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MASS SPECTROMETRY-COMPATIBLE STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC FOR ANTI-DIABETIC DRUG TENELIGLIPTIN

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ABSTRACT

Teneligliptin is a FDA approved drug for treatment of Diabetes Mellitus. Very few methods have been reported for its stability and identified degradation products. A simple, rapid, precise and accurate stability indicating RP-HPLC method was developed and validated for identification of Inertsil ODS C18 (4.5x250mm)3µm was used for the chromatographic separation at a detection wave length of 248 nm. For Buffer solution 0.1% ammonium formate in 1000ml with 3.0 ph with formic acid used and the ratio of 90:10 Buffer: ACN for mobile phase A and 30:70 Buffer: ACN selected as mobile phase B for elution and same buffer was used in the preparation of standard and sample solutions. The elution was monitored by injecting the 10µl and the flow rate was adjusted to 1ml/min. The method was validated as per ICH guidelines. Forced degradation studies of Teneligliptin were carried out under acidic, basic, neutral, peroxide, photo and thermal conditions. Degradation was observed in basic, peroxide and neutral stress samples, but not in acid, photo and thermal stress samples.

Keywords: Teneligliptin, Acn, Ammonium Formate, Inertsil Ods C18, Forced Degradation, Characterization, Dipeptidyl Peptidase-4 (Dpp-4) Inhibitors, Gliptins.

I. INTRODUCTION

Teneligliptin 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].

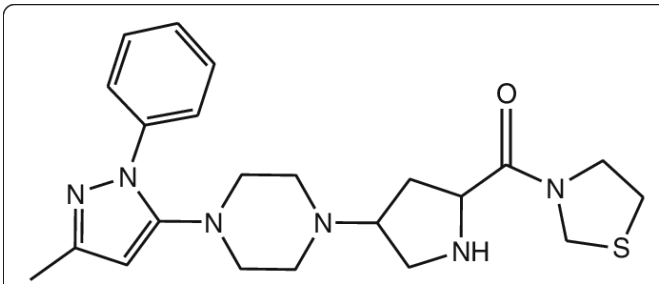

Figure 1 : Structure of Teneligliptin , 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4- [(3S,5S)-5-(1,3-thiazolidine-3-carbonyl)pyrrolidin-3- yl]piperazine (C22H30N6OS)

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II. MATERIALS AND METHODS

Instrumental condition – For the research purpose Water 2695e series HPLC instrument selected with the specification of PDA and UV detector. For the interpretations of data the Empower software is available with the instrument.

The HPLC contains loop volume of 100µl. Different type of Column like Inertsil ODS C18 (4.5x250mm)5µm Inertsil BDS C8(4.5x250mm)5µm, Inertsil ODS C18 (4.5x250mm)3µm, Hypersil BDS C18 (4.5x250mm) 5µm. is used during method development work.

Mettler toledo micro-balance is used for the weighing for the drug samples which having the range of 2mg to 200mg. (sensitivity of 0.1mg) and milligram balance is also used with the range of 50mg to 200gms.

For PH measurement work the mettler toledo PH meter is used. Ultrasonication instrument is used for degassing.

All class 1 material glassware with borosil is used during the research work.

Reagents and materials – The main component for the research work drug sample teneligliptin is purchased form Livemore pharmaceuticals. The other reagents used for mobile phase all HPLC grade methanol, acetonitrile, ortho phosphoric acid tetra hydro furan, Triethyl amine, potassium dihydrogen phosphate, ammonium acetate, ammonium formate, formic acid & Mili Q water all the material is provided by the sun pharma Vadodara.

Buffer Solution - For the final method developed buffer solution was prepared by weigh accurately 1mg of ammonium formate (0.1%) added into 1litre of mili-Q water adjust PH 3 with the help of formic acid. Sonicate the buffer solution for 5minute with the help of ultrasonication for proper mixing. The mobile phase was then filtered through 0.45 µm filter paper to remove the dissolved gases and contaminants and further used as buffer solution

Mobile Phase Preparation – Mobile phase was prepared with the above-mentioned buffer solution by adding the ratio of 90:10 buffer: acetonitrile for mobile phase A and 30:70 buffer: acetonitrile for mobile phase B. Each mobile phase was sonicated and filtered through 0.45µm membrane filter. Mobile phase A was used as diluent.

Chromatographic Condition – Inertsil ODS C18 (4.5x250mm)3µm was used for the chromatographic separation at a detection wave length of 248 nm. For Buffer solution 0.1% ammonium formate in 1000ml with 3.0 ph with formic acid used and the ratio of 90:10 Buffer: ACN for mobile phase A and 30:70 Buffer: ACN selected as mobile phase B for elution and same buffer was used in the preparation of standard and sample solutions. The elution was monitored by injecting the 10µl and the flow rate was adjusted to 1ml/min.

Sample preparation – For the purpose of method development the concentration level is stablished at 1000ppm which is prepared with the dilution of buffer solution.

Weigh 10 mg of the teneligliptin sample and added into 10 ml of volumetric flask. Add some amount of diluent approx. 5ml and sonicate the flask for 5min.or up to proper dissolution of the drug. Makeup the volume up to 5ml to archive 1000ppm concentration.

Working Stock Solution - Weighed and transferred accurately about 50 mg of Teneligliptin (TGP) drug in a 50ml volumetric flask, 35 mL of diluent was added, sonicated to dissolve and diluted up to mark with diluent.

Optimized HPLC/MS conditions - Chromatographic isolation is acquired using Inertsil ODS C18 (4.5x250mm)3µm. The mobile phase in gradient fashion consisting of 90:10 buffer: acetonitrile for mobile phase A and 30:70 buffer: acetonitrile for mobile phase B where ammonium formate (0.1%) added into 1litre of mili-Q water adjust PH 3 with the help of formic acid was used as buffer for the liquid chromatography. The temperature of the system was kept constant at 30 °C. Uniform flow rate of 1.0 mL/min is used. The eluted components were detected using photodiode array at range of 100–500 nm. The products were ionized by electrospray ionization mode for their mass data.

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Table 1: Optimized chromatographic condition for HPLC/MS

Parameters	value
Mobile phase	M.P.A – 90:10 Buffer: ACN M.P.B. – 30:70 Buffer: CAN
pump mode	gradient
diluent	Mobile phase A
column	Inertsil ODS C18 (4.5x250mm)3µm
temp.	30°C
wavelength	248nm
Injection volume	10µl
flow rate	1ml/min
Run time	35min
Typical RT	13.6

Forced degradation studies – Forced degradation studies carried out with various parameters like acidic which is used to perform with the help of HCL solution likewise for alkaline NaOH for hydrolysis and oxidation by H2O2 of different strength is used for degrade drug compound.

Acidic condition – Acid induced degradation was carried on drug sample by studying the chromatograms of solution prepared 0, 2, 5 hours. Accurately weighted bulk drug 50 mg was dissolved in 50 ml mobile phase in volumetric flask. The 5 ml of drug solution was diluted with 2.5ml of 1 N HCL in 10 ml volumetric flask. The reaction mixture was neutralized by 2.5ml of 1N NaOH after 2 hrs. and similarly prepared a sample for 5N HCL. The samples were withdrawn for Injecting sample to HPLC.

Alkaline Condition - Alkaline induced degradation was carried on drug sample by studying the chromatograms of solution prepared 0, 30min., and 2 hours. Accurately weighted bulk drug 50 mg was dissolved in 50 ml mobile phase in volumetric flask. The 5 ml of drug solution was diluted with 2.5ml of 1 NaOH in 10 ml volumetric flask. The reaction mixture was neutralized by 2.5ml of 1N HCL after 30min and 2 hrs. The samples were withdrawn for Injecting sample to HPLC.

Peroxide Condition – Peroxide induced degradation was carried on drug sample by studying the chromatograms of solution prepared 0, 15min, 30min., and 2 hours. Accurately weighted bulk drug 50 mg was dissolved in 50 ml mobile phase in volumetric flask. The 5 ml of drug solution was diluted with 2.5ml of 1%H2O2 in 10 ml volumetric flask. The samples were withdrawn for Injecting sample after 15min.,30min and 2hours to HPLC.

Thermal condition (Diluted sample) – 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 24hrs. on 80ºC and then sample of 1000 µg/ml solution was prepared and the peak area of chromatogram were read out.

Photolytic Condition - 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 for 24hrs. The samples were diluted with diluent to get concentration of 1000µg/ml.

METHOD VALIDATION - Validation of the optimized method was performed according to the ICH Q2 (R) guidelines.

LINEARITY - The linearity plot was prepared with seven concentration levels 0.1, 1, 5, 10, 50, 100, 250, 500, 750, 1000, 1250, and 1500µg/ml of teneligliptin). These concentration levels were respectively corresponding to 0.01, 0.1, 0.5, 1, 5, 10, 25, 50, 75, 100, 125, and 150 % of test solution concentration. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y =

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25073.34x + 10166.16 where x is the concentration in µg/ml and y is the peak area in absorbance units; the correlation coefficient was 1.0000.

Limit of detection and Limit of quantitation - The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal to noise (S/N) ratio of the LOD preparation and LOQ preparation. LOQ value is precised by replicate injections and checked for linear response with respect to other linearity levels by extended linearity curve.

PRECISION - Precision study was established by evaluating method precision and instrument precision study. Method precision of the analytical method was determined by analyzing six sets of sample preparation. Assay of all six replicate sample preparations was determined and mean, standard deviation, % relative standard deviation was calculated.

Instrument precision – Instrument precision study was carried out with the help of six replicate injection of standard preparation of 10µg/ml and calculate the average, standard deviation and %RSD.

Method precision – Method precision study was carried out with the help of six replicate injection of As such sample preparation of 1000µg/ml and calculate the average, standard deviation and %RSD.

Solution stability - Stability in solution was evaluated for the standard solution and the test preparation. The solutions were stored at ambient temperature. Without protection of light and tested after 12, 24, 36 and 48 h. the responses for the aged solution were evaluated by comparison with freshly prepared solutions. During study of the stability of stored solutions of standards and test preparations for assay determination the solutions were found to be stable for up to 36 h. Table shows the summary of solution stability study.

System Suitability Parameters: System suitability was performed by injecting three replicate injections of 100% test concentration, number of theoretical plates, asymmetry factor was satisfactory. The chromatographs confirm the presence of Teneligliptin at 13.66 min without any interference.

Robustness - Robustness was verified by altering the chromatographic conditions like mobile phase composition, flow rate, pH, temperature, detection wave length, etc. and the % RSD should be reported. In the operational conditions small changes were allowed and the extent to which the method was robust was determined. A deviation of ± 0.2 in the pH, and ± 0.2 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate.

III. RESULTS AND DISCUSSION

Table 2. Result Obtained During Forced Degradation

Sr. No.	Experimental condition	Sampling time	Observed degradation
1	Acidic condition 1M HCL Sample	For 2hours	0.30%
	5M HCL Sample	For 1hour	5.03%
2	Alkaline condition		
	1N NaOH	For 30min	17.36%
	1N NaOH	For 1hour	33.52%
	1N NaOH	For 2 hours	44.02 %
3	Peroxide condition		
	1% H2O2	For 15min	27.39%
	1% H2O2	For 2hours	46.42%
4	Thermal solid sample	85 degree 24hours	0.37%
5	Thermal diluted sample	85 degree 24hours	2.13%
6	Photolytic degradation	24hours	0.03 %

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Table 3. Observation Table of Linearity Study

Linearity Level	Concentration in PPM	Average area	
0.01%	0.1	958	
0.1%	1.01	11714	
0.5%	5.04	65305	
1%	10.07	107284	
5%	50.35	566989	
10%	100.7	1046158	
25%	251.75	2593829	
50%	503.5	5137767	
75%	755.25	7833637	
100.00%	1007	10269776	
125.00%	1263	12751305	
150.00%	1500	15299102	
	Slope	10166.16778	
	Correlation coefficient	1	
	Y intercept	25073.34498	

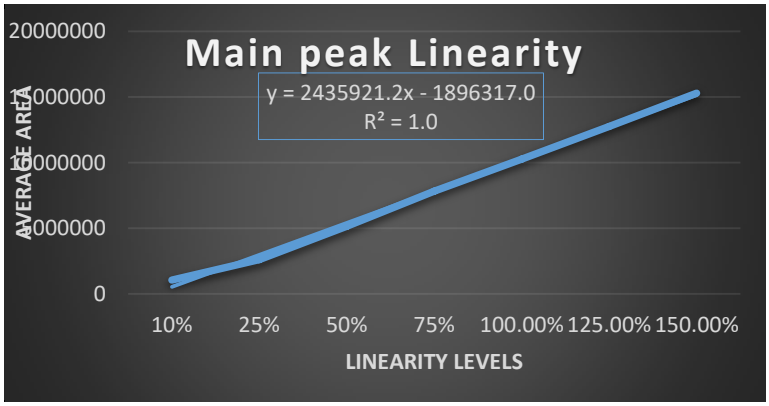

Figure 2: Main peak linearity graphical representation

Table 4: Result of LOD and LOQ by HPLC Method

Sr. No	Conc%/(PPM)	Injection 1	injection 2	injection3	average	STD.DEV.	%RSD	S/N Ratio	
1	5ppm (0.5%)	66525	63538	65851	65304.7	1566.653	2.40	383.00	LOQ
2	1ppm (0.1%)	11714	11523	11256	11497.7	230.0485	2.00	24.00	LOD
3	0.1ppm (0.01%)	810	1101	964	958.3	145.5827	15.19	4.00	

Table 5: Observation of instrument precision

Instrument precision		
Sr. No.	Inj. No.	Area
1	STD. Sample 1	99502
2	STD. Sample 2	99141

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3	STD. Sample 3	99318
4	STD. Sample 4	98513
5	STD. Sample 5	98613
6	STD. Sample 6	100499
	Average	99264.33333
	STD. Dev.	719.4046613
	%RSD	0.724736305
	Acceptance critaria	≤ 2%
	result and evaluation	complies

Table 6: Observation of method precision

Method precision		
Sr. No.	Inj. No.	Main peak area percentage
1	As such sample 1	98.88
2	As such sample 2	98.87
3	As such sample 3	98.87
4	As such sample 4	98.86
5	As such sample 5	98.90
6	As such sample 6	98.92
	Average	98.88
	STD. Dev.	0.023
	%RSD	0.023
	Acceptance critaria	≤ 2%
	Result and Evaluation	Complies

Table 7: Observation of system suitability

Parameter	**Observed value**	**limit**
No. of theoretical plates	59612	< 2000
Tailing factor	1.54	< 1.75

Table 8: Observation of solution stability

STANDARD SOLUTION STABILITY			
Injection time(Hr)	Time(Hr)	Inj. No.	Area
15-07-2023	0	Injection 1	101678
		Injection 2	100681
		Average	101179.5
16-07-2023	14	Injection 1	100562
		Injection 2	100396
		Average	100479
		% Deviation	0.69

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17/072023 18:00	51	Injection 1	100209
		Injection 2	100094
		Average	100151.5
		% Deviation	1.02

Table 9: Observation For Robustness Study

Observation for robustness study								
Sr. No.	Parameter	Condition	peak area			Average area	STD.DEV	%RSD
1	Flow Rate (ml/min) ±0.2mL	0.8	10317309	10290907	10302410	10303542	13237.35	0.13
		1	10323541			10323541		
		1.2	10303779	10292009	10297805	10297864.33	5885.224	0.06
2	Temperature (±0.2°C)	28	10307419	10322470	10327823	10319237.33	10579.15	0.10
		30	10491192			10491192		
		32	10455290	10396671	10365421	10405794	45623.8	0.44
3	pH (±0.2)	2.8	10327823	10297805	10303779	10309802.33	15889.63	0.15
		30	10299761			10299761		
		3.2	10377895	10365421	10323541	10355619	28471.89	0.27

Forced Degradation Stability Indicating Studies :

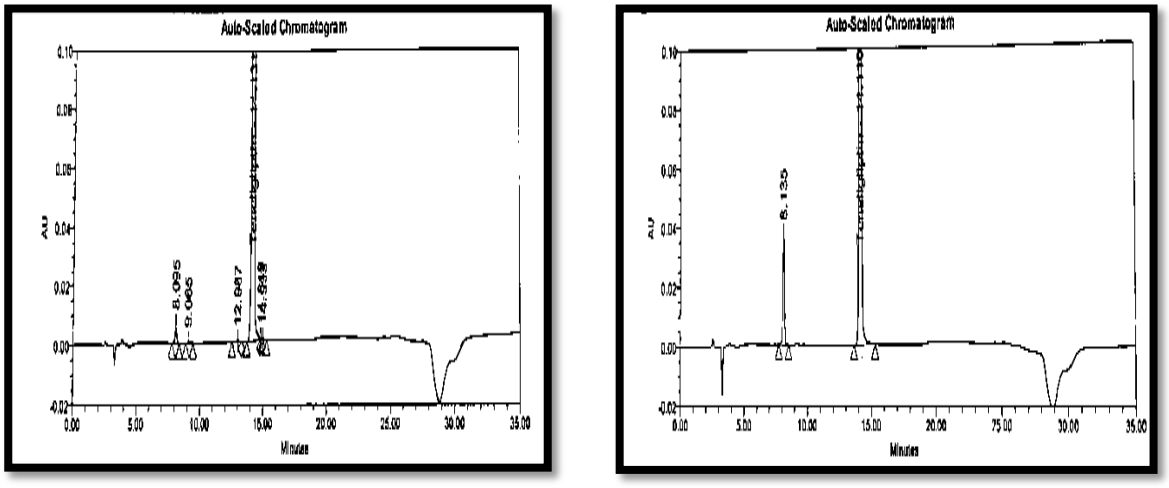

Figure 3: Acidic condition 1M and 5M HCL Sample for 2hour stress degradation chromatogram.

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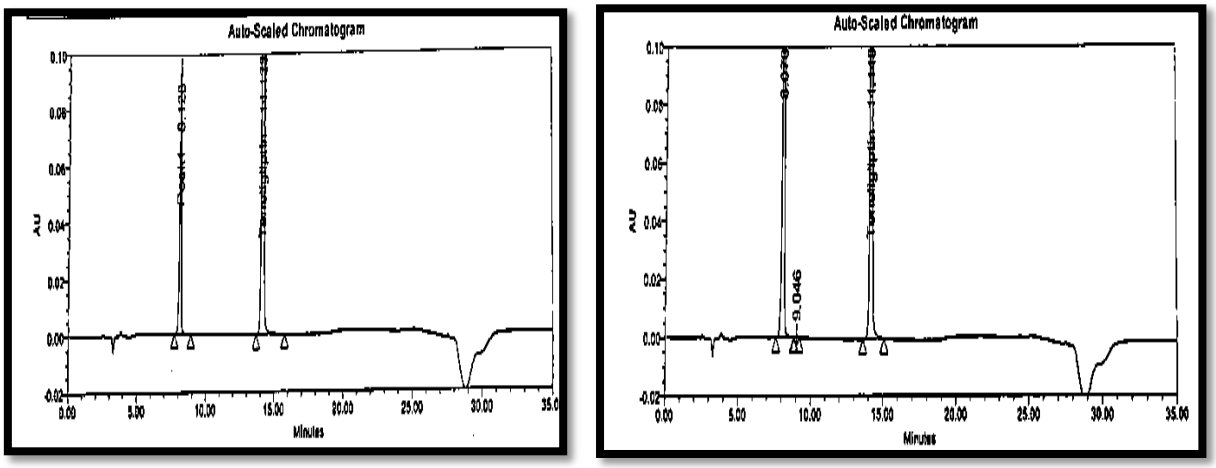

Figure 4: Alkaline condition 1N NAOH Sample for 30min and 2hour degradation chromatogram.

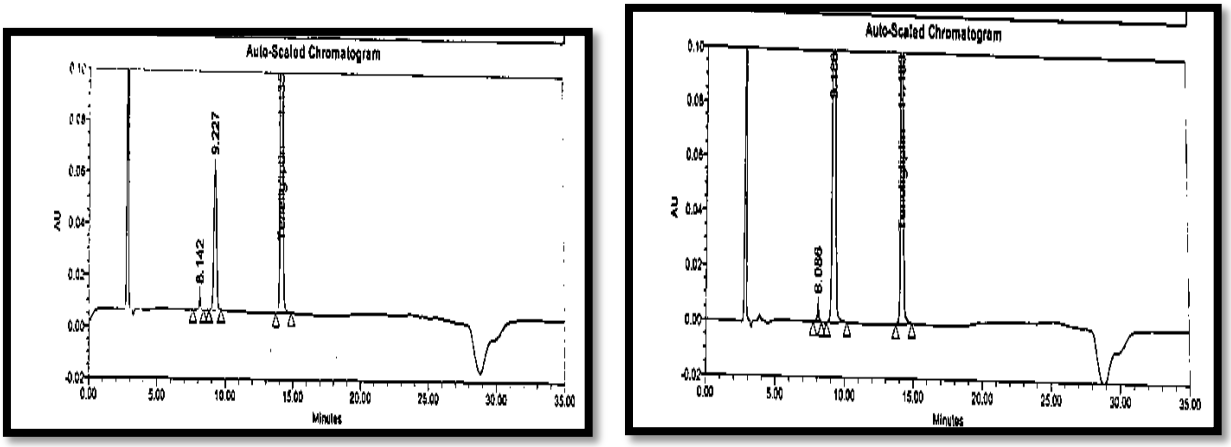

Figure 5: Peroxide condition 1% H2O2 Sample for 15min and 2hrs. degradation chromatogram.

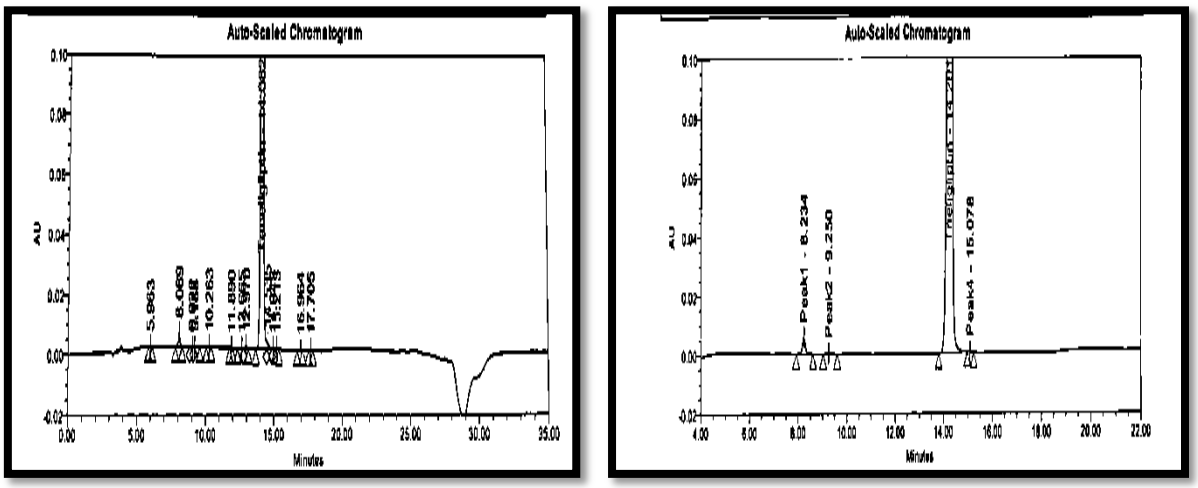

Figure 6: 24 hours thermal and photolytic degradation chromatograms

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Chromatograms obtain during Main peak linearity -

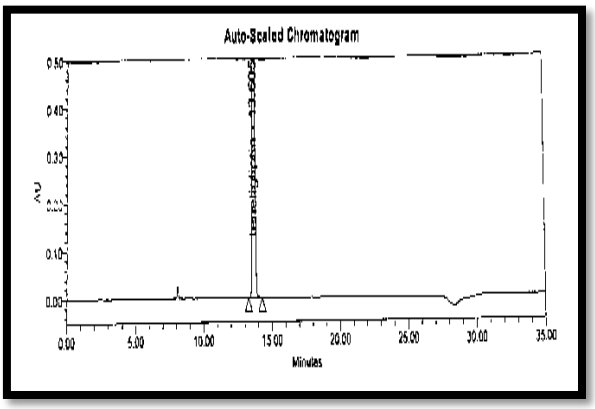

Figure 7: Linearity level 150%

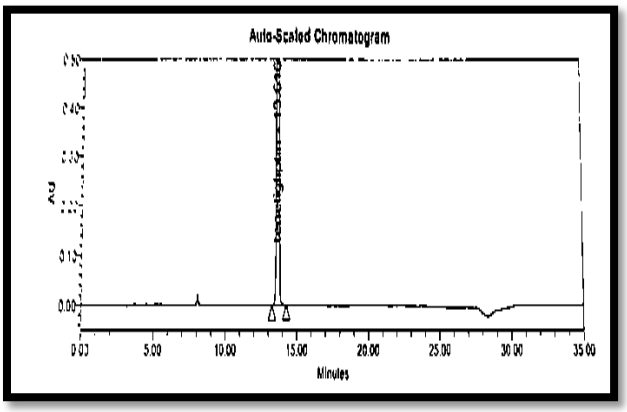

Figure 8: Linearity level 125%

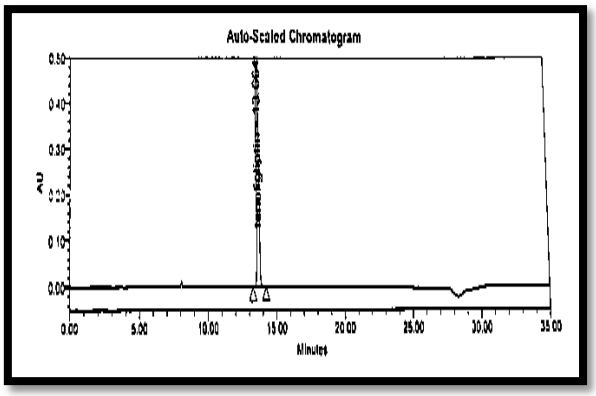

Figure 9: Linearity level 100 %

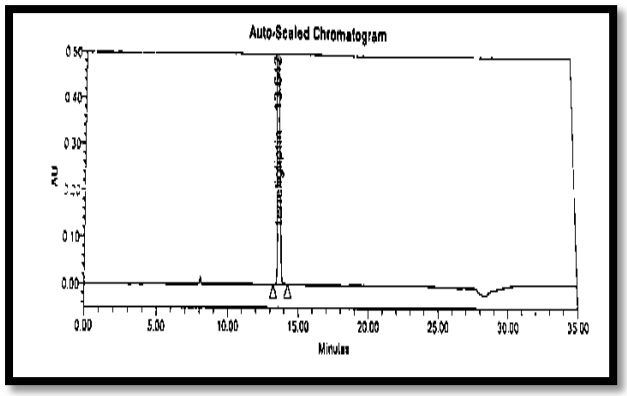

Figure 10: Linearity level 75%

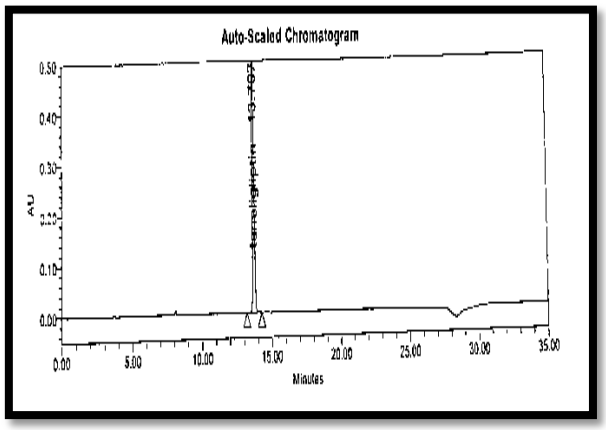

Figure 11: Linearity level 50%

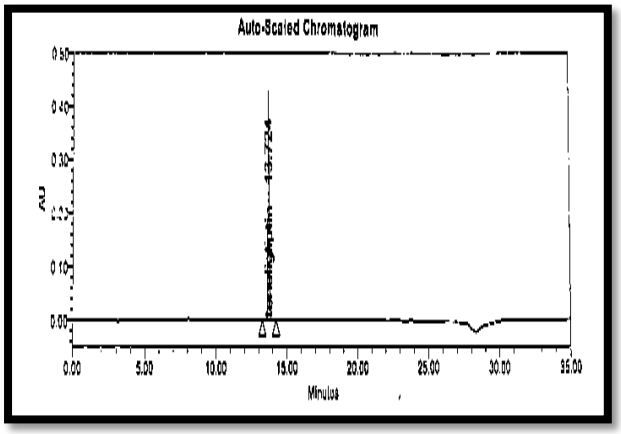

Figure 12: Linearity level 25%

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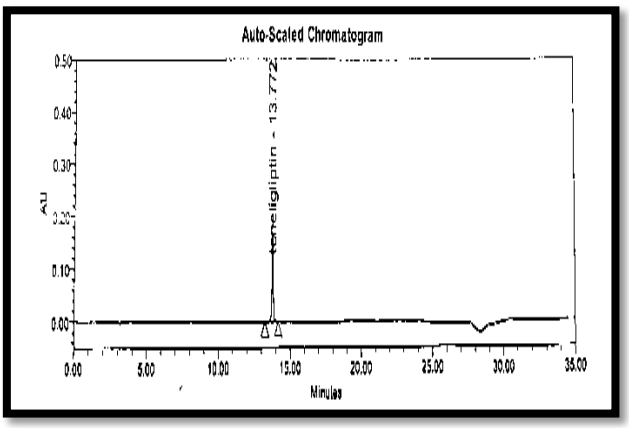

Figure 13: Linearity level 10%

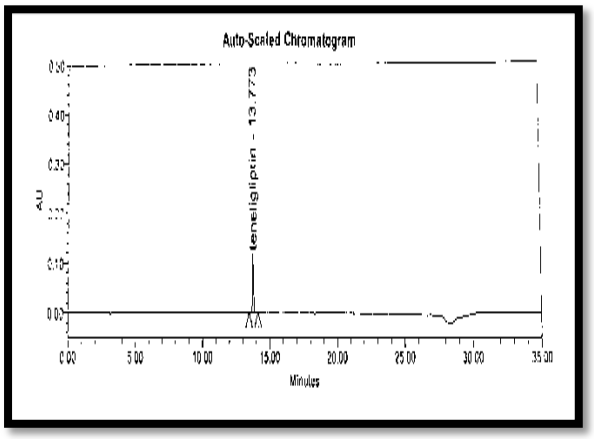

Figure 14: Linearity level 5%

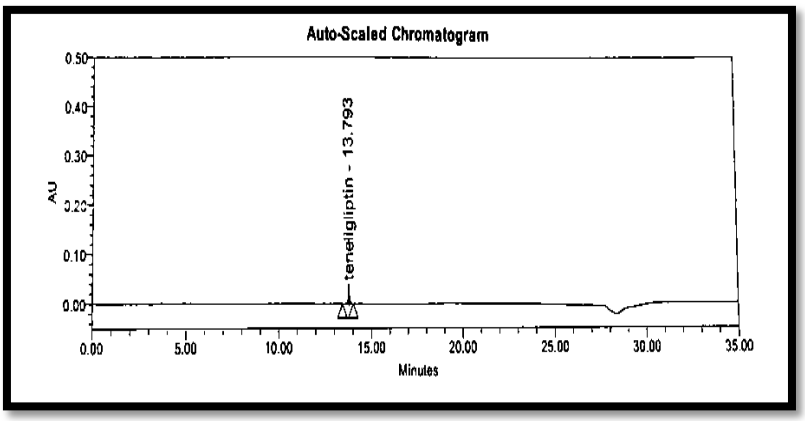

Figure 15: Linearity level 1%

CHARACTERIZATION CHROMATOGRAMS OF DEGRADATION DRUG:

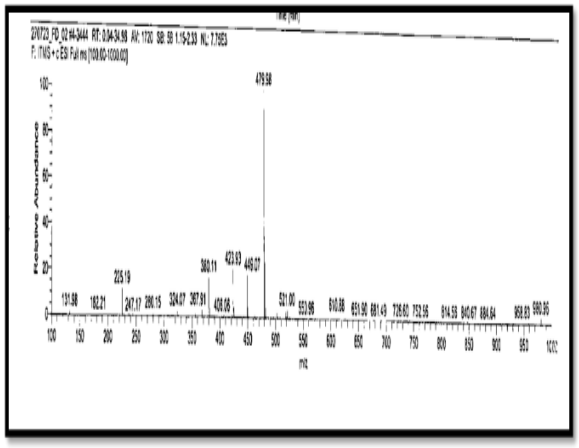

Figure 16: LC/MS/MS-ESI data of blank solution

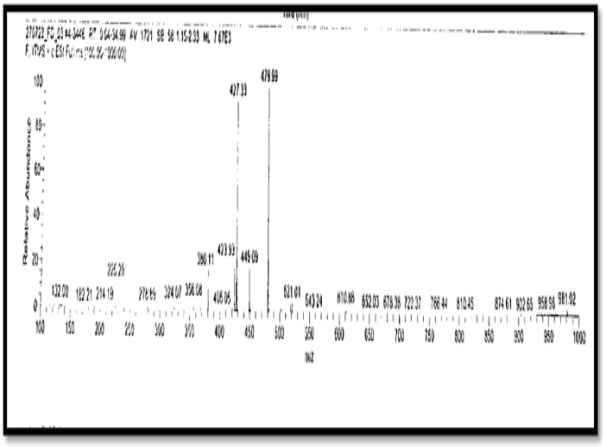

Figure 17: LC/MS/MS-ESI data of As such solution

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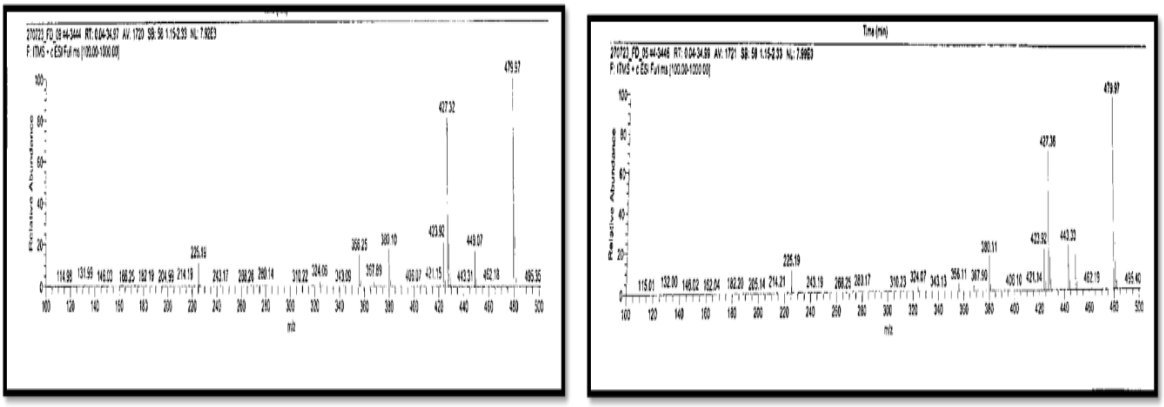

Figure 18: LC/MS/MS-ESI Data of Teneligliptin Degradation Under Base Stress and peroxide stress

IV. CONCLUSION

The method developed possesses all the qualities to be a reliable, rapid, sensitive, specific, and economical method according to the above discussed results and data. The study showed the stability indicating nature of the method with the possible short runtime. Hence, the developed method could be conveniently and effectively used for routine simultaneous estimation of Teneligliptin in bulk pharmaceutical form.

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