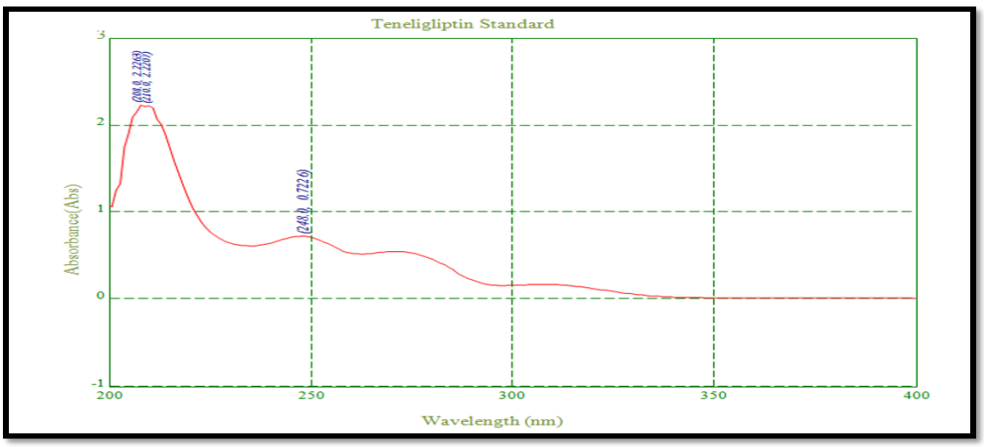


Fig. 2: Spectra of standard teneligliptin with solvent (10µg/ml).

The tablet of the teneligliptin shows maximum absorption at 248 nm.

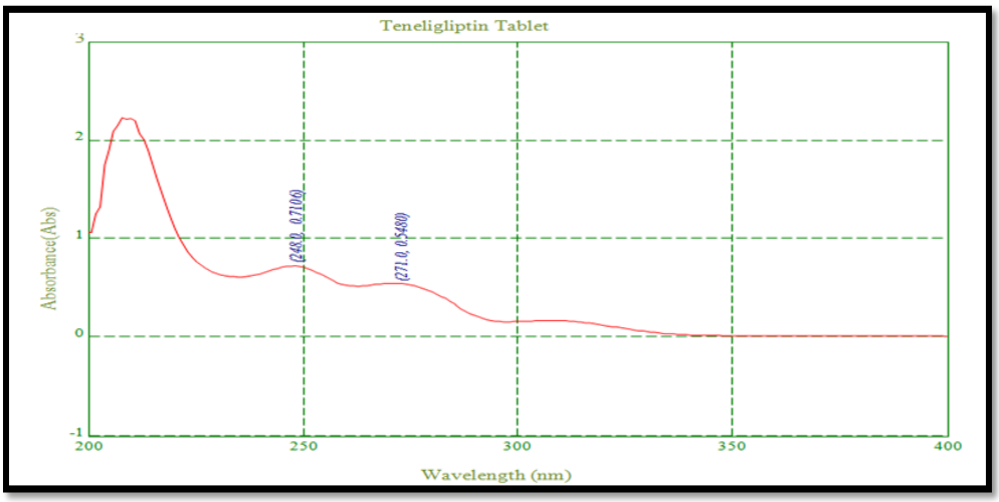


Fig. 3: Spectra of tablet teneligliptin with solvent (10µg/ml).

Overlay spectra of standard teneligliptine and tablet at 248 nm.

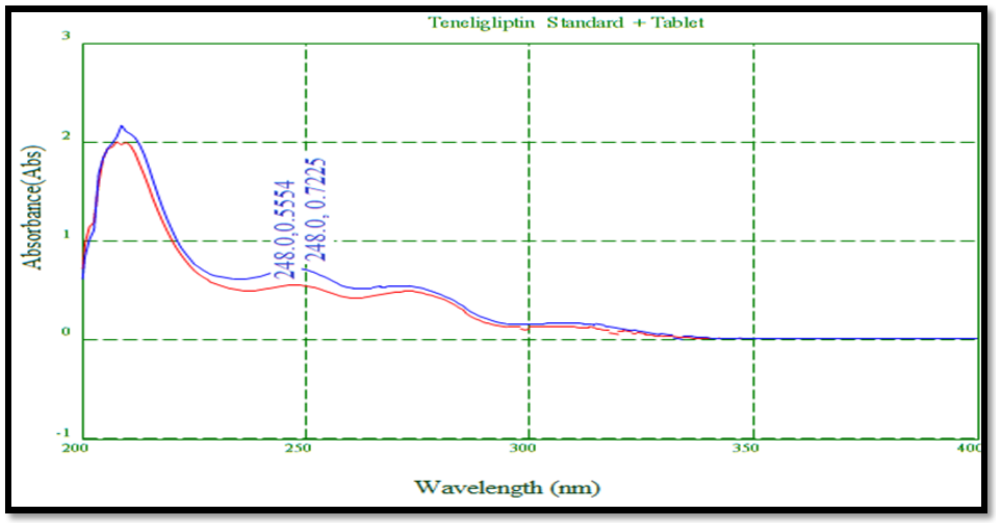


Fig. 4: Overlay spectra of standard Teneligiptin and Tablet with solvent (10µg/ml).

Calibration curve for teneligliptin

Appropriate volume of aliquots from standard Teneligliptin stock solution was transferred to different volumetric flasks. The volume was adjusted to the mark with the diluent to obtain the concentration of 2, 4, 6, 8, 10, and 12 µg/ml.

Calibration curve of each solution against the diluent was recorded at 248 nm was measured and the plot of absorbance v/s concentration was plotted. The absorptivity of drug at wavelength 248 nm was found by straight-line equation. It shows straight line meaning the calibration curve obeys Beers law.

❖ **Method validation parameter of UV**

➢ **Linearity and Range**

For linearity study, six solutions at different concentrations (2, 4, 6, 8, 10, and 12µg/ml) were prepared using six different aliquots of stock solution. The absorbance of the resulting solutions was recorded at 248 nm and the obtained data were used to plot the graph of linearity.

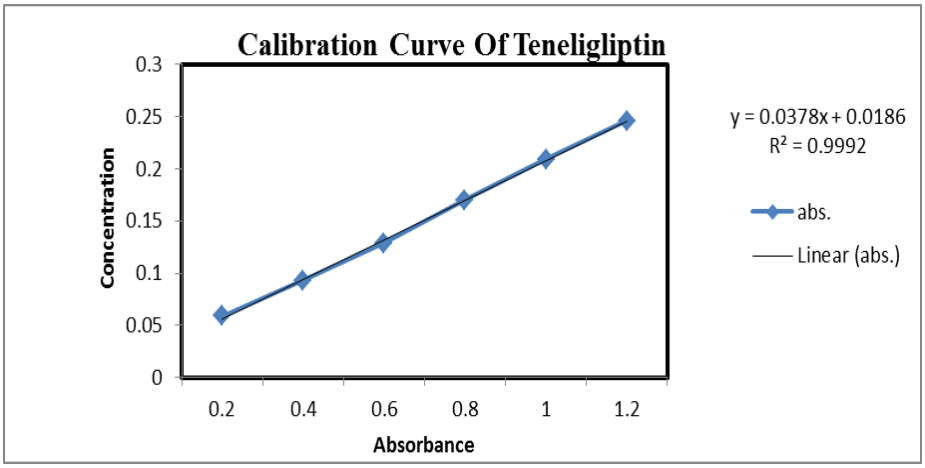


Fig. 5: Calibration curve for teneligliptin.

➢ **Accuracy**

a) Preparation of standard solution

The standard solution was prepared as per marketed formulation.

b) Preparation of sample solution

The accuracy of the analytical method for teneligliptin was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 248 nm and results were expressed in terms of % recoveries. Standard deviation and % RSD was calculated.An accurately weighed tablet powder equivalent to 10 mg of teneligliptin was transferred in series of 10.0 ml volumetric flasks and then known amount of teneligliptin was added over the range of 80%,100%, 120%. The content in the flask were shaken for 10-15 mins with methanol and volume was adjusted up to mark with same. The solutions were then filtered through Whatmann No.1 filter paper and were used as sample. Accurately measured 1.0 ml portion of filtrate was transferred to a 10.0 ml volumetric flask further diluted upto the mark with methanol to obtained final concentration of 10µg/ml. Accuracy was reported as % recovery which was calculated from the expression as equation given below,

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

Where,

B = Total amount of drug estimated

A = Amount of drug found on pre-analyzed basis

Table 7: The result of accuracy studies.

Level (%)	Formulation (µg/ml)	Added pure drug (µg/ml)	Amount recovery (µg/ml)	% Recovery	Mean	%R.S.D.
80%	8	8	15.85	99.06	99.24	0.26
80%	8	8	15.91	99.43		
100%	10	10	19.75	98.75	98.42	0.46
100%	10	10	19.62	98.10		
120%	12	12	23.91	99.62	99.08	0.77
120%	12	12	23.65	98.54		

- **Precision**

The precision of the method was checked by carrying out repeatability, intraday and interday precision. 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 the six solutions at different concentrations (2, 4, 6, 8, 10, and 12µg/ml) were prepared using six different aliquots of 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.

Intraday precision study

Table 8: The result of intraday precision study.

Sr. no.	Conc. (µg/ml)	Absorbance			Mean	S.D.	%R.S.D.
		Morning	Afternoon	Evening			
1	2	0.085	0.087	0.086	0.086	0.0010	1.162
2	4	0.093	0.093	0.091	0.093	0.0011	1.250
3	6	0.129	0.133	0.131	0.131	0.0020	1.526
4	8	0.170	0.171	0.169	0.170	0.0010	0.588
5	10	0.209	0.209	0.210	0.209	0.0005	0.275
6	12	0.247	0.248	0.245	0.246	0.0015	0.619
Average					0.155	0.0012	0.903

**(n=6)number of determination*

Interday precision study

Table 9: The result of interday precision study.

Sr. no.	Conc. (µg/ml)	Absorbance			Mean	S.D.	%R.S.D.
		Day 1	Day 2	Day 3			
1	2	0.093	0.095	0.095	0.094	0.0011	1.224
2	4	0.139	0.141	0.143	0.141	0.0020	1.418
3	6	0.169	0.172	0.173	0.171	0.0021	1.214
4	8	0.209	0.21	0.211	0.210	0.0010	0.476
5	10	0.221	0.224	0.225	0.223	0.0020	0.932
6	12	0.232	0.235	0.234	0.233	0.0015	0.653
Average					0.178	0.0016	0.986

**(n=6) number of determination*

- **Roubtness**

Sample solution of teneligliptin were prepared from stock solution and analyzed by two different analysts using similar operational and environmental conditions.

Table 10: The result of roubtness study.

Sr. no.	Analyst	Absorbance	Mean	%R.S.D.
1.	Analyst –I	0.702	0.703	1.14%
		0.696		
		0.712		
2.	Analyst -II	0.693	0.698	1.70%
		0.690		
		0.712		

**(n=2)number of determination*

- **Limit of detection (LOD) and Limit of quantification (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 × SD / Slope

LOQ = 10 × 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 5.88 ug/ml for TNG and the calculated LOQ values were found to be 17.29ug/ml. The low values of LOD and LOQ indicates the sensitivity of the method**.**

❖ **Degradation study**

Acidic hydrolytic degradation: To 1 ml of stock solution teneligliptin, 1 ml of 0.1N HCl was added into separate 10ml std flask and refluxed for 30mins at 60^0 C. The resultant solutions was diluted to obtain 100µg/ml solution of teneligliptin and then scan over a range of 400-200 nm by UV- Spectrophotometer.

Table 11: Degradation study.

Sr. no.	Stress Condition	Optimum Working Condition	Time
1	Acid Hydrolysis	1mg/ml in 1N HCL at room temp.	1hour
2	Base Hydrolysis	1mg/ml in 1N NaOH at room temp.	1hour
3	Oxidative Degradation	3% H_2O_2 at room temp. protected from light (dark solution)	12hour
4	Photolytic Degradation	Bulk sample exposed to sunlight for 24 hrs(6 hrs per day in petridish)	1hour
5	Thermal Degradation	Drug sample Placed in oven at 60^0c	1 hour

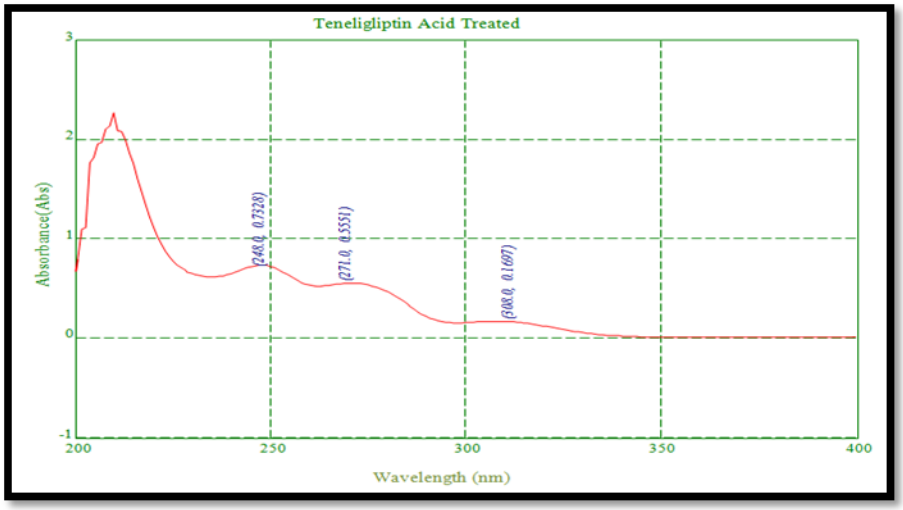


Fig. 6: Acidic hydrolytic degradation spectrum for UV of TNG.

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 30mins at 60^0 C. The resultant solutions was diluted to obtain 100µg/ml solution of teneligliptin and then scan over a range of 400-200 nm by UV- Spectrophotometer.

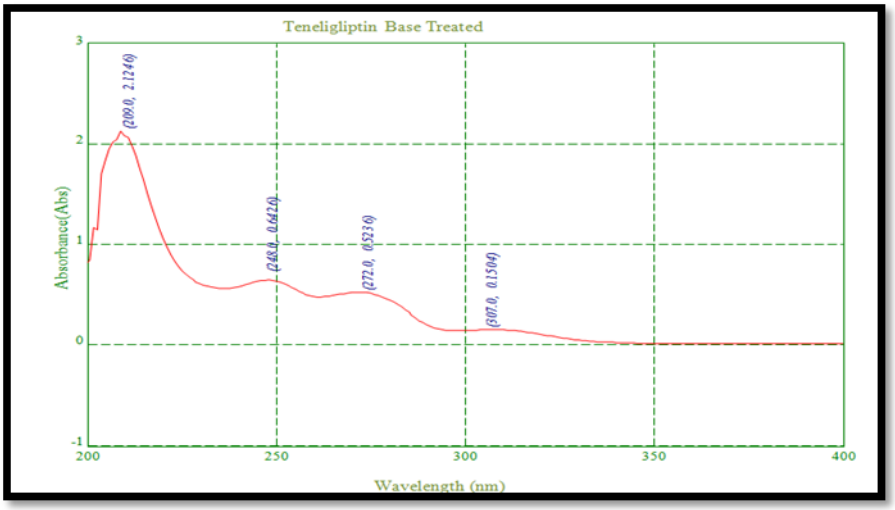


Fig. 7: Basic hydrolytic degradation spectrum for UV of TNG.

Photolytic degradation: A sample powder of Teneligliptine (1 mg) was placed in petri plate and exposed to sunlight for 30 min. Then the drug sample was transferred to 10 ml volumetric flask and diluted upto distilled water to get a conc.100µg/mL and then scan over a range of 400-200 nm.

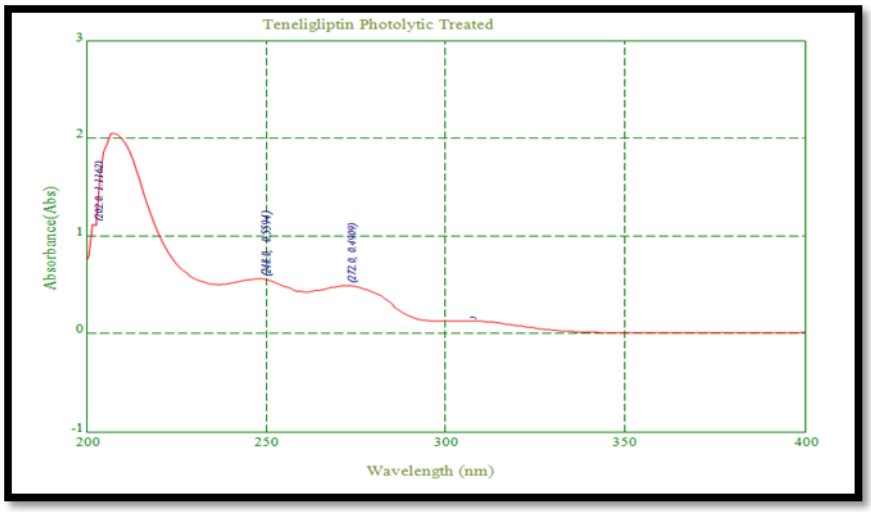


Fig. 8: Photolytic degradation spectrum for UV of TNG.

8.4.4 Oxidative degradation: To 1 ml of s tock solution teneligliptin, 1 ml of 30% H_2O_2 was added into separate 10ml std flask and refluxed for 30mins at 60 ^{0}C. The resultant solutions was diluted to obtain 100µg/ml solution of teneligliptin and then scan over a range of 400-200 nm by UV- Spectrophotometer.

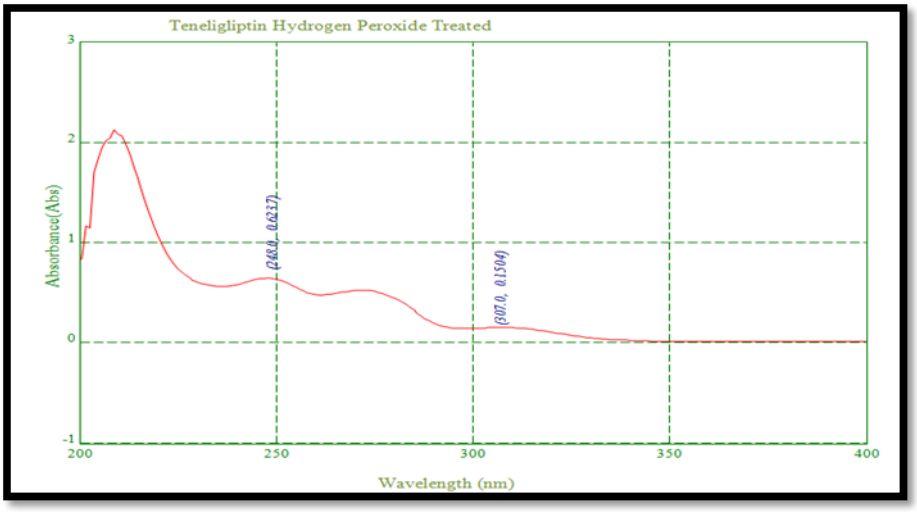


Fig. 9: Oxidative degradation spectrum for UV of TNG.

Thermal degradation: To 1 ml of s tock solution teneligliptin was added into separate 10ml std flask and refluxed for 6hrs at 80 ^{0}C. The resultant solution was diluted to obtain 100µg/ml solution of teneligliptin and then scan over a range of 400-200 nm by UV- Spectrophotometer

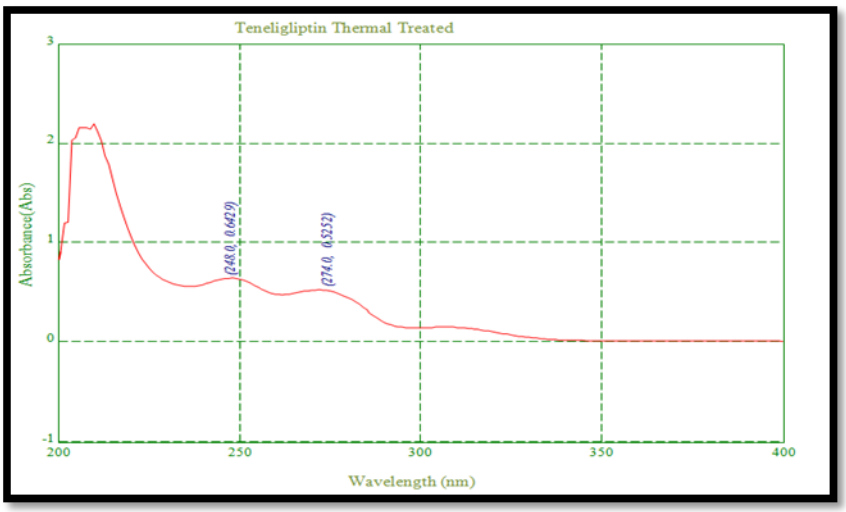


Fig. 10: Thermal degradation spectrum for UV of TNG.

CONCLUSION

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

PPM -Parts per Million

UV -Ultra violet

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

CGMP – current Good Manufacturing Process

CGLP - current Good Laboratory practice

REFERENCE

1. Potdar, M. A., Pharmaceutical Quality Assurance, Niraliprakashan, Pune, 2006; 8(1) 8.28-8.30.
2. Kasture A. V., Wadodkar S. G., Mahadik K. R., and More H. N., Textbook of Pharmaceutical Analysis – II, 11th Edition, Published By Nirali Prakashan, 1996; 1: 156-165.
3. Beckett A. H., Stenlake J. B.; Practical Pharmaceutical Chemistry, 4th Edition, Part II, CBS Publications and Distributors, New Delhi, 1997; 1: 275-300.
4. Remington's Pharmaceutical Sciences Mack Publishing Company, Pennsylvania, 1990; 18: 1513-1519.

5. Sharma J. Encyclopedia of Pharmaceutical Technology, Marcel Dekker, Inc. USA, 2001; 2: 252-254.
6. Sharma B. K.; Instrumental Method of chemical Analysis, Goel Publishing Housing, Krishna Prakashan Ltd., 2002; 21: 3.
7. Beckett A. H., Stenlake J. B.; Practical Pharmaceutical Chemistry, CBS Publications and Distributors, New Delhi, 1997; 1: 275-300.
8. Chapman, K A History of Validation in the United States. Pharm Techno Validation Comput Relat Syst, 1991; 15: 10-12.
9. Remington's Pharmaceutical Sciences; Mack Publishing Company, Pennsylvania, 1990; 18: 1513-1519.
10. Sharma B. K.; Instrumental Method of chemical Analysis, Goel Publishing Housing, Krishna Prakashan Ltd, 2002; 3: 21.
11. Skoog D. A., Leqary J. J.; Principle of Instrumental Analysis, Thomson Asia Ptd. Ltd; Singapore, 1992; 54: 580.
12. Skoog D. A., Leqary J. J.; Principle of Instrumental Analysis, 54th Edition, Thomson Asia Ptd. Ltd; Singapore, 1992; 580.
13. Beckett A. H., Stenlake J. B.; Practical Pharmaceutical Chemistry, 4th Edition, Part II, CBS Publications and Distributors, New Delhi, 1997; 1: 275-300.
14. Health Canada. Validation Guidelines for Pharmaceutical Dosage Forms, 2009.
15. Lee JY. Recent trends in Process validation. In: Berry IR, Nash RA, editors, Pharmaceutical Process Validation. 2nd edition, New York, Marcel Dekker; 1993; 582-583.
16. Maynard, DW Validation of Critical Utilities. In: Agalloco J, Carleton FJ. Validation of Pharmaceutical Processes. New York: Informa Healthcare, 2008; 51: 59.
17. GHTF study groups 3. Quality management systems – *Process validation guidance*. January, 2004; 21: 14-19.
18. Cazes, J., *Encyclopedia of Chromatography*, 2001; 691,693,695,696.
19. *The Merck Index*, Merck and company, 2001; 13: 67-777.
20. Kar, A., Pharmaceutical Drug Analysis, 2001; 570, 571,574,575,578.
21. J. M. Green, A practical guide to analytical method validation, Anal.Chem. News & Features, May 1, 1996; 305A/309A.
22. ICH/CPMP Guidelines Q2B, Validation of Analytical Procedures-Methodology, 1996.