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Simultaneous Estimation of Teneligliptin Hydrobromide Hydrate and its Degradation Product by RP-HPLC Method

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ABSTRACT:

A simple, rapid, precise and accurate reversed-phase stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of Teneligliptin hydrobromide hydrate in marketed formulation (tablets). The method has shown adequate separation for Teneligliptin hydrobromide from its associated main impurities and their degradation products. Separation was achieved on a Shisedo C18column, 5µm, 250mm × 4.6 mm i.e. column using a mobile phase consisting of Acetonitrile:Methanol: Water (30:40: 30 % v/v/v) at a flow rate of 1.0ml/min and UV detection at 246nm. The drugs are subjected to acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation to apply force degradation testing. The linearity of the proposed method was investigated in the range of 50-300µg/ml (r2= 0.9996). The limit of detection was2.78 µg/ml and the limit of quantification was 8.45 µg/ml.

KEY WORDS: Teneligliptin hydrobromide hydrate, RP-HPLC, Validation, Force Degradation..

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1. INTRODUCTION

Teneligliptin hydrobromide hydrate is a Dipeptidyl peptidase 4 (DPP-4)inhibitor is highly effective in lowering blood glucose levels.

Teneligliptin hydrobromide hydrate is a highly potent, competitive, and long-lasting DPP-4 inhibitor[1, 2, 3, 4].Glucagon like peptidase (GLP-1) a peptidase secreted from the GIT in response to food intake enhances insulin secretion and suppresses glucagon secretion from the pancreas, thereby playing an important role in controlling postprandial blood glucose level. The peptide is rapidly inactivated by degradation by DPP-4 inhibitor, an enzyme widely distributed in the body. DPP-4 inhibitor degradation, increasing the concentration of active GLP-1 in the blood, which stimulates glucose dependent insulin secretion and at the same time, suppresses glucagon secretion, thereby exhibiting a glucose lowering effect. It is effectively used to treat type 2 diabetes mellitus.

The most commonly reported adverse reactions include hypoglycemia, constipation, and feeling of enlarged abdomen, abdominal discomfort, nausea, abdominal pain, meteorism, stomatitis, eczema, rash, pruritus, dermatitis and malaise. Literature survey reveals no RP-HPLC methods have

been reported for the estimation of Teneligliptin hydrobromide hydrate in pure and tablet dosage forms.

2. MATERIALS AND METHODS

2.1. Materials:

Teneligliptin hydrobromide hydrate was obtained as gift sample from Glenmark Phramaceuticals Ltd., Mumbai, India. Methanol, Acetonitrile and Water (HPLC grade) were purchased from Merck Mumbai Ltd., India. All other chemicals and reagents employed were of analytical grade and were purchased from SD Fine Chemicals, India.

2.2 Instrumentation and Chromatographic method:

The analysis of the drug was carried out on a Peak HPLC system equipped with a reverse phase Shisedo C_{18} column, peak pump with auto samplerand a detector running on Peak LC Solution Software. The mobile phase consists of Acetonitrile:Methanol: Water (30:40: 30 % v/v/v) and the flow rate were maintained at 1.0 ml/min. The mobile phase was freshly prepared and passed through nylon membrane filter of pore size of 0.45µm and it was degassed by sonicating for 5 min. before it was used. The elution was monitored at wavelength of 246 nm with UV detector, and the injection volume was 10µl.

2.3. Determination of maximum absorbance:

The standard solutions of Teneligliptin hydrobromide hydrate were scanned in the range of 200-400 nm against mobile phase as blank. Teneligliptin hydrobromide hydrate showed maximum absorbance at 246nm. Thus the wave length selected for the determination of Teneligliptin hydrobromide hydrate is 246nm.

2.4 Preparation of stock and standard solutions:

Accurately weighed Teneligliptin Hydrobromide Hydrate 100 mg was transferred into 100 ml volumetric flask. Dissolved and dilute up to the mark with methanol to obtain final concentration of 1000 µg/ml. 100 µg/ml of Teneligliptin Hydrobromide Hydrate working standard solution was prepared by diluting 10 ml of stock solution to 100 ml with methanol.

2.5 Assay of Teneligliptin Hydrobromide Hydrate tablets:

Ten tablets of Teneligliptin Hydrobromide Hydrate were weighed and powdered to 20 mg of Teneligliptin Hydrobromide Hydrate and transferred to 100 ml volumetric flask, volume adjusted with methanol (200 µg/ml) peak area of the prepared solution was recorded and concentration of each drug was calculated using calibration curve equation.

2.6 Method development:

The RP-HPLC method developed in this study was aimed at finding the chromatographic system capable of eluting and resolving Teneligliptin hydrobromide hydrate and its degradation products with satisfying system suitability conditions. To develop the conditions various parameters such as mobile phase, pH, flow rate and solvent ratio were changed and suitable chromatographic condition has been developed for routine analysis of drug samples. Initial trails were carried out by using same column taking Methanol, Acetonitrile and Water in various proportions with flow rate of 1.0ml/min. The column was maintained with gradient phase. The chromatograms obtained after injecting drug samples and maintained with run time of 7min reported in separation and peaks were observed broad with thick peak heads and high retention time.

Further trails were carried out varying the flow rate, changing the chromatographic column, pH conditions and mobile phase composition. The best resolution was reported during a trail when Mobile phase was taken as Acetonitrile, Methanol and Water in the ratio (30 : 40 : 30% v/v/v); flow rate of 1.0ml/min, and sharp peak was depicted at retention time of 3.372min, peak was narrow, sharp and with high resolution compared to other peaks obtained in different trails. Thus, these chromatographic conditions was used for studying the different properties of drug such as degradedness and also used to validate various method parameters like linearity, precision, recovery, robustness, LOD and LOQ. Chromatographic condition was established such that it could be suitable for separation of drug and itsdegradation products separating impurities during elution from the chromatographic column. The proposed method is simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in the analysis of drugs.

2.6.1. Method validation

The Proposed method was validated according to ICH guidelines [(5)]. The parameters assessed were linearity, precision, accuracy, force degradation, LOD and LOQ.

2.6.2.Linearity:

The linearity is expressed in term of correlation co-efficient of linear regression analysis. The linearity of response for THH was assessed by analysis of five independent levels of calibration curve in range of 50-300 µg/ml for THH.

2.6.3. Precision:

I. **Repeatability:**
Solutions of 100, 150, 200 µg/ml Teneligliptin Hydrobromide Hydrate was prepared and peak area was measured with each solution and % RSD was calculated.

II. **Intraday precision:**
Solutions of 100, 150, 200 µg/ml Teneligliptin Hydrobromide Hydrate was prepared and peak area was measured containing analyzed three times on the same day and % RSD was calculated.

III. **Interday precision:**
Solutions of 100, 150, 200 µg/ml Teneligliptin Hydrobromide Hydrate was prepared and peak area was measured containing analyzed three times on different days and % RSD was calculated.

2.6.4. Recovery:

The accuracy of method was determined by recovery, by spiking of standard drug solution to pre analyzed sample at three different levels i.e., at 80, 100, and 120%. The resultant solutions were then re-analyzed by the developed method. At eachconcentration, sample was injected thrice to check repeatability and from the data it was analyzed that the method was accurate.

2.6.5. Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters

a) pH of mobile phase: ±0.2
b) Flow rate: ± 0.2 ml/min.
c) Change in the ratio of component in the mobile phase: ± 2%.

2.6.6. Limit of detection:

Limit of detection of the individual analytical method is the lowest concentration of analyte in the sample that the method can detect but not necessarily quantify under stated experimental conditions. LOD not only depend on procedure of analysis but also on type of instrument.

LOD was calculated using the formula, LOD= 3.3 * intercept / slope.

2.6.7. Limit of quantification:

Limit of quantification of the individual analytical method is the lowest concentration of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. The quantification limit is used particularly for the determination of impurities and degraded products. LOQ is calculated by the formula, LOQ= 10 * intercept /slope.

2.7. Force degradation studies

Force degradation was carried out on the drug in order to check the stability of the drug by providing various stress conditions like acid, base, oxidation and thermal degradation compared with normal conditions. The purpose of force degradation 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.

2.7.1 ACID HYDROLYSIS:

a) Preparation of standard solution of Hydrobromide Hydrate for acid hydrolysis: 2 ml of THH stock solution was transferred into 10 ml volumetric flask. Add 2 ml of 0.1N HCl in flask for acid hydrolysis. Solution was kept for 4 hours than 2 ml of 0.1N NaOH added into flask for neutralization and diluted up to mark with mobile phase (200 µg/ml).

b) Preparation of sample solution of Teneligliptin Hydrobromide Hydrate for acid hydrolysis: 2 ml of THH sample solution was transferred into 10 ml volumetric flask. Add 2 ml of 0.1N HCl in flask for acid hydrolysis. Solution was kept for 4 hours than 2 ml of 0.1N NaOH added into flask for neutralization and diluted up to mark with mobile phase (200 µg/ml).

2.7.2 ALKALINE HYDROLYSIS:

a) Preparation of standard solution of Teneligliptin Hydrobromide Hydrate for alkaline hydrolysis: 2 ml of THH stock solution was transferred into 10 ml volumetric flask. Add 2 ml of 0.1N NaOH in flask for alkaline hydrolysis. Solution was kept for 4 hours than 2 ml of 0.1N HCl added into flask for neutralization and diluted up to mark with mobile phase (200 µg/ml).

b) Preparation of sample solution of Teneligliptin Hydrobromide Hydrate for alkaline hydrolysis: 2 ml of THH sample solution was transferred into 10 ml volumetric flask. Add 2 ml of 0.1N NaOH in flask for alkaline hydrolysis. Solution was kept for 4 hours than 2 ml of 0.1N HCl added into flask for neutralization and diluted up to mark with mobile phase (200 µg/ml).

2.7.3 OXIDATIVE DEGRADATION:

a) Preparation of standard solution of Teneligliptin Hydrobromide Hydrate for oxidative degradation: 2 ml of THH stock solution was transferred into 10 ml volumetric flask. Add 2 ml of 3% H2O2 in flask for acid hydrolysis. Solution was kept for 4 hours. Solution was refluxed for 30 min at room temperature and then diluted up to mark with mobile phase (200 µg/ml).

b) Preparation of sample solution of Teneligliptin Hydrobromide Hydrate for oxidative degradation: 2 ml of THH sample solution was transferred into 10 ml volumetric flask. Add 2 ml of 3% H2O2 in flask for acid hydrolysis. Solution was kept for 4 hours. Solution was refluxed for 30 min at room temperature and then diluted up to mark with mobile phase (200 µg/ml).

2.7.4THERMAL DEGRADATION:

For thermal degradation, the standard and sample were kept individually in petri dish and placed in Hot air oven for 1hour at 40 ˚C. Then after sample and standard solution are prepared and final dilution are made for standard of THH and its sample.

3. RESULTS AND DISCUSSION

3.1. Linearity:

The calibration curve showed (Fig.1) good linearity in the range of 50-300µg/ml, for Teneligliptin hydrobromide hydrate with correlation coefficient (r^2) of 0.9996. A typical calibration curve has the regression equation of y = 2.4262x + 2.0508. Results are given in Table 1.

3.2. Precision:

Intraday precision was carried out using test samples prepared and analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The low % RSD values below 2 indicate that the method is precise. The results are given in table 2&3.

3.3. Recovery:

At each concentration, sample was injected thrice to check repeatability and from the RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.02% to 102.02% at three different concentrations 80, 100 and 120µg/ml.

3.4. Robustness:

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (± 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in favor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Teneligliptin hydrobromide hydrate. The results are given in table 6**.**

3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD of was found to be2.78 µg/ml and the LOQ 8.45 µg/ml estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration.

3.6. Force Degradation studies:

RP-HPLC study of samples obtained on stress testing of Teneligliptin hydrobromide hydrate under different conditions using mixture of methanol and acetonitrile in the ratio 65:35 (v/v) with pH5.3 as a mobile solvent system suggested the following degradation behavior.

The chromatograms obtained on stress degradation, like photolytic degradation and similarly other conditions were shown in figure.

3.7 DISCUSSION

- RP-HPLC method was found to be linear over the range of 50-300 µg/ml for Teneligliptin Hydrobromide Hydrate. The method has been validated for linearity,

accuracy and precision, LOD, LOQ and system suitability according to ICH guideline.

- The force degradation study for Teneligliptin Hydrobromide Hydrate indicates that the drug significantly degrade under acidic and oxidative conditions.

4. CONCLUSION:

- The study shows that the developed HPLC Method is simple, precise, specific, and accurate.
- In this developed method linearity of RP-HPLC was observed in the conc. range of 50-300 µg/ml for Teneligliptin Hydrobromide Hydrate with co-efficient of correlation (r^2) =0.9992 respectively.
- % RSD for intraday and interday precision was found to be less than 2.
- The LOD of the TTH was found to be 2.278 µg/ml and LOQ of the TTH was found to be 8.45 µg/ml.
- % Recovery is greater than 98 but less than 102 for both the methods which shows that the method is accurate and free from the interference of excipients used in formulation.
- So, the developed method can be used for routine analysis and quality control test.
- The developed method can be successfully applied to the market formulation. The results obtained were in good agreement with label claimed.
- Forced degradation study was carried out in various stressconditions like ACID, BASE, OXIDATION and THERMAL.
- The maximum degradation of Teneligliptin Hydrobromide Hydrate was observed in Oxidativedegradation i.e. 9.26 % for standard and 7.74 % for tablet.
- The peak of degraded component were in resolved from the peak of main component and do not interfere with the API peak.
- The forced degradation study gave future scope that the degraded product can be separated in sufficient quantity and characterized. Then it can be studied for its safety profile.

5. REFERENCES:

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6. LIST OF FIGURES & TABLES:

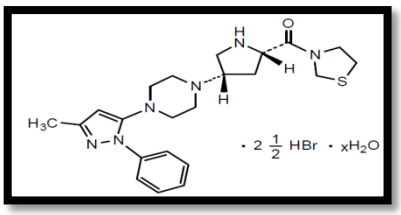

Fig. 1: Molecular structure of Teneligliptin hydrobromidehydrate

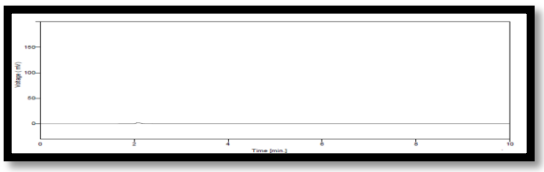

Fig. 2 Chromatogram for mobile phase

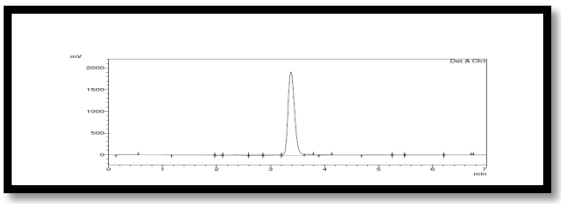

Fig.3 Chromatogram of standard

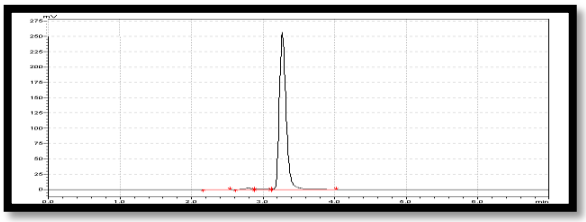

Fig.4 Chromatogram of sample

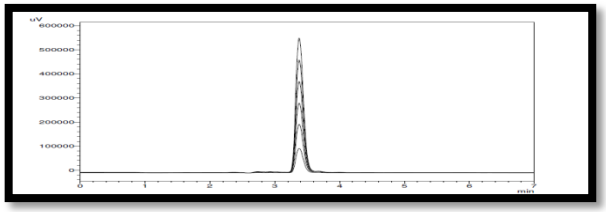

Fig. 5Calibration curve of Teneligliptin hydrobromide hydrate

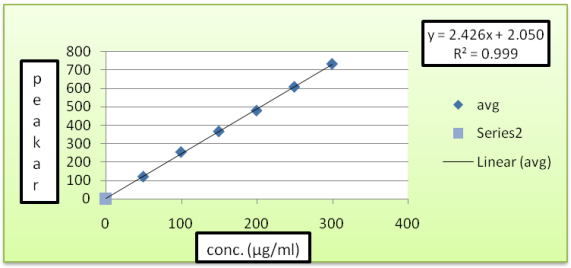

Fig. 6 Linearity results of Teneligliptin hydrobromide hydrate

Acid Hydrolysis:

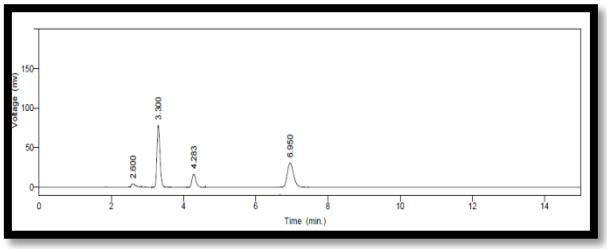

Fig.7 Chromatogram of Teneligliptin Hydrobromide Hydrate under Acid Hydrolysis of standard

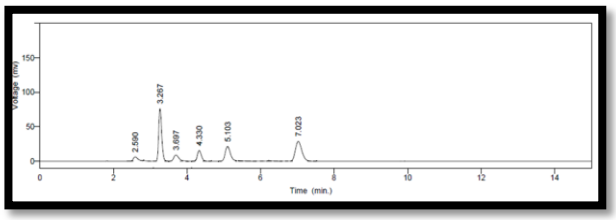

Fig.8 Chromatogram of Teneligliptin Hydrobromide Hydrate under Acid Hydrolysis of sample

Alkaline Hydrolysis:

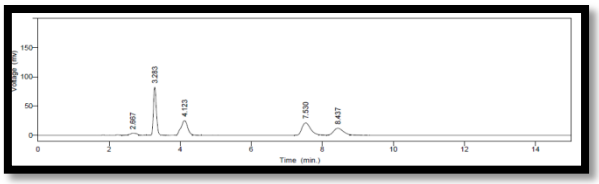

Fig.9 Chromatogram of Teneligliptin Hydrobromide Hydrate under Alkaline Hydrolysis of standard

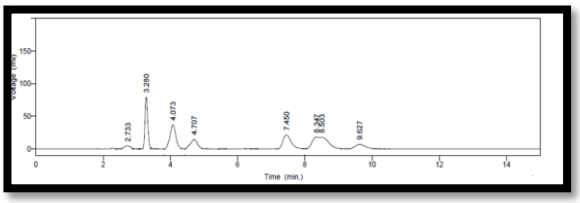

Fig.10 Chromatogram of Teneligliptin Hydrobromide Hydrate under Alkaline Hydrolysis of sample

Oxidative Degradation:

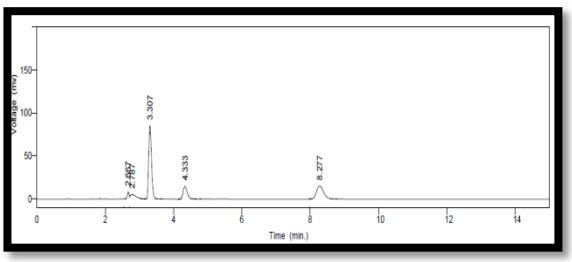

Fig.11 Chromatogram of Teneligliptin Hydrobromide Hydrate under Oxidative Degradation of standard

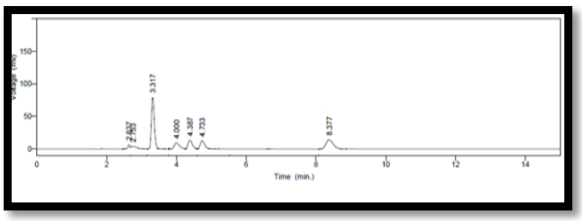

Fig.12 Chromatogram of Teneligliptin Hydrobromide Hydrate under Oxidative Degradation of sample

Thermal Degradation:

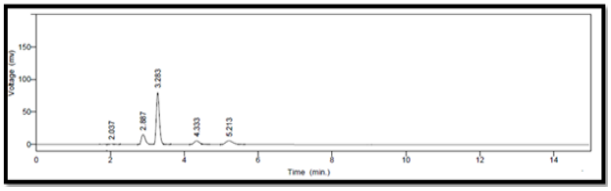

Fig.13 Chromatogram of Teneligliptin Hydrobromide Hydrate under Thermal Degradation of standard

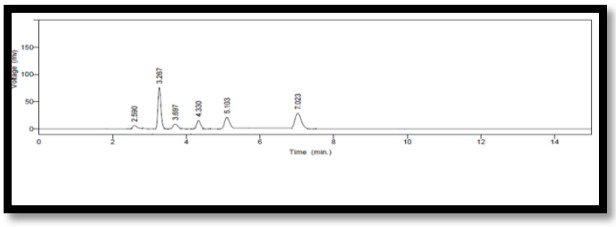

Fig.14 Chromatogram of Teneligliptin Hydrobromide Hydrate under Thermal Degradation of sample

Table1: Table showing values of concentration vs. area

S.NO	Concentration in µg/ml	Mean±SD	%RSD
1	50	121.069 ± 1.816	1.500
2	100	254.392 ± 4.415	1.735
3	150	366.516 ± 4.278	1.167
4	200	479.176 ± 7.929	1.654
5	250	608.103 ± 6.786	1.115
6	300	732.610 ± 11.187	1.527
	Slope	**2.4262**	-
	Intercept	**2.0508**	-
	Cc	**0.9996**	-

Intraday Precision:

Table 2: Table showing results of intraday precision

S.NO	Concentration in µg/ml	Mean ± SD	%RSD
1	100	245.110 ±4.462	1.820
2	150	364.649 ±4.821	1.322
3	200	490.014 ±9.674	1.974

Interday Precision:

Table 3: Table showing results of interday precision

S.NO	Concentration in µg/ml	Mean ±SD	%RSD
1	100	248.815 ±6.086	2.446
2	150	356.460 ±10.016	2.810
3	200	472.805 ±9.285	1.963

Repeatability:

Table 4: Table showing results of repeatability

S.NO	Concentration in µg/ml	Mean ±SD	%RSD
1	100	244.812 ± 1.00	0.411
2	150	362.656 ± 2.195	0.605
3	200	482.603 ± 1.871	0.387

Table 5: Table showing results of recovery

	%Recovery							
% of Recovery	**Target Conc., (µg/ml)**	**Spiked conc., (µg/ml)**	**Final Conc., (µg/ml)**	**Conc., Obtained**	**% of Assay**	**Avg**	**SD**	**%RSD**
80%	50	40	90	90.63	100.7	99.97	0.859	0.860
	50	40	90	89.12	99.02			
	50	40	90	90.17	100.18			
100%	50	50	100	100.03	100.03	101.03	0.995	0.984
	50	50	100	101.06	101.06			
	50	50	100	102.02	102.02			
	50	60	110	111.9	101.72	101.26	0.824	0.813

120%	50	60	110	111.92	101.74
	50	60	110	110.34	100.30

Table 6: Table showing results of Robustness

Sr. no	pH(+0.2 units)	pH (-0.2units)	Flow rate(+0.2 units)	Flow rate(-0.2units)	Mobile phase (+2%)	Mobile phase (-2%)
1	274.814	245.014	232.622	232.321	247.148	236.481
2	269.271	244.414	239.536	238.884	246.843	233.127
3	275.483	239.214	235.931	231.873	239.843	227.438
Avg	273.189	242.880	236.029	234.359	244.570	232.348
SD	3.409	3.189	3.458	3.924	4.099	4.571
%RSD	1.248	1.313	1.465	1.674	1.676	1.967

Table 7: Result of forced degradation studies of Teneligliptin Hydrobromide Hydrate

Sr. No.	Type of Degradation	Teneligliptin Hydrobromide Hydrate (R_t min)	% Degradation
1	Acid	3.300	5.95
2	Alkaline	3.283	7.68
3	Oxidative	3.307	9.26
4	Thermal	3.283	6.19

Table 8: Result of forced degradation studies of Teneligliptin Hydrobromide Hydrate in formulation

Sr. No.	Type of Degradation	Teneligliptin Hydrobromide Hydrate (R_t min)	% Degradation
1	Acid	3.267	5.22
2	Alkaline	3.280	6.74
3	Oxidative	3.317	7.74
4	Thermal	3.287	5.46

Table 9: Summary of Forced Degradation study of Teneligliptin Hydrobromide Hydrate

Sr. No.	Type of Degradation	Optimized Condition	Matrix	% Degradation
1	Acid	0.1 N HCl at 40˚C for 4 h.	THH	5.95
			THH (sample)	5.22
2	Alkaline	0.1 N NaOH at room temperature for 4 h.	THH	7.68
			THH (sample)	6.74
3	Oxidative	3 % H_2O_2 at room temperature for 4 h.	THH	9.26
			THH (sample)	7.74
4	Thermal	60 ˚C for 1 h.	THH	6.19
			THH (sample)	5.46