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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE ACTIVE PHARMACEUTICAL INGREDIENT BY USING RP-HPLC

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ABSTRACT

Teneligliptin Hydrobromide Hydrate is an Anti-Diabetic drug used in the treatment of Diabetes. From the literature survey it was found that many methods are available for determination assay of Teneligliptin Hydrobromide Hydrate. In the proposed study an attempt will be made to develop HPLC for the estimation of related substances. In HPLC the chromatographic separation was achieved with L1 stationary phase (YMC Triart C18 150 x 4.6mm; 3µm). The mobile phase involved a variable composition of solvent A (Buffer: Acetonitrile: Methanol (50:40:10) % v/v/v), solvent B (Acetonitrile:water in the ratio of 80:20 v/v). The mobile phase was pumped through the column with at a flow rate of 1ml/min. The detection was carried out at wavelength 210nm and column oven temperature at 45°C, the method was established from the peak purity induces obtained with the aid of PDA detection. Regression analysis shows an 'r' value (correlation coefficient) is 0.999 for Teneligliptin Hydrobromide Hydrate in both HPLC. Robustness against small modification column oven temperature, flow rate and percentage of the

mobile phase composition was ascertained. The method was validated for accuracy, precision, specificity, robustness, and detection and Quantitation limits, in accordance with ICH guidelines. Statistical analysis proved that the method was precise, reproducible, selective, specific, and accurate for analysis of Teneligliptin Hydrobromide Hydrate.

Keywords: Teneligliptin Hydrobromide Hydrate, Method validation, Method development, RP-HPLC, Active pharmaceutical ingredient, Regression

INTRODUCTION

Teneligliptinis chemically 3-{(2*S*,4*S*)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl)-1–piperazinyl] -2- pyrrolidinyl}carbonyl(-1,3-thiazolidine,hydrobromide (2:5) hydrate **[1, 2]**. Its molecular formula is $C_{22}H_{30}N_6OS.21/2HBr.H_2O$and molecular weight is 646.85 g/mol **[3, 4] (Figure 1)**. Teneligliptin used for the treatment of type 2 diabetes mellitus **[5, 6]**. It belongs to the class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptin". Teneligliptin hydroromide hydrate is a novel, potent, peptidomimetic, and long-acting DPP-4 inhibitor that inhibits human dipeptidyl peptidase-4 (DPP-4) **[6]** enzyme activity with the IC50 =1nM, more than 150 fold selectivity against DPP-8 and DPP-9 .By DPP-4 inhibition, Teneligliptin prevented the degradation of incretins GLP-1, GIP and promoted insulin release which prevented blood glucose increase after food intake.

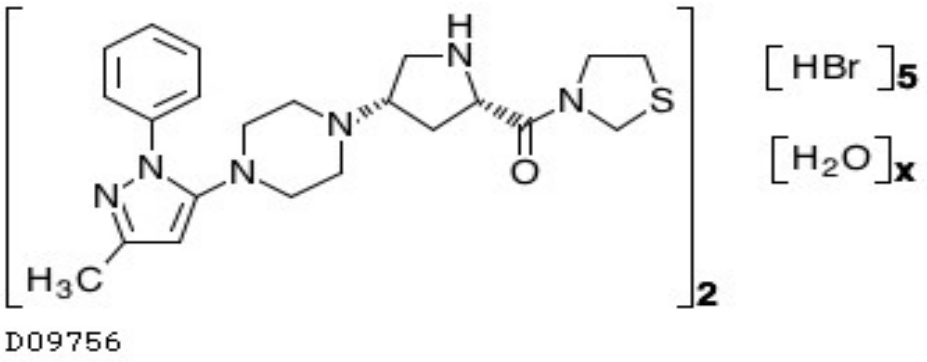

Figure 1: Structure of Teneligliptin hydroromide hydrate [3]

MATERIALS & METHODS

A pharmaceutical grade sample of Teneligliptin Hydro bromide Hydrate along with the solvents like methanol, acetonitrile, Orthophosphoric acid, Perchloric acid of A.R grade were used. The instrument used was

HPLC with L1 stationary phase (YMC Triart C18 150 x 4.6mm; 3µm) column. An electronic balance of Metler Toledo micro balance XP-105, DR. Sartorius, Ultra sonicator and a p^H Meter **[7]**.

METHOD DEVELOPMENT

Determination of absorption maxima (λ_{max}) [8]:

Teneligliptin working standard of 10ppm concentration was prepared along with Placebo and blank solutions. All these solutions were scanned between 200 to 400nm using UV-Visible spectrophotometer. From the UV spectrum the drug showed maximum absorbance at 210 nm. After reviewing the chromatograms and peak purity chromatograms a wavelength of 210 nm was selected as the optimum wavelength for this drug **(Figure 2, 3)**.

Preparation of Diluent:

Teneligliptin Hydrobromide Hydrate is freely soluble in methanol and Acetonitrile. Buffer: Methanol: Acetonitrile (50:40:10 %v/v/v) is selected as diluent.

Preparation of Mobile phase: [9]

Preparation of Buffer: Accurately weigh about 2.30gm of 1-Octane sulfonic acid Sodium salt and 1.36gm of potassium dihydrogen phosphate in 1000ml of Milli-Q-water and adjust to pH 3.5±0.05 with dilute H3PO4 solution, filtered and degas through 0.45µ membrane filter.

Solution-A: Buffer, Methanol and Acetonitrile in the ratio of 50:40:10 (% v/v/v)

Solution-B: Acetonitrile and water in the ratio of 80:20(% v/v).

Gradient programme:

Time	% A	% B
0.0	100	0
10	100	0
30	40	60
45	40	60
46	100	0
60	100	0

Preparation of Standard solution: [8]

Weigh accurately about 50.0mg of standard into a 50ml volumetric flask, add 20.0ml of diluent and sonicate to dissolve and make up to the mark with diluent. Transfer 5ml of above solution in 25ml volumetric flask, dissolve ad make up to volume with diluent.

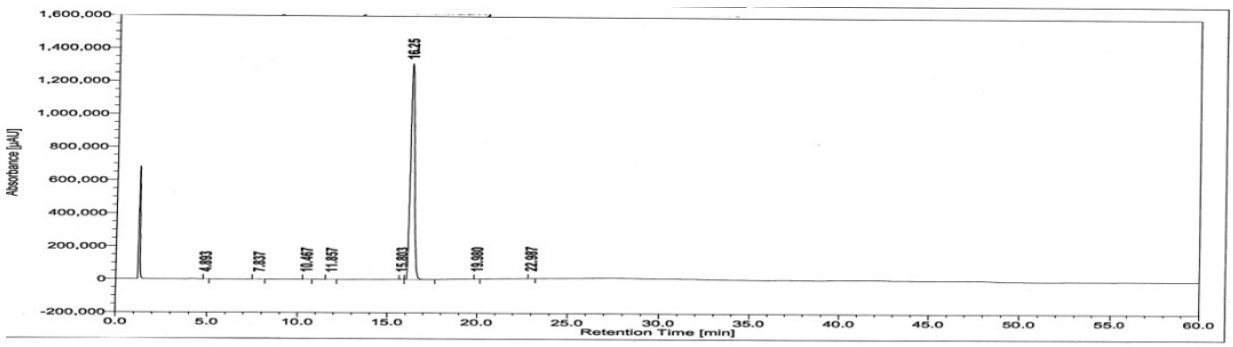

Figure 2: Chromatogram of Standard

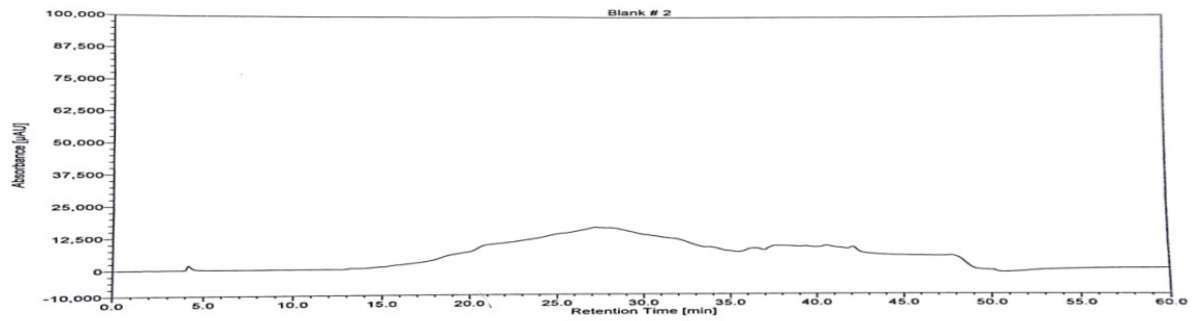

Figure 3: Blank Chromatogram

METHOD VALIDATION

1. SPECIFICITY: [10]

Preparation of sample solution (1mg/ml):

Weighed 50 mg of Teneligliptin hydro bromide hydrateand transferred into a 50ml volumetric flask. Dissolve and made up to mark the volume with diluent. Transfer 5ml of above solution in 25ml volumetric flask, dissolve ad make up to volume with diluent.

Acceptance criteria:

Teneligliptin hydro bromide hydrate and its impurities should be well separated from each other.

2. LINEARITY: The linearity of the method was done over a concentration range of 25%-150% that were prepared from the standard stock solution **[11, 12]**.

Preparation Linearity solution:

Transfer 60.0 µl of standard solution into a 20ml volumetric flask containing about 10ml of diluent. Mixed well and made up to the mark with diluent. Further dilute the solutions to 25%-150% concentration.

Acceptance criteria:

i) The correlation coefficient should be not less than 0.999.

ii) Y-intercept value shall be NMT ± 5% of the response at 100% level

3. RANGE: **[12]**

Lower Level: Injected LOQ Solution (which was prepared in LOQ) for 6 times in to the liquid chromatographic system.

Upper Level: Injected 150% Solution (which was prepared in linearity-150%) for 6 times in to the liquid chromatographic system.

Acceptance criteria: % RSD for peak areas of Teneligliptin hydro bromide hydrate from LOQ level should not be more than 10.0

4. METHOD PRECISION: [8, 11]

Procedure:

Method precision was evaluated by injecting six assay samples of drug product and Injected each sample preparation once into the chromatographic system and calculated the % RSD for single maximum unknown impurity content and total impurities.

Acceptance criteria: % RSD of single maximum unknown impurity content and total impurities should not be more than 10.0.

5. LIMIT OF DETECTION:

Transfer 10.0 µl of system suitability solution into a 10ml volumetric flask containing about 5ml of diluent. Mixed well and made up to the mark with diluent.

Acceptance criteria: The S/N ratio should be in between 2.0 to 4.0.

6. LIMIT OF QUANTIFICATION:

Transfer 30.0µl of system suitability solution into a 10ml volumetric flask containing about 5ml of diluent. Mixed well and made up to the mark with diluent.

Acceptance criteria: The S/N ratio should be in between 9.0 to 11.0.

7. ROBUSTNESS: [7, 8, 11]

EFFECT OF FLOW VARIATION:

The robustness of the HPLC method was demonstrated by changing the flow rate from 1.0ml/min to 1.2ml/min and 0.8ml/min. Injected reference solution and system suitability solution and calculated the system suitability parameters at each flow rate.

Standard solution preparation:

Weighed 49.942 mg of Teneligliptin hydro bromide hydrate standard into a 50ml volumetric flask. Dissolved in about 20ml of diluent and then made up to the mark with diluent.

EFFECT OF COLUMN OVEN TEMPERATURE VARIATION:

The robustness of the HPLC method was demonstrated by changing the column oven temperature from 30°C to 25°C and 35°C. Injected reference solution and system suitability solution and calculated the system suitability parameters at each column oven temperature.

Standard solution preparation:

Weighed 50.057 mg of Teneligliptin hydro bromide hydrate standard into a 50ml volumetric flask. Dissolved in about 20ml of diluent and then made up to the mark with diluent.

8. RUGGEDNESS: [7, 8, 12]

Method is validated for ruggedness by different analysts on different instrument with different column.

Standard solution preparation:

Weighed 50.219 mg of Teneligliptin hydro bromide hydrate standard into a 50ml volumetric flask. Dissolved in about 20ml of diluent and then made up to the mark with diluent.

Procedure:

Injected each sample preparation once, into the chromatographic system and calculated the % RSD for single maximum unknown impurity content and total impurities.

Acceptance criteria:

% RSD of single maximum unknown impurity content and total impurities should not be more than 10.0.

RESULTS AND DISCUSSION

METHOD VALIDATION

1. SPECIFICITY:

The specificity of the developed HPLC method for Teneligliptin Hydro bromide Hydrate Developed HPLC method was found to be specific **(Table 2, Figure 5)**.

2. LINEARITY

Linearity of Teneligliptin Hydro bromide Hydrate was determined over a range of obtained limit of quantification (LOQ) to 150% of specification limit (range was inclusive of concentrations at 25,50, 75, 100, 125 and 150%). The linear regression data for the calibration plot were indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance **(Table 3, Figure 6)**.

3. RANGE

From the linearity taken lower and upper level concentrations and performed range according to the guidelines laid by ICH. The %RSD of upper and lower concentrations was found to be within the limits **(Table 4, Figure 7)**.

Lower level (LOQ Level) (Figure 7)

Upper level (150%): (Table 5, Figure 8)

4. PRECISION:

The precision of the related substances method verified by repeatability and by intermediate precision. Repeatability was checked by injecting six individual preparations of Teneligliptin Hydro bromide Hydrate **(Table 6, 7)**.

System precision (Table 6)

Method precision (Table 7)

5. LIMIT OF DETECTION:

Performed the Limit of Detection Parameter for Teneligliptin Hydro bromide Hydrate as per the procedure mentioned in the protocol. The results are shown in **Table 8, Figure 9**.

6. LIMIT OF QUANTITATION: (Table 9, Figure 10)

7. ROBUSTNESS:

To determine the robustness of the developed method, experimental conditions weredeliberately altered and the resolution between critical pair, i.e. Impurity-C andImpurity-D was recorded in table-7. The parameters selected were mobile phasecomposition (±2% of gradient composition), pH of the mobile phase (±0.2 units), flowrate (± 10%), wavelength (± 5 nm) and column temperature (± 5°C). The effect of thepercent organic strength on the resolution was studied by varying acetonitrile

by -5 to+5% while other mobile phase components were held constant.

Variation in Flow rate (Table 10)

Variation in Oven temperature (Table 11)

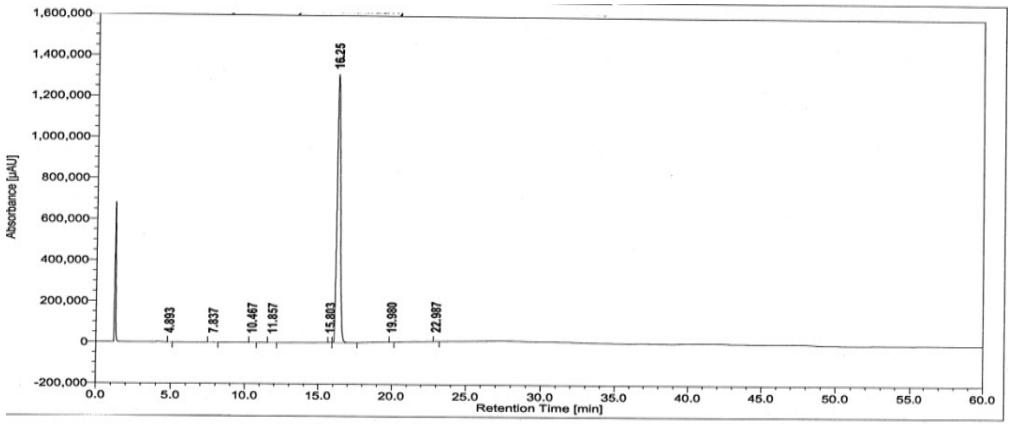

Figure 4: System suitability chromatogram

Table 1: Data of System suitability

S.NO	Name	Retention Time	Peak Area	Tailing Factor	Theoretical plates
1	Teneligliptin Hydro bromide Hydrate	16.25	21321545	1.22	52591

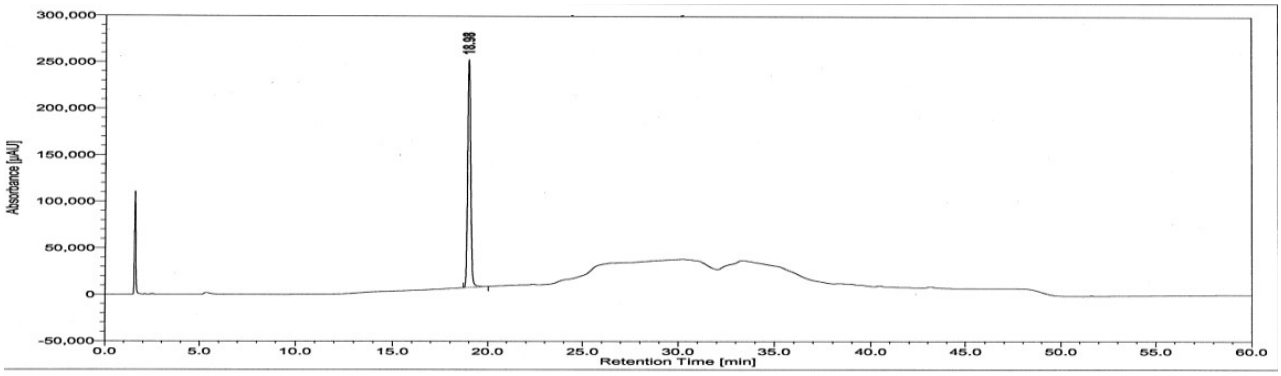

Figure 5: Specificity Chromatogram

Table 2: Data of Specificity

S.NO	Name	Retention time of injection	
		From each individual standard solution	From SST Solution
1	Teneligliptin Hydro bromide Hydrate	16.27	16.25

Table 3: Data of Linearity

S.NO	Name	Volume taken from system suitability solution (ml in 20ml)	W.r.to test conc. (%)	Area of Tenelıgliptin Hydro bromide Hydrate
1	LOQ solution	-	0.030	6787
2	25% Solution	0.25	0.125	28754
3	50% Solution	0.50	0.250	56395
4	75% Solution	0.75	0.375	83119
5	100% Solution	1.00	0.500	112182
6	125% Solution	1.25	0.625	140556
7	150% Solution	1.50	0.750	164312
Correlation Coefficient				1.000
y-Intercept				968.37
Slope				220564.06
% of y-Intercept				0.86

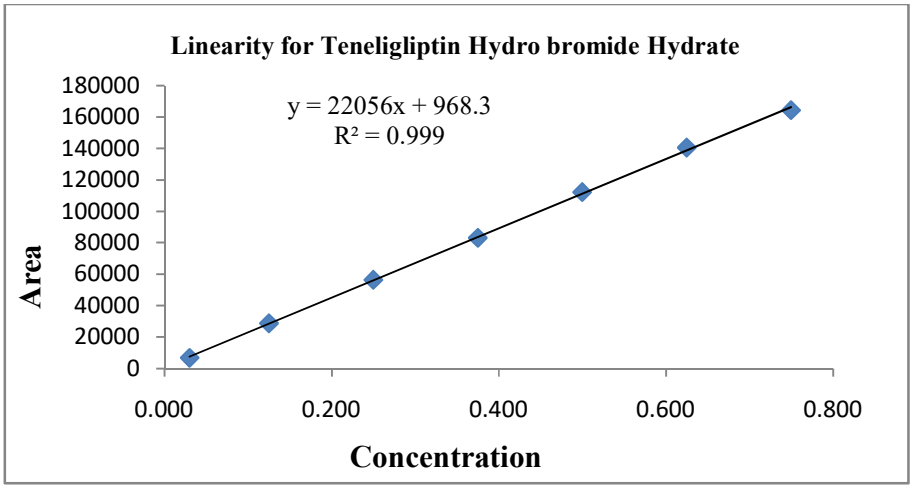

Figure 6: Linearity graph of Teneligliptin Hydro bromide Hydrate

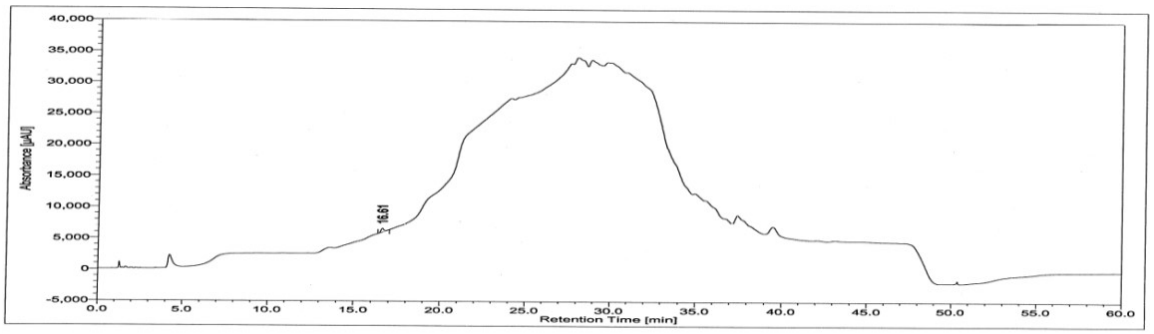

Figure 7: Range at Lower level (LOQ Level) Chromatogram

Table 4: Data of Range at Lower Level (LOQ Level)

S.NO	Sample name	Area of Teneligliptin Hydro bromide Hydrate
1	LOQ Solution-1	6691
2	LOQ Solution-2	6551
3	LOQ Solution-3	6411
4	LOQ Solution-4	6397
5	LOQ Solution-5	6403
6	LOQ Solution-6	6524
Average		6496
STDEV		116.20
%RSD		1.79

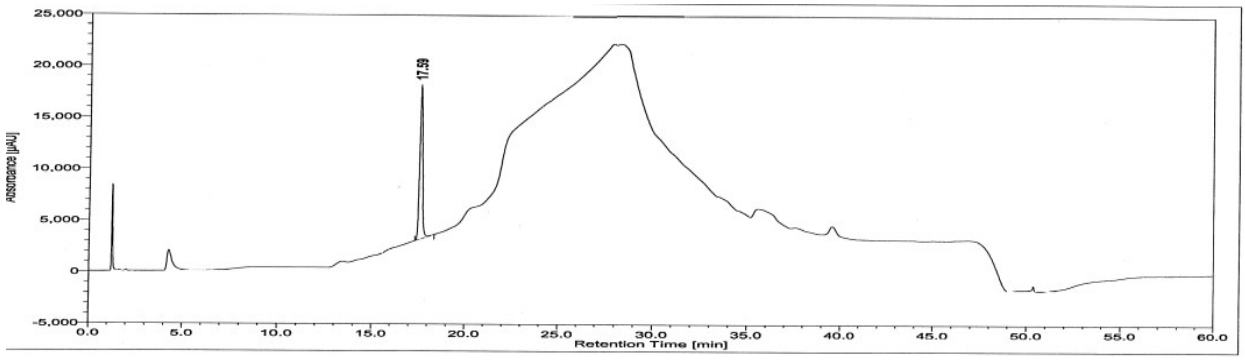

Figure 8: Range at Upper level (LOQ Level) Chromatogram

Table 5: Data of Range at Upper Level (LOQ Level)

S.NO	Sample name	Area of Teneligliptin Hydro bromide Hydrate
1	150% Solution-1	164312
2	150% Solution-2	165501
3	150%Solution-3	164211
4	150% Solution-4	163297
5	150%Solution-5	165403
6	150%Solution-6	165824
Average		164758
STDEV		116.20
%RSD		1.42

Table 6: Data of System precision

S.NO	Sample name	Area of Teneligliptin Hydro bromide Hydrate
1	LOQ Solution-1	6691
2	LOQ Solution-2	6551
3	LOQ Solution-3	6411
4	LOQ Solution-4	6397
5	LOQ Solution-5	6403
6	LOQ Solution-6	6524
Average		6496
STDEV		116.20
%RSD		1.79

Table 7: Data of Method precision

S.NO	Sample name	Area of Teneligliptin Hydro bromide Hydrate
1	Sample solution inj-1	109521
2	Sample solution inj-2	109759
3	Sample solution inj-3	109488
4	Sample solution inj-4	109300
5	Sample solution inj-5	109773
6	Sample solution inj-6	109093
Avg. area		109489
Standard Deviation		263.48
%RSD		0.24

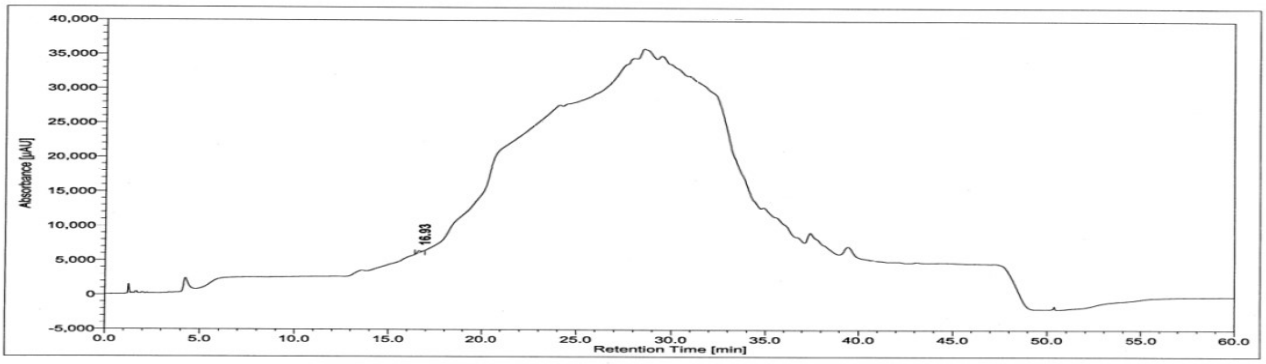

Figure 9: LOD Chromatogram

Table 8: Data of LOD

S.NO	Name	Volume taken from system suitability solution (µL in 10mL)	LOD (%)	S/N Ratio
1	Teneligliptin Hydro bromide Hydrate	15.0	0.015	3.7

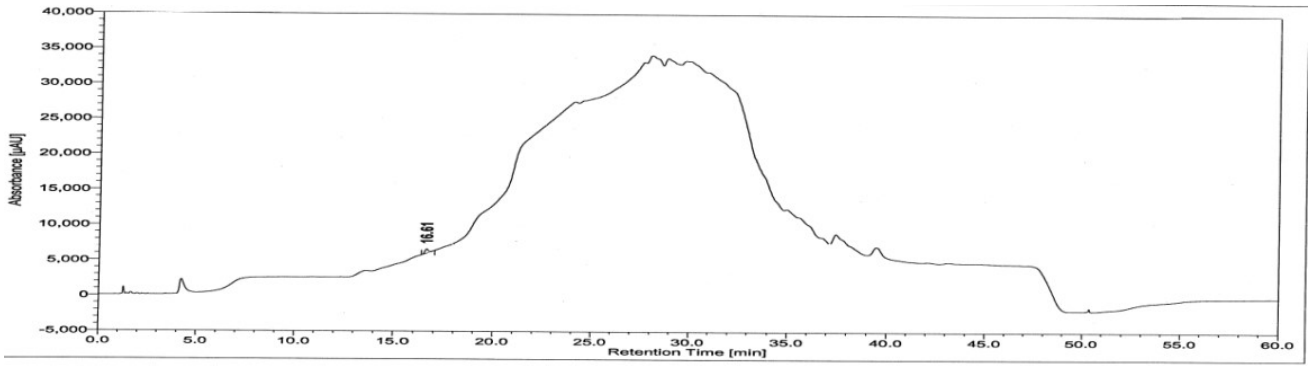

Figure 10: LOQ Chromatogram

Table 9: Data of LOQ

S. NO	Name	Volume taken from system suitability solution (µL in 10mL)	LOQ (%)	S/N Ratio
1	Teneligliptin Hydro bromide Hydrate	30.0	0.030	10.8

Table 10: Data of Robustness at flow rate 0.8ml/min & 1.2ml/min

S. NO	System suitability parameter	Result			Acceptance criteria
		1.0mL/min	1.2mL/min	0.8mL/min	
1	The theoretical plates for principle peak from system suitability solution	43313	23152	68639	NLT 3000
2	The tailing factor for principle peak in system suitability solution	1.24	1.22	1.23	NMT 2.0
3	% RSD for six replicate Reference solution injections	0.90	0.78	0.72	NMT 5.0

Table 11: Data of Robustness at variation in temperature 25°C and 35°C

S. NO	Systemsuitability parameter	Result			Acceptance criteria
		At 30°C	At 25°C	At 35°C	
1	The theoretical plates for principle peak from system suitability solution	43317	59230	27337	NLT 3000
2	The tailing factor for principle peak in system suitability solution	1.24	1.26	1.23	NMT 2.0
3	% RSD for six replicate Reference solution injections	0.90	0.92	1.06	NMT 5.0

CONCLUSION

A stability study was found to be simple and have short run time which makes the method rapid and efficient HPLC method for the quantification of Teneligliptin Hydrobromide hydrate in drug product was developed and validated. Several studies in the literature for the determination of the tested compound depend on HPLC method. Nevertheless, the results of the study indicate that the developed RP-HPLC method was found to be simple, selective, precise, accurate, Linear and robust. Therefore, this method can be used for routine testing of production sample as well as stability analysis of Teneligliptin Hydrobromide hydrate drug substances. All statistical results were well within the acceptance criteria. The results of the stress testing of the drug, undertaken according to the ICH guidelines.

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