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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR THESIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND ROSUVASTATIN IN TABLET DOSAGE FORM

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ABSTRACT

A simple, economical, precise, and accurate reverse phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated to estimate teneligliptin and rosuvastatin in a tablet dosage form. A RP-HPLC analysis was performed on ECO-C18 5µ (15mm*4.6mm*5µ (particle size) column with using mobile phase buffer pH 6.8: ACN 75:25 (v/v) and 1 ml of triethylamine at 280 nm detection wavelength with the flow rate of 1.0 mL/min. The analytical method was validated as per International Council for Harmonization (ICH) guidelines. The linearity was observed in the Limit of Quantitation (LOQ)- 0.258 µg/ml range for teneligliptin. Similarly, the LOQ-0.129 µg/ml range was observed linearity for rosuvastatin. The Limit of Detection (LOD) value was found to be 0.853 µg/mL for teneligliptin and 0.427 µg/mL for rosuvastatin. The correlation coefficient was 0.99 for both teneligliptin and rosuvastatin. The %recovery value was found to be a minimum of 100.27% and a maximum of 101.64% for teneligliptin. Similarly, the %recovery value was found to be a minimum of 100.31% and a maximum of 101.60% for rosuvastatin. The relative standard deviation value for repeatability, interday precision, and intraday precision was less than 2%. Forced degradation studies was also applied to these drugs in a various condition like acidic, basic, oxidation, thermal and photolytic. During stability studies, it was found that

Teneligliptin is degraded more in oxidative condition (11.33%) and Rosuvastatin in acidic condition. (20.31%). The proposed method was found to be specific, sensitive, precise, accurate, and robust in nature

Keywords: Teneligliptin, Rosuvastatin, stability indicating RP-HPLC method, validation

INTRODUCTION:

Teneligliptin and Rosuvastatin both in combination used for the treatment of diabetes and Cholesterol **[1-2]** teneligliptin and rosuvastatinCombination was approved by CDSCO at 12/01/2022. Gliptins also known as DPP-IV(Dipeptidyl peptidase-4) Inhibitors a new class of medication for the treatment of Type 2 Diabetes. Now a days gliptins are the focal point for research work **[3-4]**. Teneligliptin **(Figure 1)** is the DPP-4 inhibitor which is extremely potent, focused, and long-lasting **[5]**. As ingestion of food Glucagon-like peptide-1 (GLP-1), a peptide released from the GIT improves insulin release and inhibits pancreatic glucagon release, playing a crucial role in regulating postprandial blood sugar level **[6-8]**. Natural molecule, DPP-4 inhibitor degrades the peptide, and inactive them. DPP-4 inhibitor degradation increases the blood's concentration of GLP-1, which strengthens the release of glucose-dependent insulin and, at the same time, stifles glucagon emission, demonstrating a glucose-lowering effect **[9-11]**. Type 2 diabetes is successfully treated with it. The most common negative reactions are hypoglycemia, congestion, and sensation of the developing stomach area, nausea, stomach pain, meteorism, stomatitis, skin irritation, rash, pruritus, dermatitis, and unease **[12]**.

Rosuvastatin **(Figure 2)** has antilipidemic effects **[13]**. The enzyme that catalysis the conversion of HMG-CoA to mevalonate **[14]**, a precursor to cholesterol, is known as hepatic hydroxymethyl-glutaryl coenzyme A (HMG-CoA) Reductase, and rosuvastatin specifically and competitively binds to it and inhibits it **[15-16]**. As a result, the level of hepatic cholesterol falls and LDL (low density lipoprotein) cholesterol is taken up more readily **[17-19]**. There is no analytical method for simultaneously estimating teneligliptin and rosuvastatin in a formulation, according to literature review. A straightforward, accurate, and exact stability indicating RP-HPLC technique was developed.

To determine the stability of molecule, studies of degradant product is the most important and force degradation study involves the degradation of drug product and drug substance according to ICH guidelines **[20-21]**. according to this guideline stress testing is used to determined degradation product which further helps to determine

intrinsic stability of drug **[22-23]** and identified the possible degradation pathways **[24-25]**. Knowledge about the stability helps to choose proper ingredients for formulation, packaging and providing proper storage condition it is also used for the determination of shelf life of drugs which is necessary for the regulatory documentation **[26-28]**. ICH guideline includes the force degradation study under certain conditions like pH, light, oxidation, acidic, basic and hydrolysis etc. it's also given guidance for the separation drug from the impurities **[29-31]**.

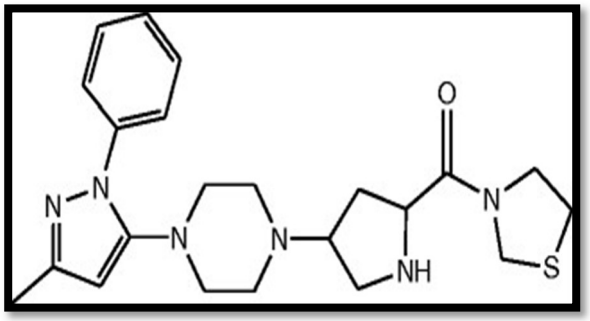


Figure 1: Structure of Teneligliptin

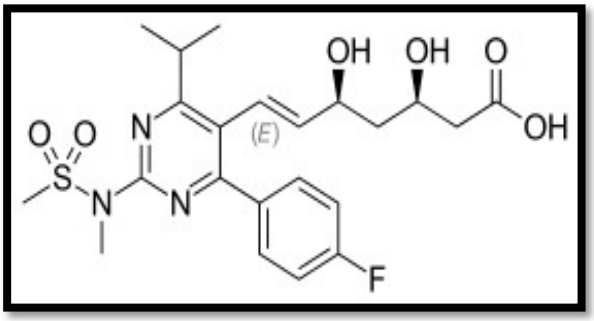


Figure 2: Structure of Rosuvastatin

EXPERIMENTAL

Materials

Raw material of rosuvastatin and teneligliptin were gifted by the Glenmark., HPLC grade methanol, acetonitrile and water purchase from Finar Pvt. Ltd

Instrumentation

Analytical MC-20 AT HPLC chromatographic system, Agilent digital weighing balance (ATX 224), JSK Scientific Pvt. Ltd pH meter, Frontline Ultrasonic Cleaner ultra-Sonicator, hot air oven, and Thermolabile pune, were used for the method development. A 0.45µ Millipore filter was used for filtration

Selection and Detection of Wavelength

The selectivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the selected drug that is to be detected not interference of solvent effect. It gives maximum absorbance at 280nm in ACN: Methanol (50:50 %v/v) at 280nm drugs gives good peak point and not interference of solvent effect so; this wavelength was selected for estimation of teneligliptin and rosuvastatin. Chromatogram shown in **(Figure 3)**.

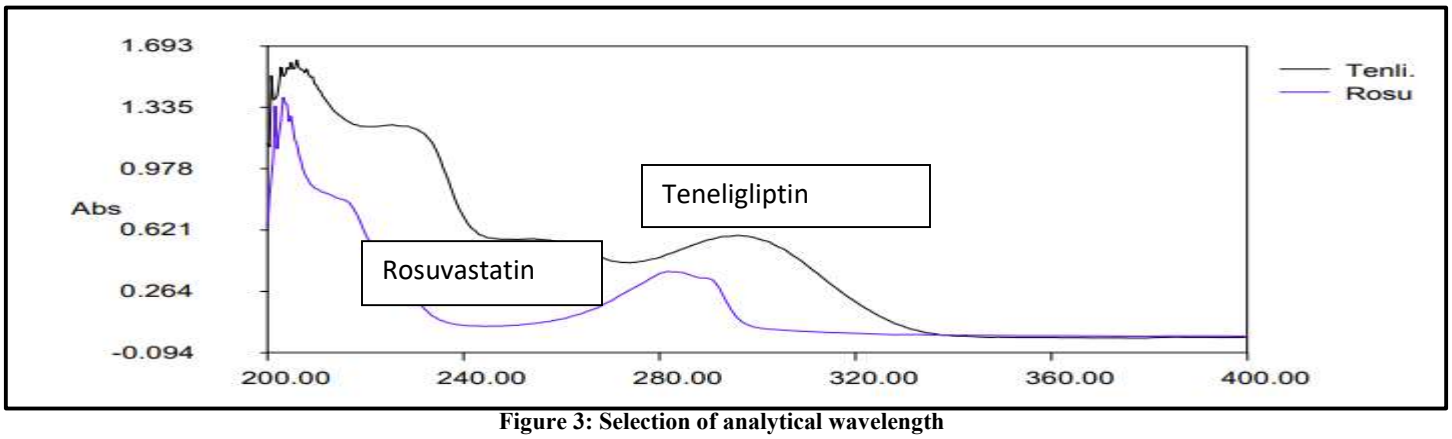


Figure 3: Selection of analytical wavelength

Selection of mobile phase

The potassium dihydrogen phosphate was prepared by dissolving accurately weight 6.8gm of potassium dihydrogen phosphate in 1000ml HPLC grade water in a 1L volumetric flask and pH was adjusted to pH 6.8 with ortho phosphoric acid. The prepared buffer pH was checked by using pH meter by ultra sonicating for 5 min solution degassed and obtained solution was filtered through 0.45µ millipore filter and mobile phase is prepared with ratio of buffer (pH 6.8): Acetonitrile (75:25 v/v).

Standard solution preparation

Preparation of standard stock solution of Teneligliptin

Preparation of Standard Stock Solution of Teneligliptin (25 ppm): Prepared the standard stock solution of teneligliptin by accurately weighing 20 mg of teneligliptin bulk drug into a 100 mL volumetric flask and dissolved in solvent (50:50 ACN: Methanol), and it make up to the mark to get concentration of 200 µg/mL of teneligliptin Standard Stock Solution. For the preparation of 20µg/ml (20 ppm) of teneligliptin Working Standard Solution. The teneligliptin Standard stock solution accurately took 1 mL into 10 mL volumetric flask, and the volume was made up with the mobile phase

Preparation of standard stock solution of Rosuvastatin

Preparation of Standard Stock Solution of rosuvastatin (10 ppm): Prepared the standard stock solution of rosuvastatin by accurately weighing 10 mg of rosuvastatin bulk drug into a 100 mL volumetric flask and dissolved in solvent (50:50 ACN: Methanol), and it make up to the mark to get concentration of 100 µg/mL of rosuvastatin Standard Stock Solution. For the preparation of 10µg/ml (10 ppm) of rosuvastatin Working Standard Solution. The rosuvastatin Standard stock solution accurately took 1 mL into 10 mL

volumetric flask, and the volume was made up with the mobile phase

Method validation

The purposed method was validated as per ICH guidelinesQ2 (R1) **[32]**

The method was validated by the different parameters such as Accuracy, Precision, Linearity, LOD, LOQ, Robustness.

Linearity and Range

The linearity of a method is measured to see how well a calibration plot of response vs. concentration approximates a straight line. Calibration curve from working solution containing 200µg/mL teneligliptin and 100µg/mL rosuvastatin Aliquots0.5 ml, 0.75 ml, 1 ml, 1.25 ml and 1.5 ml were transferred in clean and dry 10mL volumetric flask respectively and sonicated. The volume was made up to the mark with diluents. This yielded solution of 10, 15, 20, 25 and 30ppm for teneligliptin and 5, 7.5,10, 12.5 and 15 ppm for rosuvastatin. An aliquot (20 µl) of each solution was injected under the operating chromatographic condition as described earlier calibration curve was prepared by plotting peak areas versus concentration and the regression equation was calculated each response was average of three determinations.

Precision

System precision was performed by injecting six replicates of a standard solution containing teneligliptin (20.0 µg/mL) and rosuvastatin (10.0 µg/mL) and chromatograms were recorded and areas of peaks were measured to calculate results of repeatability. A standard solution containing (10, 20 and 30 µg/mL) of teneligliptin as well as standard solution containing (5, 10, 15 µg/mL) of rosuvastatin were assessed on different day for three times in interday precision and the same day in intraday precision and %RSD was calculated.

Accuracy

To check the accuracy of the proposed method for determination of teneligliptin and rosuvastatin recovery studies were carried out at80, 100 and 120% of the test concentration according to ICH guidelines. The recovery study was performed three times at each level.

LOD and LOQ

LOD (Limit of Detection) and LOQ (Limit of Quantification) for both the drugs were estimated using the linearity data. Then LOQ and LOD were calculated with formula given below.

LOD=3.3(SD/Slop of calibration curve), LOQ=10 (SD/Slop of calibration curve)

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing three small changes.

- Change flow rate by 10%. (i.e., 0.8ml/min and 1.2 ml/min)
- Change in mobile phase by 2ml
- Change in pH by 0.2unit (i.e., pH 6.6. and pH 7)

Force degradation studies

Force degradation study was intended to ensure the effective separation of teneligliptin and rosuvastatin and their potential degradation products which are generated under different condition as like acid hydrolysis, alkali hydrolysis, and oxidative degradation, photolytic and thermal degradation.

Acid hydrolysis

Individually and combination 20 mg and 10mg accurately weighed amount of teneligliptin and rosuvastatin were transferred in to 100 mL volumetric flask. Dissolve with 5mL of ACN: methanol (50:50 v/v) and dilute up to with 0.1N HCl (200µg/ml and 100 µg/ml) and it was kept at R.T. for 4 hr. from this solution.1ml was taken and transferred into 10 ml volumetric flask and neutralized with 0.1 N NaOH and diluted up to with mobile phase (20ppm and10ppm). The peak area and shape were observed under optimized chromatographic conditions.

Alkali Hydrolysis

Individually and combination 20 mg and 10mg accurately weighed amount of teneligliptin and rosuvastatin were transferred in to 100 mL volumetric flask. Dissolve with 5mL of ACN: methanol (50:50 v/v) and dilute up to with 0.1N NaOH (200µg/ml and 100 µg/ml).and it was kept at room temperature for 4 hr. from this solution.1ml was taken and transferred into 10ml volumetric flask and neutralized with 0.1 N HCl and diluted up to with mobile phase (20ppm and10 ppm). Chromatogram was recorded to check the peak shape and peak area.

Oxidative Hydrolysis (3%H_2O_2)

Individually and combination 20 mg and 10mg accurately weighed amount of teneligliptin and rosuvastatin were transferred in to 100 mL volumetric flask. Dissolve with 5mL of ACN: methanol (50:50 v/v) and dilute up to with 3% H_2O_2 (200µg/ml and 100 µg/ml) and it was kept at R.T. for 8 hr. from this solution.1ml was taken and transferred into 10ml volumetric flask and diluted up to with mobile phase (20ppm and10 ppm). Chromatogram was recorded to observed peak shape and peak area.

Thermal degradation

Individually and combination 20 mg and 10mg accurately weighed amount of teneligliptin and rosuvastatin were transferred in to 100 mL volumetric flask. Dissolve with 5mL of ACN: methanol (50:50 v/v) and it was kept at 80°C. For 12 hr. from this solution 1ml

was taken and transferred into 10ml volumetric flask and diluted up to with mobile phase (20ppm and10 ppm). Chromatogram was recorded to check peak area and peak shape.

Photo stability

Individually and combination 20mg and 10mg accurately weighed amount of teneligliptin and rosuvastatin were transferred in to Petri dish and it was kept in UV chamber 254nm for 24 hr. After it were Dissolve and make up to with ACN: methanol (50:50 v/v). From this solution 1ml was taken and transferred into 10ml volumetric flask and diluted up to with mobile phase. This generated to 20ppm and10ppm. Chromatogram was recorded.

RESULTS AND DISCUSSION

Method Validayion

The proposed method was validated with linearity, accuracy, precision, specificity, system suitability, LOD and LOQ, and robustness.

After a series of trials, the final chromatographic conditions were determined as follows in **Table 1**.

Table 1: Optimized Chromatoraphic Condition

Parameters	Conditions
Mobile Phase	Buffer pH 6.8: ACN 75:25 (v/v) + 1ml of triethylamine
Stationary Phase	ECO-C18 5µ (15mm*4.6mm*5µ (particle size)
Flow rate	1 ml/min
Run time	15 min
Volume of injection	20 µl
Detection of wavelength	280 nm

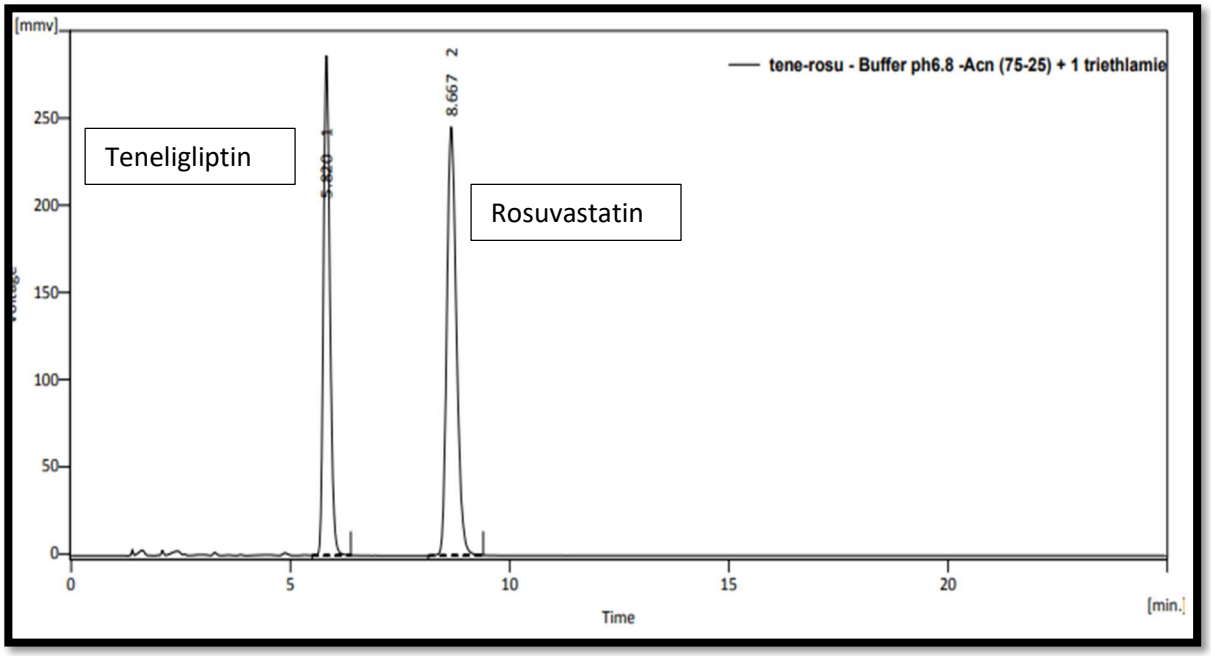


Figure 4: Optimized Chromatogram of Teneligliptin and Rosuvastatin

System Suitability

The System Suitability was calculated from different parameters like retention time, theoretical plates, resolution, and tailing factor. System suitability was used to verify the repeatability and resolution of the system were sufficient for the analysis intended. The system suitability parameters observed for teneligliptin and rosuvastatin shown in **(Table 2)**.

Specificity

Specificity was established by studying the resolution factor of drug peaks from the nearest resolving peak and among all other peaks. The specificity of method was evaluated by comparison between chromatogram of standard and test solution. There should be absence of any interfering peak with analyze peak **(Figure 5, 6 and 7)**.

Linearity

The linearity for teneligliptin and rosuvastatin were assessed by analysis of combined standard solution in a range of 10 to 30 µg/mL and 5 to 15 µg/mL, respectively. The correlation coefficient for the calibration curve of teneligliptin and rosuvastatin was found to be NLT 0.999, respectively **(Table 3)** and calibration curve of teneligliptin and rosuvastatin shown in **(Figure 8 and 9)**.

Table 2: System suitability parameters of Teneligliptin and Rosuvastatin

Parameters	Teneligliptin	Rosuvastatin
Retention time	5.820	8.667
Theoretical plates	7330	7224
Tailing factor	1.34	1.35
Resolution	8.37	

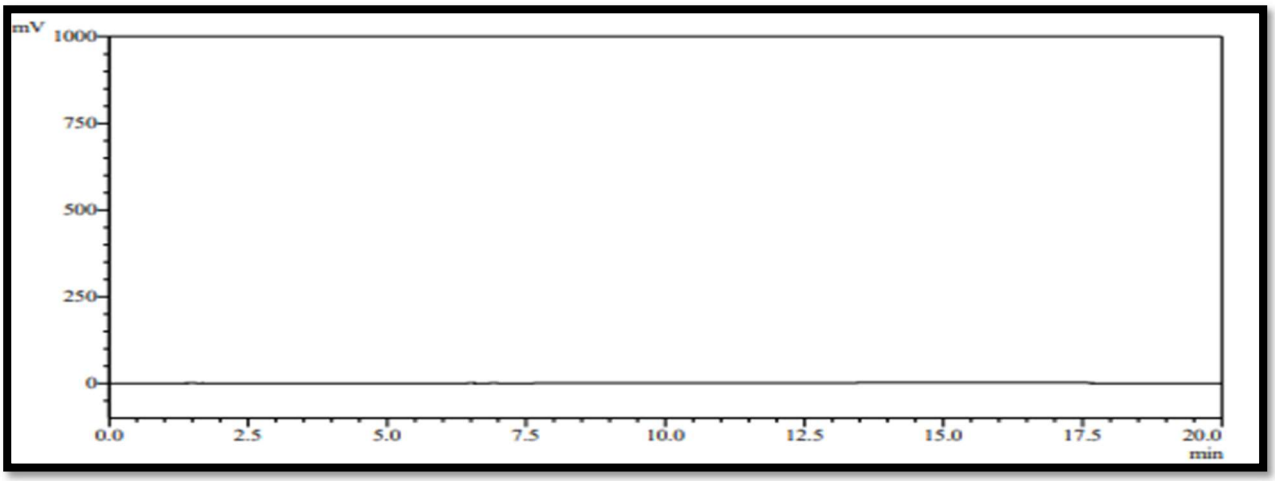


Figure 5: Blank Chromatogram of Teneligliptin and Rosuvastatin

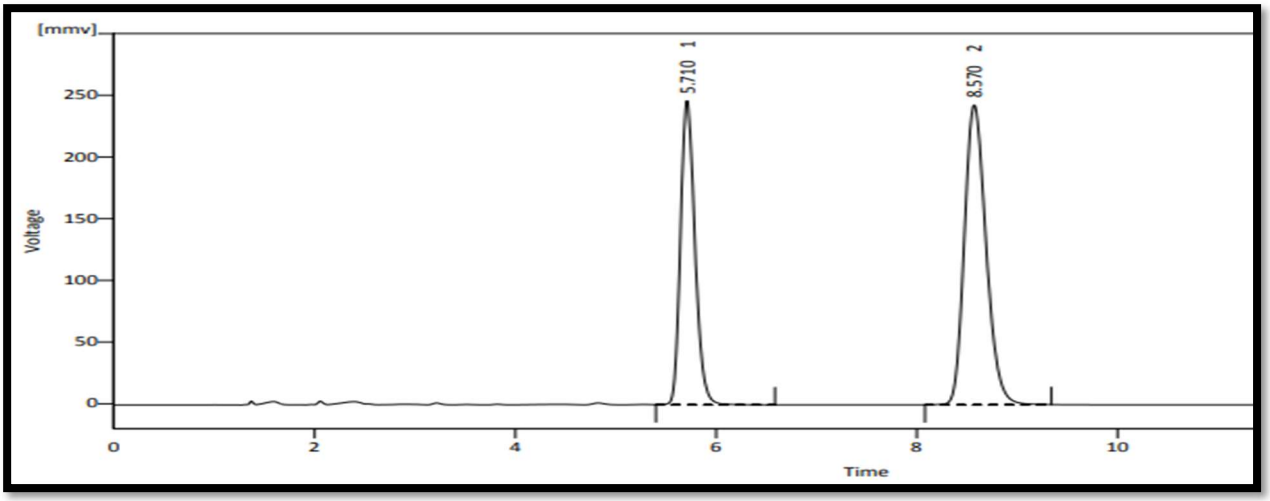


Figure 6: Chromatogram of teneligliptin and rosuvastatin Standard

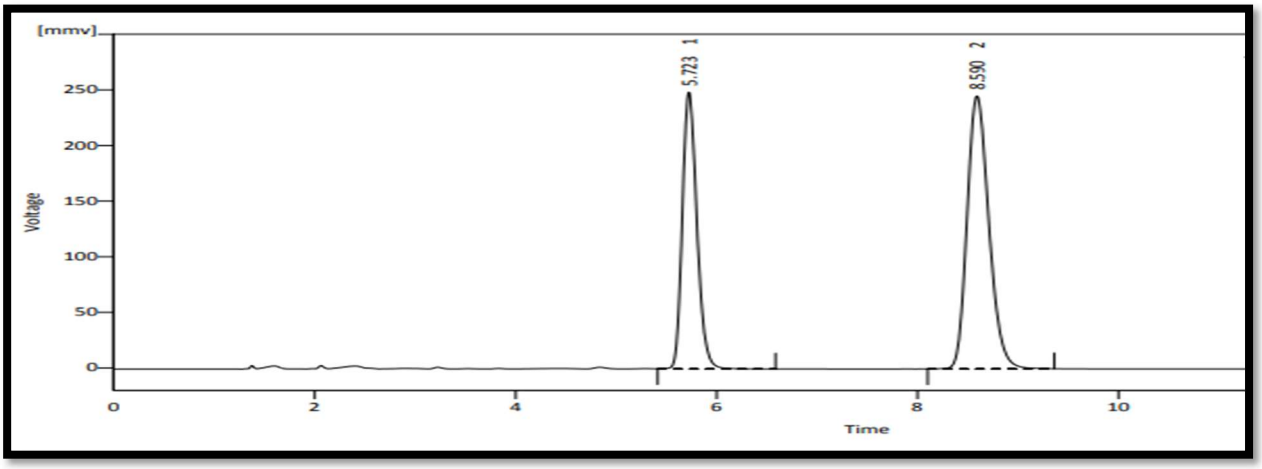


Figure 7: Chromatogram of teneligliptin and rosuvastatin Sample

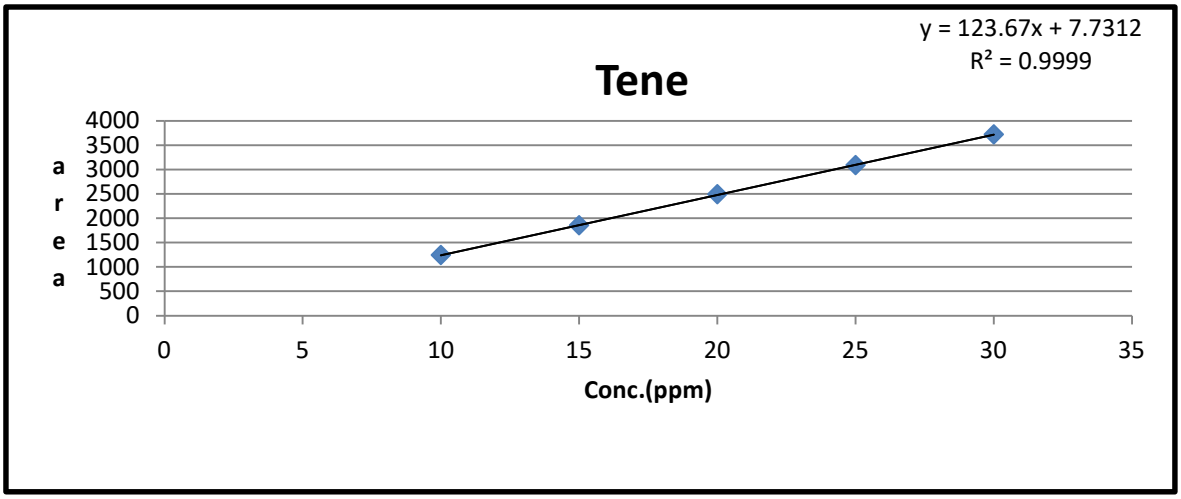
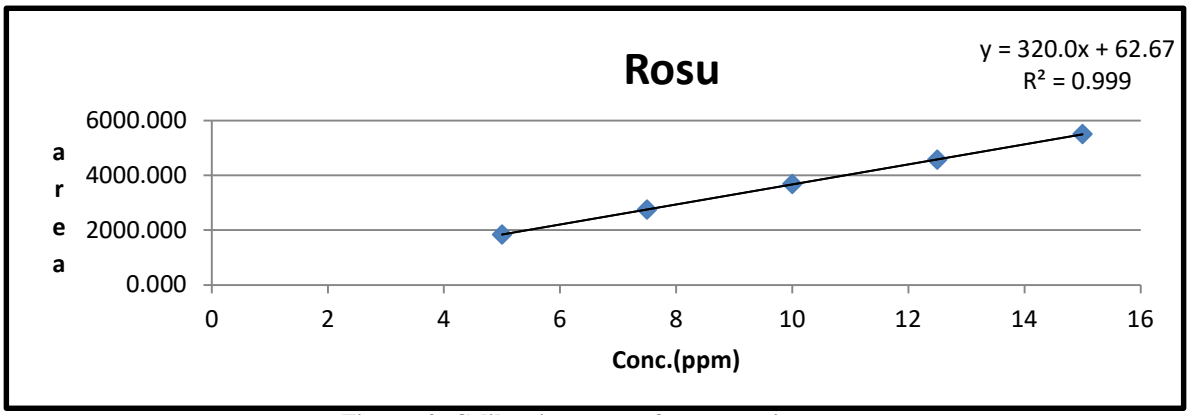


Figure 8: Calibration curve of teneligliptin



Figurer 9: Calibration curve of rosuvastatin

Table 3: Analytical data of calibration curve

Teneligliptin		Rosuvastatin	
Concentration (µg/ml)	Area ± S. D	Concentration (µg/ml)	Area± S. D
10	1245.432± 7.53	5	1841.110± 0.97
15	1856.420± 3.09	7.5	2744.700±1.88
20	2493.344± 2.45	10	3686.669± 0.34
25	3090.268± 1.06	12.5	4569.312± 0.57
30	3720.28± 0.90	15	5500.779± 0.74
SD	9.687	SD	14.343
Correlation Coefficient (r)	0.99	Correlation Coefficient (r)	0.99

Precision

Repeatability

The repeatability data of peak area measurement for teneligliptin and rosuvastatin based on six measurements of same solution. The mean area observed 2656.79 for teneligliptin and 3928.63 for rosuvastatin with %RSD 0.35 for both. The repeatability shows that the % RSD values observed within the acceptance limit of NMT 2% results shown in **(Table 4)**.

Interday and intraday precision

The data for inter-day precision as well as intraday precision for teneligliptin and rosuvastatin is shown in **(Table 5 and 6)**. The %RSD calculated and all values are within the acceptance limit. Hence the method is précised.

Accuracy

To check the accuracy of the proposed method for determination of teneligliptin and rosuvastatin, recovery studies were carried out at 80, 100, and 120% of the test concentration according to ICH guidelines. The method accuracy was established by a recovery study from marketed formulation at three levels of standard addition. The percentage recovery for teneligliptin was 100.27 to 101.64%, and the Percentage recovery for rosuvastatin was 100.31 to 101.60% **(Table 7)**.

LOD and LOQ

Limit of detection and limit of quantitation for both the drugs were estimated using the linearity data **(Figure 5 and 6)**. Repeated calibration curve 5 times and calculated standard deviation of the intercepts. The limit of detection for teneligliptin was observed 0.258 µg/mL, for rosuvastatin observed 0.129 µg/mL, However, the LOQ for teneligliptin observed 0.853 µg/mL, for rosuvastatin observed 0.427 µg/mL. Results were shown in **(Table 8 and 9)**.

Robustness

The robustness study was carried out to assessed the influence of small but deliberate variations in the chromatographic conditions. The chromatographic factors as ratio of Mobile phase were changed ±2mL, flow rate of mobile phase ± 0.2 mL/min, was changed and pH was changed ±2 and effect were observed on system suitability for standard preparation. The results show that the changing effect was found to be within the acceptance criteria and the % RSD values were observed within the standard limit of not more than 2% **(Table 10)**.

Forced Degradation Studies

The sample was injected under various stress conditions. The acidic degradation, base degradation, oxidative degradation, thermal degradation and photodegradation were performed as per procedure, and %degradation was calculated from the chromatographic peaks shown in **(Figure 10 to 14)**. The details of %degradation is given in the **(Table 11)**. However, Teneligliptin degraded more in oxidative degradation and rosuvastatin degraded more in acidic condition.

Analysis of marketed formulation

An aliquot of 20µL from sample solution was injected under chromatographic condition and peak area measured and % assay was calculated from regression equation. Response was average of six determinations. The results are shown in **Table 13**.

Table 4: Repeatability Data

Teneligliptin					Rosuvastatin				
Sr no.	Conc.	Area	Mean ± S. D (n=6)	% RSD	Sr no.	Conc.	Area	Mean ± S. D (n=6)	% RSD
1	20µg/ml	2646.654	2656.79 ±9.26	0.348	1	10 µg/ml	3913.566	3928.63 ± 13.71	0.349
2		2652.821			2		3922.744		
3		2646.578			3		3913.544		
4		2666.994			4		3943.698		
5		2662.808			5		3937.563		
6		2664.885					3940.640		

Table 5: Analytical data of intraday precision

Teneligliptin				Rosuvastatin			
Sr no.	Conc. (µg/ml)	Area Mean ± S. D (n=6)	% RSD	Sr no.	Conc. (µg/ml)	Area Mean ± S. D (n=6)	% RSD
1	10	1282.44 ± 1.85	1.45	1	5	1895.807 ±2.79	0.148
2	20	2528. 37 ± 0.97	0.039	2	10	3738.513 ± 1.447	0.039
3	30	3757.044 ± 1.378	0.037	3	15	5555.109 ± 2.11	0.038

Table 6: Analytical data of Interday precision

Teneligliptin				Rosuvastatin			
Sr no.	Conc. (µg/ml)	Area Mean ± S. D (n=6)	% RSD	Sr no.	Conc. (µg/ml)	Area Mean ± S. D (n=6)	% RSD
1	10	1279.819 ± 1.360	0.106	1	5	1891.945 ±2.027	0.107
2	20	2565.208 ± 5.232	0.204	2	10	3793.018 ±7.684	0.203
3	30	3862.879 ± 6.315	0.163	3	15	5711.691 ± 9.401	0.165

Table 7: Analytical data of Recovery study of Teneligliptin (tene) and Rosuvastatin (rosu)

Spike Level	Amount of test solution (mcg/ml)		Amount of sample taken (mg)		Mean area		Amount of standard recovery		% Recovery		%RSD	
	tene	rosu	tene	rosu	tene	rosu	tene	rosu	tene	rosu	tene	rosu
80 %	20	10	8	4	1061.01	1576.21	8.02	4.01	100.27	100.31	0.40	0.40
100%	20	10	10	5	1350.33	1995.66	10.2	5.08	101.64	101.60	0.15	0.12
120%	20	10	12	6	1609.596	2381.085	12.11	6.06	100.96	101.02	0.46	0.46

Table 8: LOD and LOQ data teneligliptin

LOD	3.3*Standard deviation (SD)/slope of calibration curve	3.3*9.687/123.671	0.258
LOQ	10* Standard deviation (SD)/slope of calibration curve	10*9.687/123.671	0.853

Table 9: LOD and LOQ data of rosuvastatin

LOD	3.3*Standard deviation (SD)/slope of calibration curve	3.3*14.343/320	0.129
LOQ	10* Standard deviation (SD)/slope of calibration curve	10*14.343/320	0.427

Table 10: Statistical data for Robustness

Drug	Variation		Mean area±SD	%RSD
Teneligliptin	Flow rate	1.2 ml	2631.096 ± 4.44	0.169
		0.8 ml	2523.377 ± 3.47	0.138
	Mobile Phase	72:28 %v/v	2569.120 ± 5.841	0.227
		68:32 %v/v	2575.425 ± 4.35	0.169
	pH	6.6	2614.378 ± 4.39	0.168
		7	2662.851 ± 6.71	0.252
Rosuvastatin	Flow rate	1.2 ml	3891.680 ± 6.75	0.174
		0.8 ml	3731.610 ± 4.46	0.120
	Mobile phase	72:28 %v/v	3798.748 ± 8.59	0.226
		68:32 %v/v	3808.183 ± 6.46	0.170
	pH	6.6	3865.781 ± 6.60	0.171
		7	3937.595 ± 9.99	0.254

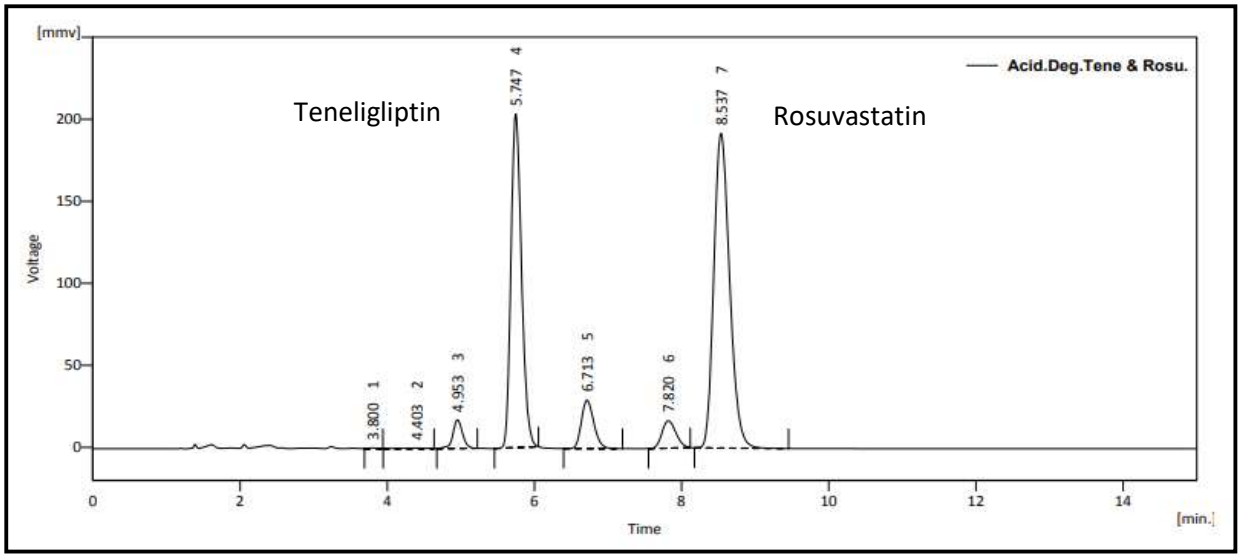


Figure 10: Chromatogram of Acid Hydrolysis of Teneligliptin and Rosuvastatin at 280nm

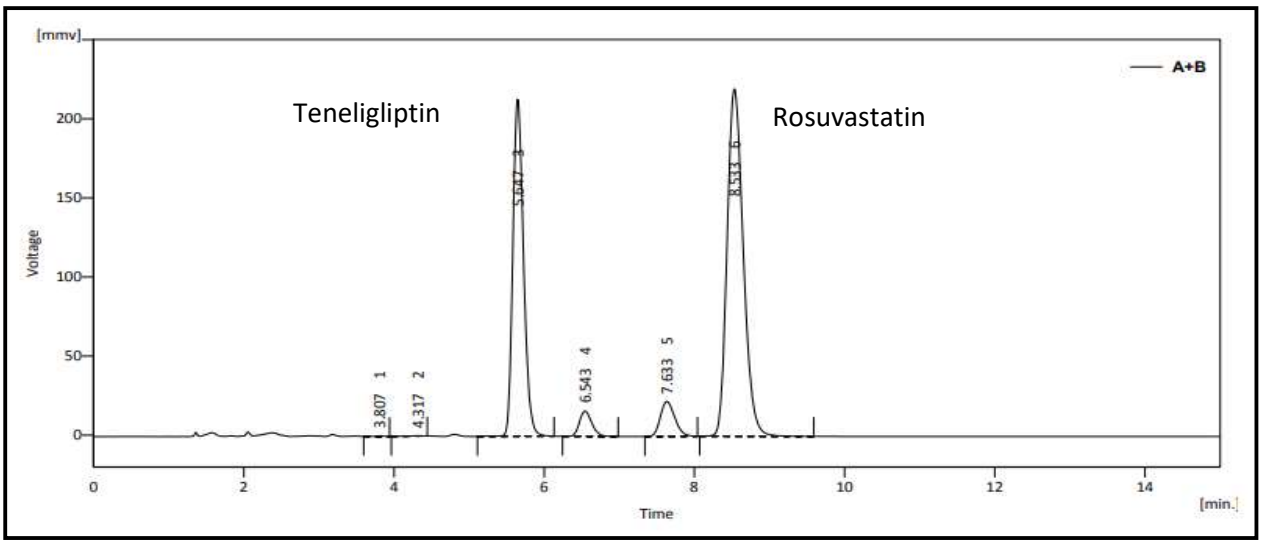


Figure 11: Chromatogram of Base Hydrolysis of Teneligliptin and Rosuvastatin at 280nm

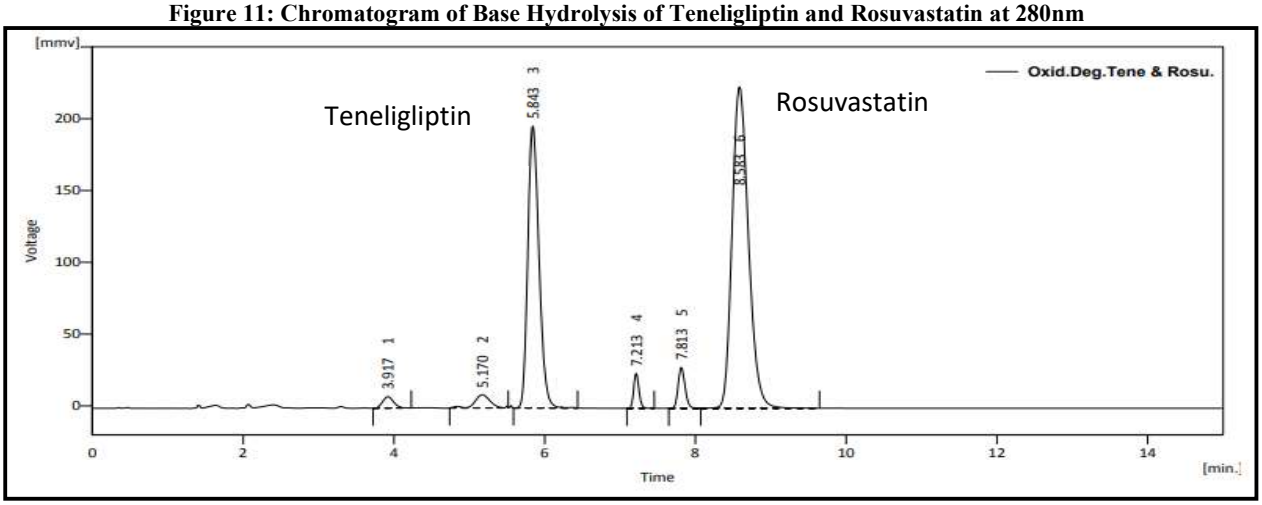


Figure 12: Chromatogram of Oxidative Hydrolysis of Teneligliptin and Rosuvastatin at 280nm

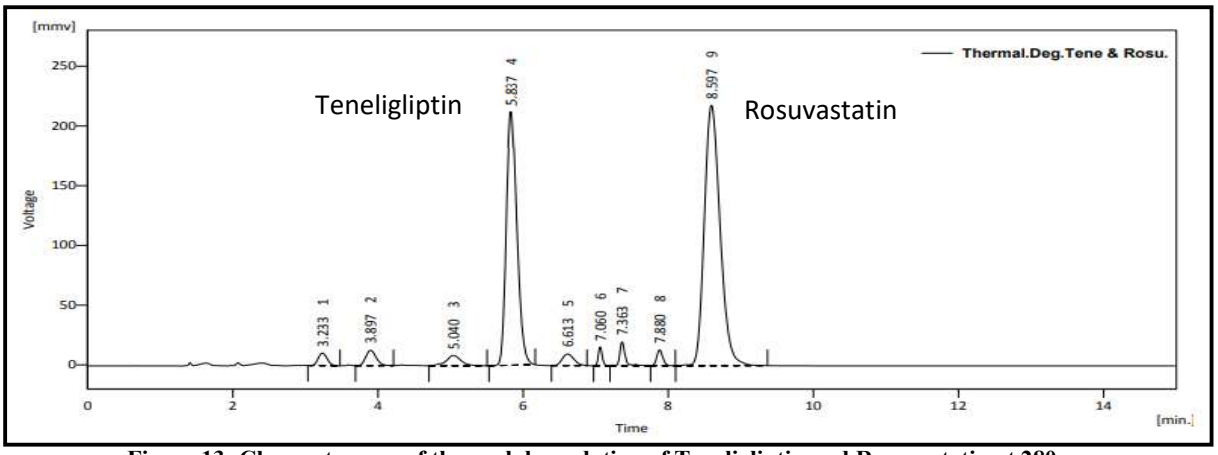


Figure 13: Chromatogram of thermal degradation of Teneligliptin and Rosuvastatin at 280nm

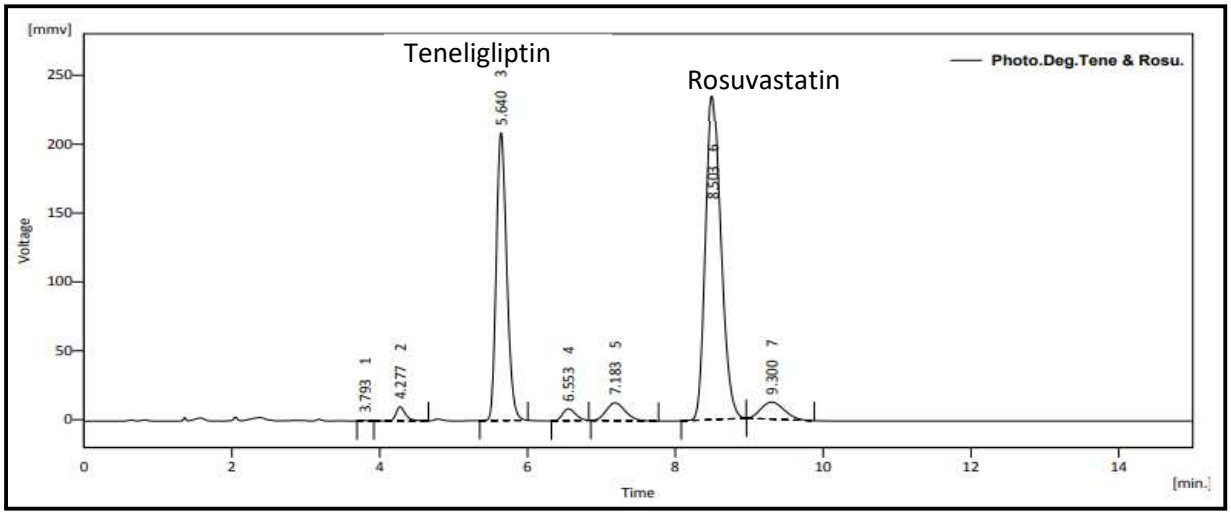


Figure 14: Chromatogram of Photo degradation of Teneligliptin and Rosuvastatin at 280nm

Table 11: Summary data of forced degradation study

Conditions	Retention time		% Degradation	
	Teneligliptin	Rosuvastatin	Teneligliptin	Rosuvastatin
0.1 N HCL	5.474	8.537	10.48	20.31
0.1 N NaOH	5.647	8.553	6.91	10.81
3% H_2O_2	5.843	8.583	11.33	8.44
Thermal degradation (80°C)	5.837	8.597	5.08	10.54
Photo degradation	5.640	8.503	9.31	6.20

Table 12: Summary of validation parameter

Parameter	Teneligliptin	Rosuvastatin
Linearity (Regrassion Value)	10-30 µg/ml (0.99)	5-15 µg/ml (0.99)
%Recovery	100.27-101.64 %	100.31-101.64%
Repeatability (%RSD, n=6)	0.348	0.349
Precision (RSD) Intra-day (n=3) Inter-day (n=3)	0.037-1.45 0.106-0.204	0.038-0.148 0.107-0.203
Limit of Detection	0.258	0.129
Limit of Quantification	0.853	0.427
Robustness	Robust	Robust

Table 13: Analysis of marketed tablet formulation

Drug name	Labeled (mg)	% Label claimed (% Assay* ± SD)	%RSD
Teneligliptin	**20**	**101.076 ±0.115**	**0.113**
Rosuvastatin	**10**	**101.077±0.115**	**0.114**

***Average of six determinations**

CONCLUSION

Teneligliptin and Rosuvastatin could be separated and precisely quantified using an RP-HPLC method that was designed and validated to be accurate, precise, robust, and stability indicating. The knowledge acquired through method development and robustness experiments allowed for the selection of tailing and resolution criteria for system compatibility, which ensured the separation of all relevant medicines from their degradation products. With no influence from co-formulated additives, the approach is used to quantify the active peaks in the commercial formulation. Therefore, the proposed method can be suggested for assay content assessment of teneligliptin and rosuvastatin in pharmaceutical dosage form in the presence of its degradation products.

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