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Development and Validation of Stability Indicating RP-HPLC method for Teneligliptin Hydrobromide Hydrate

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ABSTRACT

Teneligliptin hydrobromide hydrate is a new FDA approved drug for treatment of Diabetes Mellitus. Very few methods have been reported for its identified degradation products and their effects on human. A simple, rapid, precise and accurate stability indicating RP-HPLC method was developed and validated for identification of Teneligliptin hydrobromide hydrate and its degradants on Kromacil C18 column using pH 5.5 phosphate buffer and methanol (75:25v/v) as a mobile phase in isocratic mode of elution at a flow rate 1.2 ml/min. The column effluents were monitored by a variable wavelength UV detector at 270 nm. The method was validated as per ICH guidelines. Forced degradation studies of Teneligliptin hydrobromide hydrate were carried out under acidic, basic, neutral, peroxide, photo and thermal conditions. Degradation was observed in basic and neutral stress samples, but not in acid, peroxide, photo and thermal stress samples.

Keyword: Teneligliptin hydrobromide hydrate, RP-HPLC, validation, stability, degradation

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INTRODUCTION

Teneligliptin hydrobromide hydrate is a novel drug, which is used for the treatment of type 2 diabetes mellitus. It is an antidiabetic drug that belongs to dipeptidyl peptidase-4 inhibitors or "gliptins"[1]. Chemically, it is {(2S, 4S)-4- [4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]-pyrrolidinyl} (1, 3-thiazolidin-3-yl) methanone (Figure 1). Teneligliptin exerts its activity for 24 hrs with elevation of activated glucagon-like peptide 1 (GLP-1) levels by suppressing postprandial hyperglycemia after the meals[2,3]. Significant decrease in hemoglobin A1c (HbA1c), fasting blood glucose, and postprandial blood glucose levels were observed in type 2 diabetic patients taking teneligliptin hydrobromide hydrate for 12 weeks[2]. This drug showed a promising effect in stabilizing the glycemic fluctuations throughout the day and suppressing the diabetic complications[3]. Teneligliptin hydrobromide hydrate is approved for use in India, Japan, and Korea in 2012. Although the drug entered the market, there is no much information available about its degradation studies and its degraded products. Few methods have been reported for its metabolism and pharmacokinetic studies[4,5,6,7]. Identification of the degraded products helps in future metabolic studies and also related impurity determination during its bulk synthesis. In the present study, mainly focused on development and validation of a RP-HPLC method for identifying the teneligliptin hydrobromide hydrate and its degradation products formed during various forced conditions as per the ICH guidelines[8].

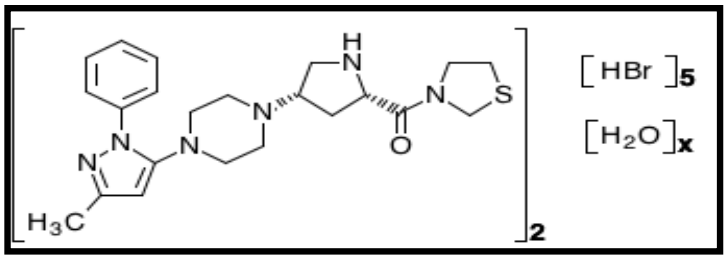


Figure 1: Structure of Teneligliptin Hydrobromide Hydrate

MATERIALS AND METHOD

Materials and reagents:

Teneligliptin hydrobromide hydrate was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad. The chemicals used in study like methanol, water (HPLC grade), potassium dihydrogen o-phosphate and sodium hydroxide were obtained from Loba Chemicals Ltd. Mumbai, India. Teneligliptin hydrobromide hydrate tablets were obtained from Glenmark Pharmaceuticals Ltd. Mumbai, India.

Instrument:

The analysis of drug was carried out on a peak HPLC system equipped with a reverse phase C18 column, peak pump with auto sample and a detector running on peak software.

Chromatographic conditions:

Chromatographic separation was achieved on Kromasil 100-5-C18 using a mobile phase consisting of a mixture of pH 5.5 phosphate buffer and methanol (75:25 v/v) under isocratic mode of elution. The mobile phase was prepared and filtered through whatmann filter paper 42 and sonicated for 30 min prior to use. Separation was performed using 1.2 mL/min flow rate at room temperature, and the run time was 11.5 min. The injection volume was 20 µL and the detection wavelength set at 270 nm.

Determination of maximum absorbance:

The standard solution of Teneligliptin hydrobromide hydrate was scanned in the range of 200-400 nm against mobile phase as blank. Teneligliptin hydrobromide hydrate showed maximum absorbance at 270 nm. Thus, the wavelength selected for the determination of Teneligliptin hydrobromide hydrate was 270 nm.

Preparation of stock and standard solution:

An accurately weighed quantity of 50 mg Teneligliptin hydrobromide hydrate was dissolved in methanol in 50 ml volumetric flask and volume was made upto the mark, to get final concentration 1000 µg/ml. The 1.0 ml portion of standard stock solution of Teneligliptin hydrobromide hydrate was diluted up to 100 ml with mobile phase to get final concentration of 10µg/ml.

Assay of Teneligliptin hydrobromide hydrate:

Ten tablets of Teneligliptin hydrobromide hydrate were weighed and average weight of a single tablet was calculated. Tablets were crushed and mixed using a mortar and pestle. Then drug sample equivalent to 100 mg of Teneligliptin hydrobromide hydrate is accurately weighed and transferred into the 100 ml volumetric flask and mixed with known amount of methanol and mixing followed by ultrasonication and then filter through a whatmann filter paper 42. The drug sample was diluted by adding methanol to obtain a stock solution of 100 µg/ml.

Validation

System suitability:

The system suitability was determined by six injection of Teneligliptin hydrobromide hydrate (100 µg/ml). The developed method was found to be suitable for use as the tailing factor and peak resolution for Teneligliptin hydrobromide hydrate were within the limits.

Linearity:

Linearity Teneligliptin hydrobromide hydrate was studied from the standard concentration ranging from 80 to 120 µg/ml. The calibration curve of peak intensity versus concentration was plotted, and correlation coefficient and regression line equation were determined.

Accuracy:

The recovery of the method was determined by adding a known amount of the drug standard concentration. The recovery was performed at three levels of 80, 100 & 120 % of Teneligliptin hydrobromide hydrate standard concentration. The three samples were prepared for each recovery levels & recoveries were calculated.

Precision:

The precision of the method was determined by six (n=6) injections of Teneligliptin hydrobromide hydrate (100 µg/ml) and the % RSD of peak area were calculated. The obtained RSD was within the range (≤2).

Ruggedness:

The degree of reproducibility obtained by analyzing the same under variety of normal test conditions such as different days, different analyst etc. Comparison of reproducibility of test result to the precision of assay is the direct measure of ruggedness of the method.

Stress degradation study:

Stress degradation study was carried out on the drug in order to check the stability of the drug by providing various stress conditions like light, heat, acid, base and oxidation compared against blank solution stored under normal condition. The purpose of stability indicating assay method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products.

Hydrolytic study:

Hydrolytic study under acidic basic condition involves catalyzation of ionisable functional groups present in the molecule. HCl and N_aOH are employed for generating acidic and basic stress samples, respectively.

Acid Hydrolysis

Acid induced degradation was carried on drug sample by studying the chromatograms of solution prepared 0, 2, 5, 8, hours. Accurately weighted bulk drug 50 mg was dissolved in 50 ml mobile phase in volumetric flask. The 5 ml of this solution was diluted with 50 ml of 0.1 N HCL in 250 ml dry round bottom flask. The reaction mixture was refluxed for 8 hrs on the water bath. The samples were withdrawn at 0, 2, 5, 8 hrs interval and further diluted with mobile phase to get concentration of 10 ug/ml.

Alkali hydrolysis

To study the base hydrolysis, the drug samples were prepared at 0, 2, 5, 8 hours. The preparation of base hydrolyzed sample involves, accurately weighted bulk drug 50 mg was dissolved in 50 ml mobile phase in volumetric flask. The 5 ml of this solution was diluted with 50 ml of 0.1 N NaOH in 250 ml dry round bottom flask. The reaction mixture was refluxed for 8 hr. on the water bath. The samples were withdrawn at 0, 2, 5, 8 hrs interval and further diluted with mobile phase to get concentration of 10 ug/ml.

Neutral hydrolysis

To study the neutral hydrolysis, the drug samples were prepared at 0, 2, 5, 8 hours. The preparation of base hydrolyzed sample involves, accurately weighed quantity of bulk drug 50 mg was dissolved in 50 ml mobile phase in volumetric flask. The 5 ml of this solution was diluted with 50 ml water in 250 ml dry round bottom flask (RBF). The reaction mixture was refluxed for 8 hr on the water bath. The samples were withdrawn at 0, 2, 5, 8 hrs interval and further diluted with mobile phase to get concentration of 10 µg/ml.

Oxidation studies:

Many drug substances undergo autoxidation i.e. oxidation under normal storage condition and involving ground state elemental oxygen. Therefore it is an important pathway of many drugs. Autoxidation is free radical reaction that requires free radical initiator to begin that chain reaction. Oxidative degradation was performed using hydrogen peroxide.

Accurately weighed 50 mg of bulk drug was dissolved in 50 ml of mobile phase in volumetric flask. The 5 ml of this solution was diluted with 50 ml of 3 % hydrogen peroxide solution in 50 ml volumetric flask. The reaction mixture of teneligliptin hydrobromide hydrate bulk drug was kept for 1 and 6 hrs interval and further diluted to get concentration of 10µg /ml.

Photolytic Degradation (UV Light):

Photolytic degradation is carried out by exposing the drug substance in UV light. To study the photolytic degradation of drug sample, the sufficient amount of bulk drug was spread on the petri plates, as a sample. The petri plate was placed inside the photo stability chamber under UV light exposure as per ICH guideline for 15 days. The samples were withdrawn at 0, 5^{th} and 15^{th} day of the study for analysis and further diluted to get concentration of 10µg/ml.

Thermal stability studies (Dry heat):

In general, rate of reaction increases with increase in temperature. Hence, the drugs are susceptible to degradation at higher temperature. Thermal degradation study is carried out at 40-80ºC.

In order to study the thermal degradation, drug samples were placed in an oven for 3 months at 50^0C and then sample of 10 µg/ml solution was prepared and the peak area of chromatogram were read out.

RESULTS AND DISCUSSION

Table 1: System suitability test

Sr. No.	Peak area	Retention time	Capacity factor	No. of Theoretical plates	Asymmetry
1	111457	2.517	0.0416	5997.15	1.30
2	111471	2.51	0.0438	5964.59	1.33
3	111465	2.53	0.0513	6060.02	1.34
4	111423	2.53	0.0513	6060.02	1.34
5	111440	2.51	0.0438	5964.59	1.51
Mean	111451.2	2.5194	0.04636	6009.42	1.364
±S.D.	18.41467	0.010089	4.59815	48.0648	8.3246
R.S.D.	0.001652	0.004004	99.1832	0.00799	6.103
C.V.	0.016522	0.040045	9918.35	0.79982	610.30

Linearity

Table 2: Observations of linearity study

Sr. No.	Concentration in ug/ml	Peak area of Teneligliptin
1	0	0
2	80	74051
3	90	802134
4	100	867900
5	110	967870
6	120	1032404

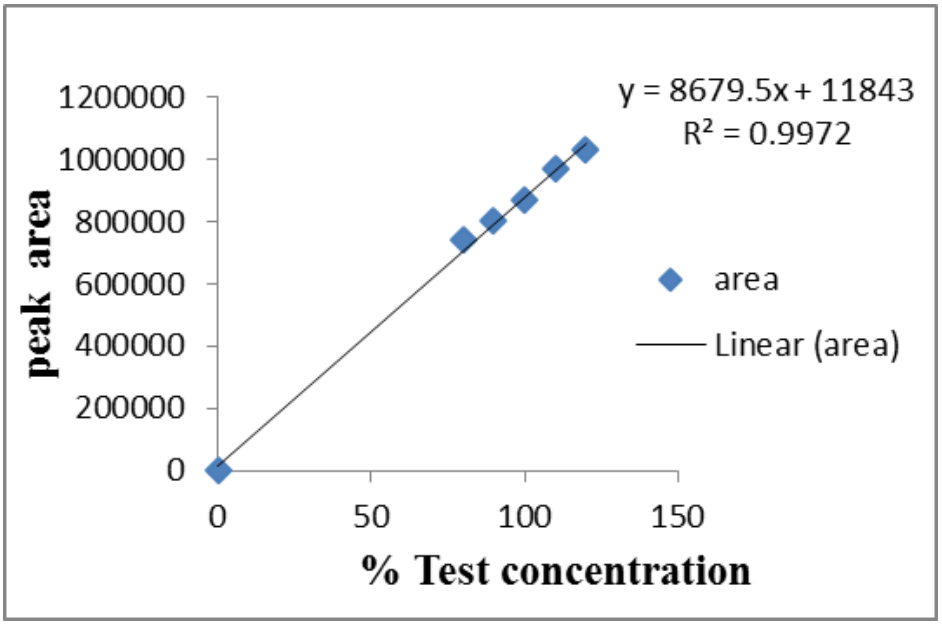


Figure 2: Plot of linearity and range for teneligliptin hydrobromide hydrate

Accuracy

Table 3: Observations of Accuracy study

Sr. No.	Level of recovery	Weight of drug taken (mg)	% amount of drug found on preanalysed basis	Amount of pure drug added (mg)	Peak area	% Recovery
1.	80%	20 mg	99.51	16 mg	1696919	99.90
2.	100%	20 mg	99.41	20mg	1870546	98.30
3.	120%	20 mg	99.93	24 mg	2080513	100.45
					Mean	99.5
					± S.D.	1.1169
					R.S.D	0.011
					C.V.	1.121

Ruggedness

Table 4: Intraday study

Sr. No.	Drug	Hours	Weight taken (mg)	Peak area	% estimation
1	Standard	-	20 mg	944437	-
2	Sample	0	20 mg	931230	98.60
		3	20 mg	929230	98.38
		6	20 mg	928230	98.28
				Mean	98.42
				±S.D.	0.163
				R.S.D.	0.0016
				C.V.	0.165

Table 5: Intraday (different days) study

Sr. No.	Drug	Day	Weight taken (mg)	Peak area	% estimation
1	Standard	-	20 mg	944437	-
2	Sample	1st	20 mg	931233	98.74
		2nd	20 mg	930233	98.64
		3rd	20 mg	941233	99.80
				Mean	99.39
				±S.D.	0.6423
				R.S.D.	0.0064
				C.V.	0.643

Table 6: Different analyst study

Sr. No.	Drug	Hours	Weight taken (mg)	Peak area	% estimation
1.	Standard	0	20 mg	944437	-
2.	Analyst	1	20 mg	931566	98.63
		2	20 mg	935170	99.01
				Mean	98.82
				±S.D.	0.2687
				R.S.D.	0.00271
				C.V.	0.2719

Stability indicating study:

RP-HPLC study of samples obtained on stress testing of Teneligliptin hydrobromide hydrate under different conditions using mixture of phosphate buffer and methanol in the ratio (75:25v/v) as a mobile phase solvent system suggested the following degradation behavior.

Acidic hydrolysis:

HPLC Report

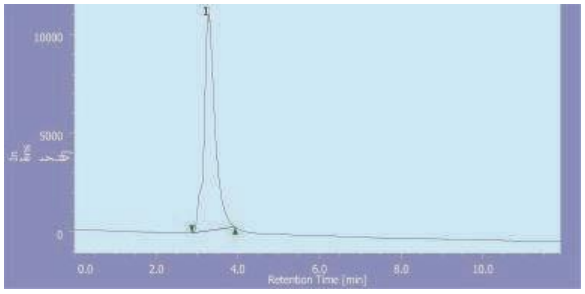


Figure 3: Chromatogram after acid hydrolysis (0 hr)

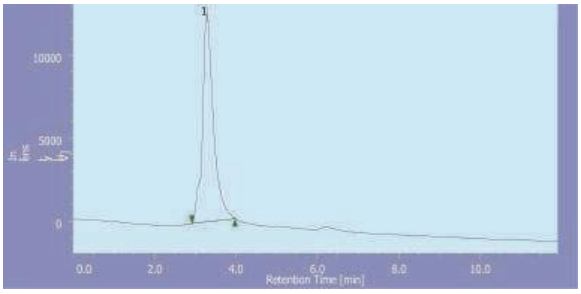


Figure 4: Chromatogram after acid hydrolysis (2 hr)

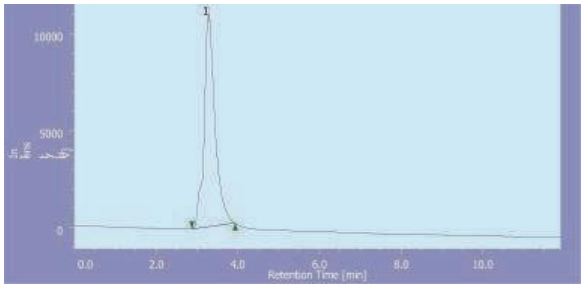


Figure 5: Chromatogram after acid hydrolysis (5 hr)

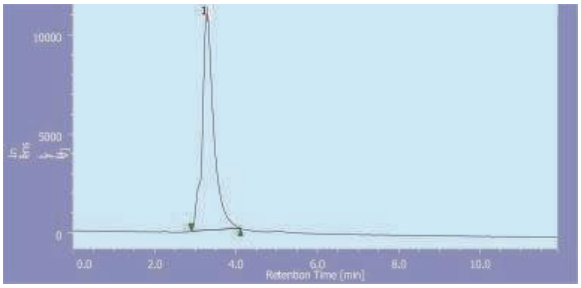


Figure 6: Chromatogram after acid hydrolysis (8 hr)

Alkali hydrolysis:

HPLC Report

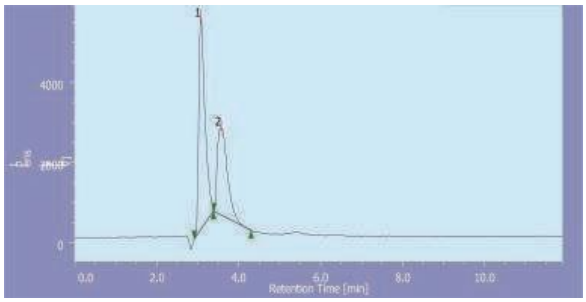


Figure 7: Chromatogram after alkali hydrolysis (0 hr)

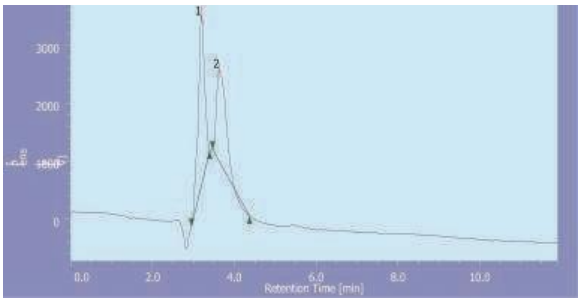


Figure 8: Chromatogram after alkali hydrolysis (2 hr)

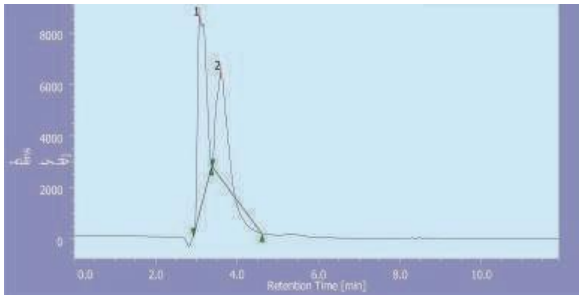


Figure 9: Chromatogram after alkali hydrolysis (5 hr)

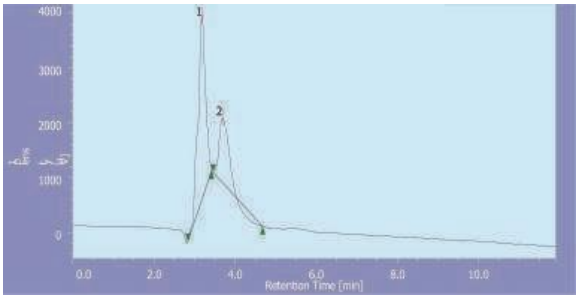


Figure 10: Chromatogram after alkali hydrolysis (8 hr)

Neutral hydrolysis:

HPLC Report

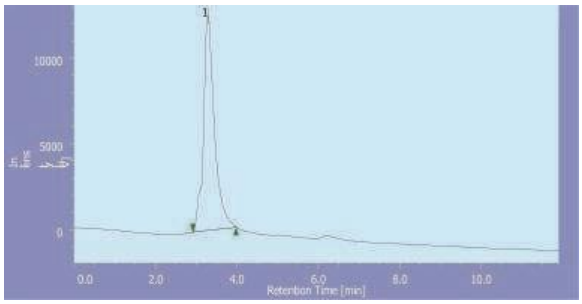


Figure 11: Chromatogram after neutral hydrolysis (0 hr)

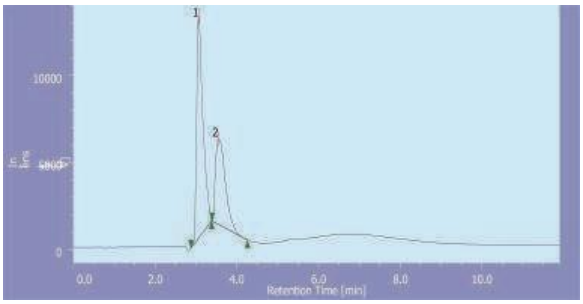


Figure 12: Chromatogram after neutral hydrolysis (2 hr)

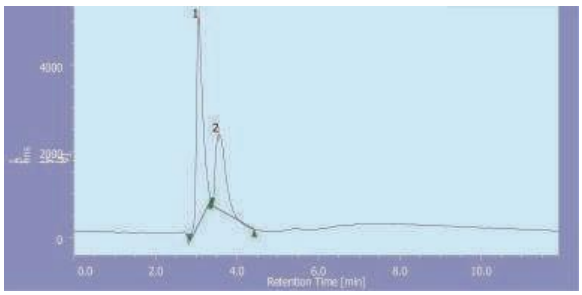


Figure 13: Chromatogram after neutral hydrolysis (5 hr)

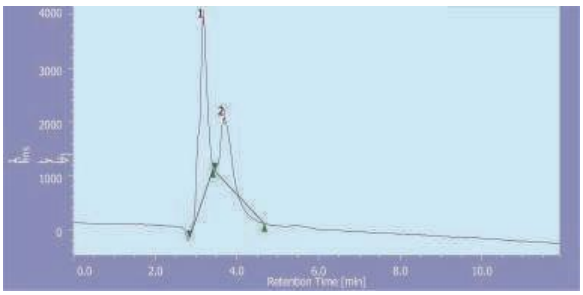
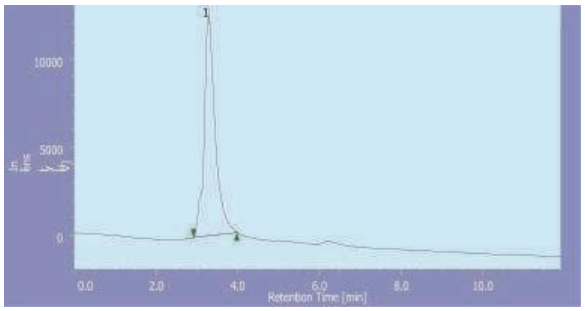


Figure 14: Chromatogram after neutral hydrolysis (8 hr)

Oxidation studies:

HPLC Report



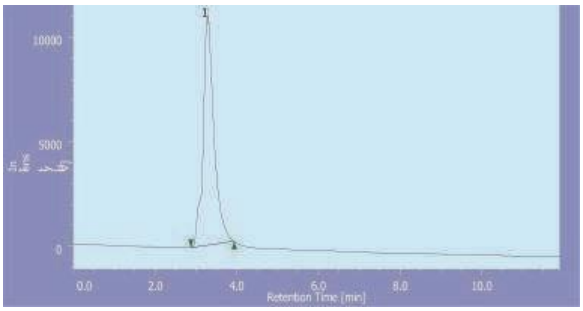


Figure 15: Chromatogram after peroxide study (0 hr)

Figure 16: Chromatogram after peroxide study (1 hr)

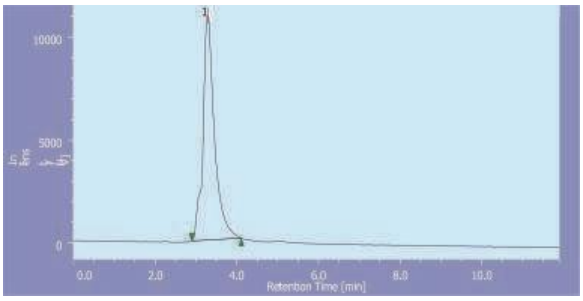


Figure 17: Chromatogram after peroxide study (6 hr)

Photolytic Degradation (UV Light):

HPLC Report

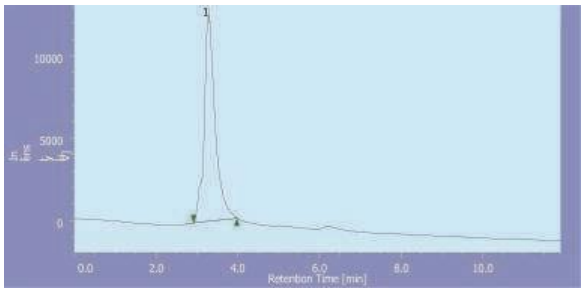


Figure 18: Chromatogram after photolytic study (0 day)

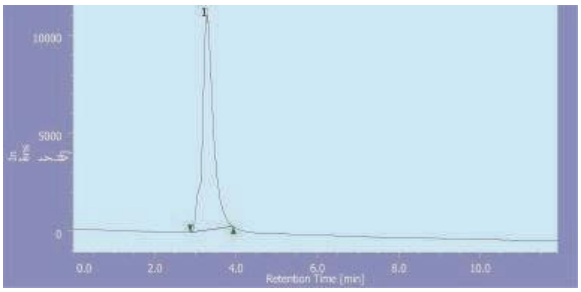


Figure 19: Chromatogram after photolytic study (5th day)

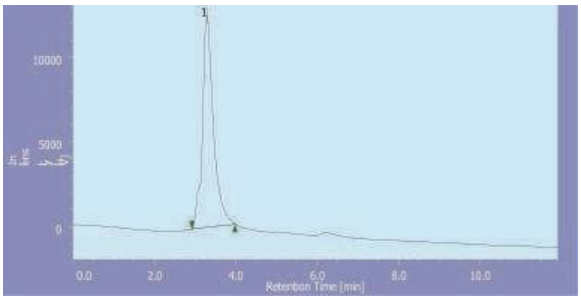
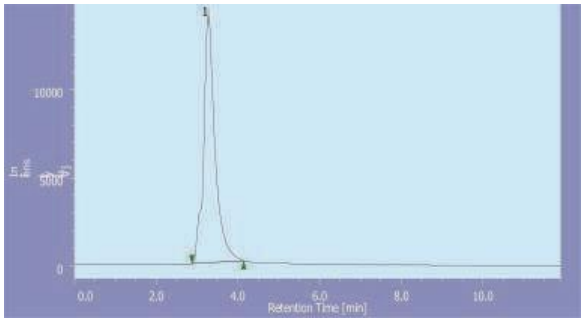


Figure 20: Chromatogram after photolytic study (15th day)

Thermal stability Studies (Dry heat):

HPLC Report



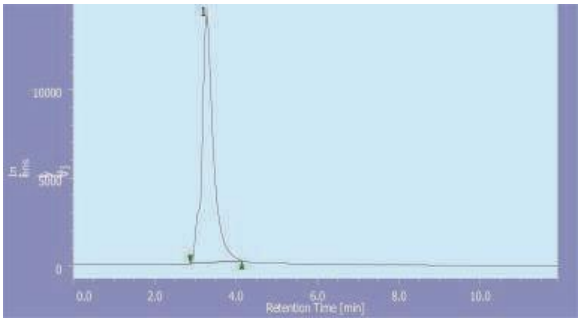


Figure 21: Chromatogram after thermal study (0 day)

Figure 22: Chromatogram after thermal study (90th day)

HPLC Report:

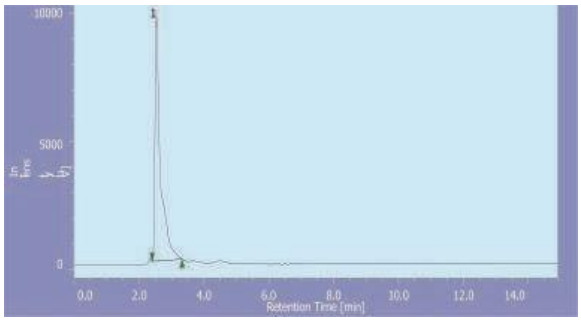


Figure 23: Standard chromatogram of teneligliptin hydrobromide hydrate (R_t = 2.51 min) with methanol: phosphate buffer (75:25 %v/v) as mobile phase at 270 nm.

CONCLUSION

The study shows that developed RP- HPLC method is simple, precise, specific, accurate and stability indicating. The stability- indicating method resolved the drug peak and also the peak of degradation products formed under variety of conditions. After exposure of Teneligliptin hydrobromide hydrate to stress conditions, it was concluded that the drug is susceptible to acid, base, neutral, oxidation, photolysis, thermal degradation, but peaks were unaffected in spite of degraded products. Therefore, this method can be employed for monitoring the stability of Teneligliptin hydrobromide hydrate and can be used for the routine analysis of the drug in pure and tablet dosage forms.

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