METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION BEHAVIOR OF TENELIGLIPTIN AND REMOGLIFLOZIN ETABONATE IN COMBINED DOSAGE FORM BY RP-HPLC METHOD

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Abstract

A simple, accurate, precise method was developed for the simultaneous estimation of the Teneligliptin (TEN) and Remogliflozin etabonate (REM) in tablet dosage form. Chromatogram was run through Standard kromasil C_{18} (4.6x150mm,5µm). Mobile phase containing Acetonitrile: KH_2PO_4 taken in the ratio 65:35 v/v was pumped through column at a flow rate of 1 mL/min. Buffer used in this method was Phosphate buffer and pH was adjusted to 5.4 by adding 0.1% Formic acid. Temperature was maintained at 30°C. Optimized wavelength selected was 228 nm. Retention time of Remogliflozin etabonate and Teneligliptin were found to be 2.263 min and 2.994 min. %RSD of the Remogliflozin etabonate and Teneligliptin were and found to be 0.9 and 0.4 respectively. %Recovery was obtained as 100.21% and 100.08% for Remogliflozin etabonate and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Remogliflozin etabonate and Teneligliptin were 0.26, 0.78 and 0.03, 0.09 respectively. The developed method was validated as per ICH Q2 (R1) guidelines. Regression equation of Remogliflozin etabonate was y = 24560x + 3087 and y = 40746x + 617.4 for Teneligliptin. The method developed was found to be robust. The drug samples were also subjected to forced degradation conditions such as acid, base, peroxide, thermal, UV and water.Retention times were decreased and that run time was also decreased. Hence the proposed simultaneous estimation was accurate, specific, reproducible and economical that can be adopted in regular Quality control analysis in Pharmaceutical Industries.

Keywords: Teneligliptin, Remogliflozin etabonate, Degradation Behaviour, RP-HPLC.

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1. Introduction

Teneligliptin TEN, (Figure 1) is chemically [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3yl) piperazin-1yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl)methanone] is a novel oral dipeptidyl peptidase-4 (DPP-4) inhibitor which is used in the treatment of Type II Diabetes mellitus.[1] It is a potent and selective agent, acts by increasing incretin levels GLP-1 and GIP, which in turn increases insulin secretion and thus decreases blood glucose level.[2]

Remogliflozin etabonate REM, (Figure 2) is chemically [ethyl[(2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-[5-methyl-1-propan-2-yl-4-[(4-propan-2-yloxyphenyl)methyl]pyrazol-3yl]oxyoxan-2yl]methyl carbonate] is the latest addition to the sodium glucose co-transporter [SGLT] inhibitor classes of drugs that have been approved for the management of Type II Diabetes mellitus. REM is a pro-drug of Remogliflozin. It inhibits SGLT, which are responsible for glucose reabsorption in the kidney. It causes blood glucose to get eliminated through the urine and thus lowers the level of glucose.[3]

Analytical method validation is a set of procedures which ensures that the developed new analytical method shall give reliable and repeatable outcomes.[4] Information about validation parameters such as accuracy, precision, linearity, LOD, LOQ, specificity, range and robustness helps in formulation of new dosage forms. Validation is done based on the ICH guidelines.[5]

Literature survey revealed few analytical methods for the estimation of TEN and REM individually or in combined dosage form or in combination with other anti diabetic drugs.

As per the review of literature, Ultra Violet Spectrophotometry (UV)[6-9], Reversed Phase High Performance Liquid Chromatography (RP-HPLC)[10-12], Reversed Phase Ultra Performance Liquid Chromatography (RP-UPLC), Liquid Chromatography-Mass Spectroscopy (LC-MS)[13] and High Performance Thin Layer Chromatography (HPTLC)[14] were found to be the most prominent methods used for the estimation of selected drugs.

In this research, a new simple, precise, accurate and rapid RP-HPLC method was developed with effective solvent ratio for the simultaneous estimation of Teneligliptin and Remogliflozin etabonate in combined dosage form. The developed method was validated as per ICH Guidelines and subjected to Forced degradation conditions for stability assessment.

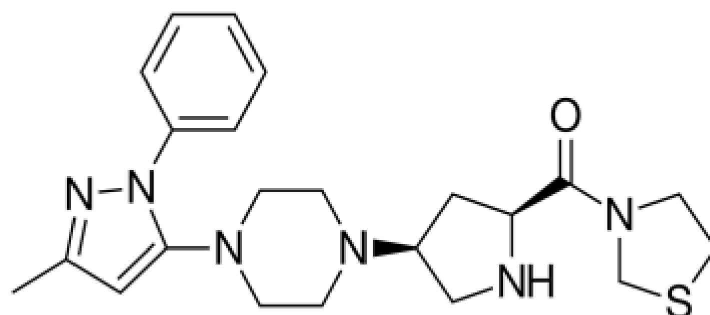

Figure 1 : Teneligliptin

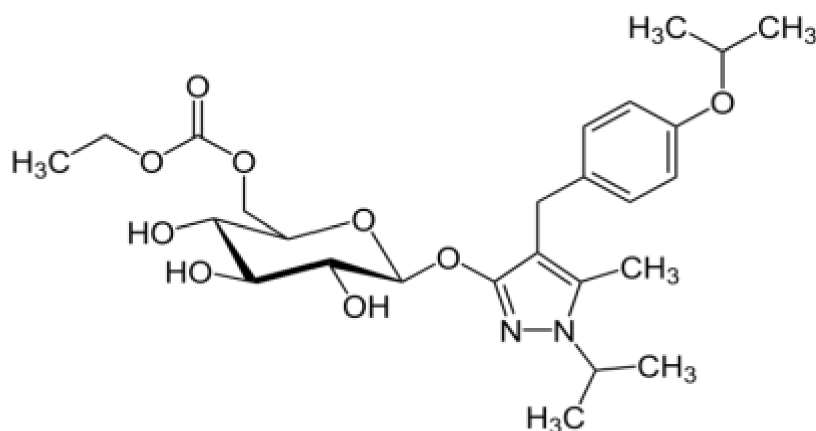

Figure 2 : Remogliflozin etabonate

2. Materials And Methods

Materials and reagents:

Pure drug samples of Teneligliptin and Remogliflozin etabonate (API) were received as a gift from spectrum Pharma labs. Commercially available tablet formulations of Zita Plus-R (Combination of Teneligliptin and Remogliflozin etabonate tablets) purchased from local market for assay purpose. Reagents used in this method distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen Ortho Phosphate buffer and Formic acid were received from Rankem. Approval from ethical committee was not required for our research study.

Instrumentation:

The development and validation of analytical method were done using Waters HPLC 2695 system equipped with quaternary pumps. Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with spectral bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of TEN and REM solutions.

Methodology:

Preparation of Standard stock solution:

Accurately weighed 25 mg of REM, 2.5 mg of TEN and transferred to 50 mL volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (500 µg/mL of REM and 50 µg/mL of TEN).

Preparation of Sample stock solution:

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (1000 µg/mL of REM and 100 µg/mL of TEN).

Preparation of Buffers:

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36 gm of Potassium dihydrogen Ortho phosphate in a 1000 mL of Volumetric flask add about 900 mL of Milli-Q water added and degassed to sonicate and finally make up the volume with water then pH adjusted to 5.4 with dilute Formic acid.

0.1% Formic acid Buffer**:** 1 mL of Conc. Formic acid was diluted to 1000 mL with water.

Mobile phase:

Solvents such as Acetonitrile and Potassium dihydrogen Ortho phosphate were taken in the ratio of 65:35 (v/v) and used as mobile phase.

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of REM (50ppm) and TEN (5 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The results were compared with the standard limits as per ICH guidelines.

Specificity:

Specificity is the checking for the interferences in optimized method. Interference peaks in blank and placebo at retention time of selected drugs should not be observed. Then the proposed method said to be specific.

Linearity:

The linearity of the method was obtained by preparing the calibration standards of 6 different concentrations (25%,50%,100%,125%,150%) for both the drugs. The calibration curve plots for TEN and REM were obtained by plotting the peak areas on y-axis and concentrations on the x-axis over the concentration ranges of 1.25-7.5 µg/mL for TEN and 12.5-75 µg/mL for REM. The correlation coefficient should be greater than 0.99.

Accuracy:

The accuracy of the method developed was assessed by determining recovery% of the two drugs. Recovery experiments are done by adding known quantity of pure standard drug to the sample drug solution and recovering the same in terms of its peak areas.

The sample was spiked with standard at levels of 50%, 100% and 150% of the test concentration for the selected drugs. The recovery% for each level should be between 98.0% to 102%.

Precision:

The Precison of an anlytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample under prescibed conditions. Precision may be measured of either the degree of repeatability or reproducibility of the analytical method. It is measured in terms of standard deviation (SD) or relative standard deviation (RSD).

System Precision:

Sample solutions were injected 6 times under optimized and their peak areas were recorded. Average area, SD, %RSD were calculated for two drugs. As the limit of precision less than 2, then the system precision was considered to be pass.

Method Precision:

Six working sample solutions of same concentration were prepared, and injected 6 six times on different days and their peak areas were measured for two drugs. As the limit of precision less than 2, then the method precision was considered to be pass.

Intermediate Precision:

Six replicates of sample solutions were injected under optimized conditions on the same day and peak areas were recorded for both the drugs. The results should not be greater than 2.

Sensitivity:

Limit of quantitation (LOQ) and Limit of detection (LOD) were determined by analysing different solutions of TEN and REM and signal-to-noise ratio. The LOD is the smallest concentration that can be detected but not necessarily be quatified as an exact value. LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy.

Robustness:

The robustness of the method was determined by making small deliberate changes in the method like flow rate, mobile phase ratio and temperature. Changes was made to evaluate its effect on the method. Obtained data for each case was validated by calculating %RSD and %recovery.

Forced degradation behavior:

TEN and REM standard drug sample were subjected to forced degradation under different stress conditions like acidic, alkali, peroxide, thermal, UV and water. For acid and alkali degradation, samples were refluxed for 30 min at 60°C with 2N Hcl and 2N NaOH respectively. For oxidative degradation, 20% v/v H_2O_2 was used and the sample was placed in an oven at 105°C for 6 hours for thermal degradation study. The drug sample was exposed to UV radiation by keeping the sample in the UV chamber for 7 days or 200 Watt h/m^2. Neutral degradation studies were observed by refluxing the selected drugs in water for 6 hours at 60°C. The samples were diluted and injected into the system and the resulting chromatograms were recorded to indicate stability.

3. Results

Method development and optimization:
The optimized chromatographic conditions were obtained, given in the Table 1. (Figure 3) gives optimized chromatogram of resolved peaks of the selected drugs.

Table 1: Optimized chromatographic conditions

Mobile phase	Acetonitrile : KH_2PO_4 (65:35)
Flow rate	1 mL/min
Column used	Kromasil C_{18} (4.6 x 150 mm, 5 µm)
Detector wavelength	228 nm
Column temperature	30°C
Injection volume	10 µL
Run time	6.0 min

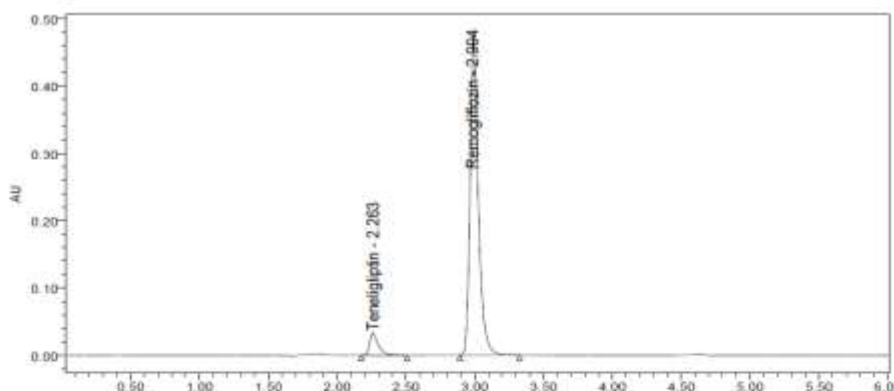

Figure 3 : Optimized chromatogram of TEN and REM

Method validation:

System suitability parameters:

The system suitability parameters such as tailing factor, retention time, USP Plate count and resolution were found to be in the acceptance criteria.

We did not find any interfering peaks at the retention time of the selected drugs, which reveals that the method developed is specific. The quantification was linear in the concentration range of 1.25-7.5 µg/mL for TEN with the correlation coefficient of 0.999 (Figure 4) and 12.5-75 µg/mL for REM with the correlation coefficient of 0.999 (Figure 5). The results of linearity were tabulated in Table 2.

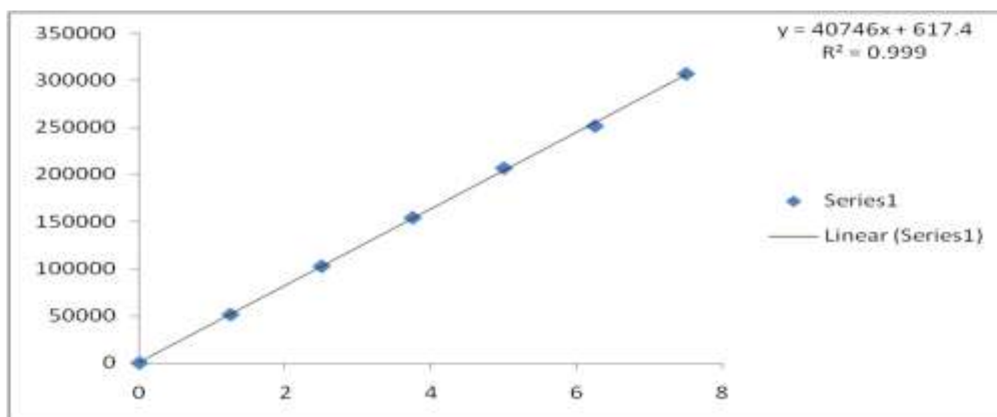

Figure 4 : Calibration curve of TEN

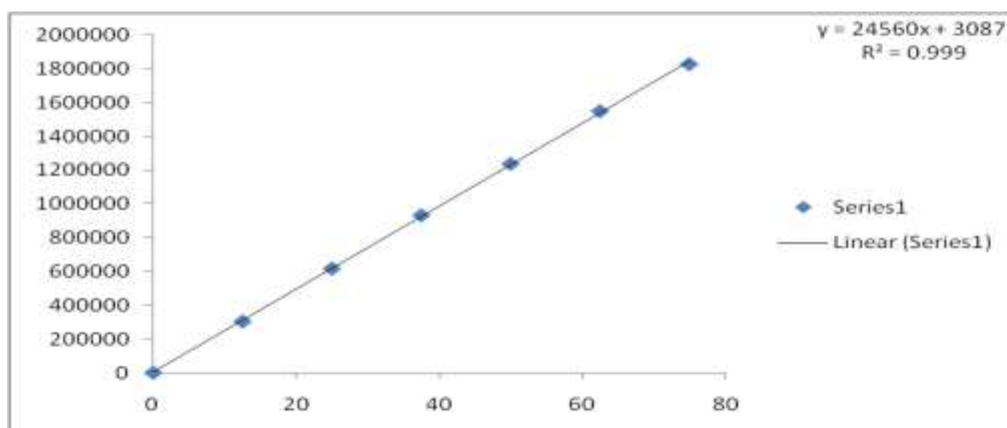

Figure 5: Calibration curve of REM

Table 2: Results of Linearity of TEN and REM drugs.

TEN		REM	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
1.25	51388	12.5	303780
2.5	102689	25	617095
3.75	154191	37.5	932656
5	206858	50	1237495
6.25	251616	62.5	1549699
7.5	307161	75	1827944

The recoveries of TEN and REM were in the range of 100.30% and 100.21% respectively given in Table 3.

Table 3 : Recovery data of TEN and REM

Drug	%Level	Amount Spiked (μg/mL)	Amount recovered (μg/mL)	%recovery	Mean %recovery
TEN	50%	2.5	2.502591	100.106	100.30%
	100%	5	5.009635	100.19	
	150%	7.5	7.545164	100.603	
REM	50%	25	25.041506	100.16	100.21%
	100%	50	50.171966	100.34	
	150%	75	75.100503	100.13	

The precision of method was satisfactory as %RSD is NMT 2. The system precision of method was also satisfactory as %RSD is NMT 2. The results are given in Table 4 and 5.

Table 4 : Method precision table of TEN and REM

Drug	Mean Area	SD	%RSD	Acceptance criteria
TEN	204854	1380.9	0.7	%RSD NMT 2
REM	1245203	7467.3	0.6	

Table 5 : System precision table of TEN and REM

Drug	Mean Area	SD	%RSD	Acceptance criteria
TEN	204278	878.2	0.4	%RSD NMT 2
REM	1239474	10933.5	0.9	

The assay results were compared with the labeled claim of TEN and REM marketed formulations and the results are given in Table 6 and Table 7. The resultant chromatogram were shown in (Figure 6 and 7).

Table 6 : Assay results of TEN in marketed formulation (Zita plus-R tablets)

Drug	Label claim	Parameters	Standard area	Sample area	% Assay
TEN	10 mg	Mean	204278	204854	100.08
		S.D	878.2	1380.9	0.67
		%RSD	0.4	0.7	0.7

Table 7 : Assay results of REM in marketed formulation (Zita plus-R tablets)

Drug	Label claim	Parameters	Standard area	Sample area	% Assay
REM	100 mg	Mean	1239474	1245203	100.26
		S.D	10933.5	7467.3	0.601
		%RSD	0.9	0.6	0.6

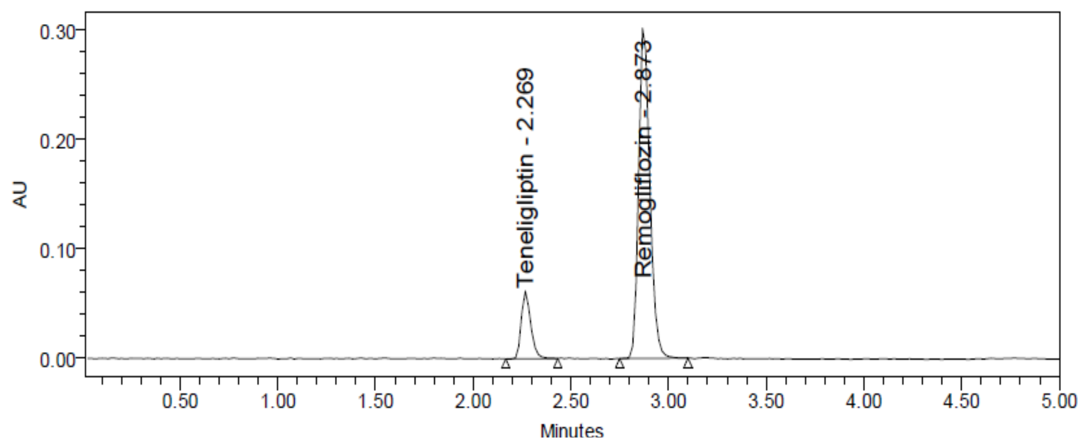

Figure 6 : Chromatogram of working standard solution

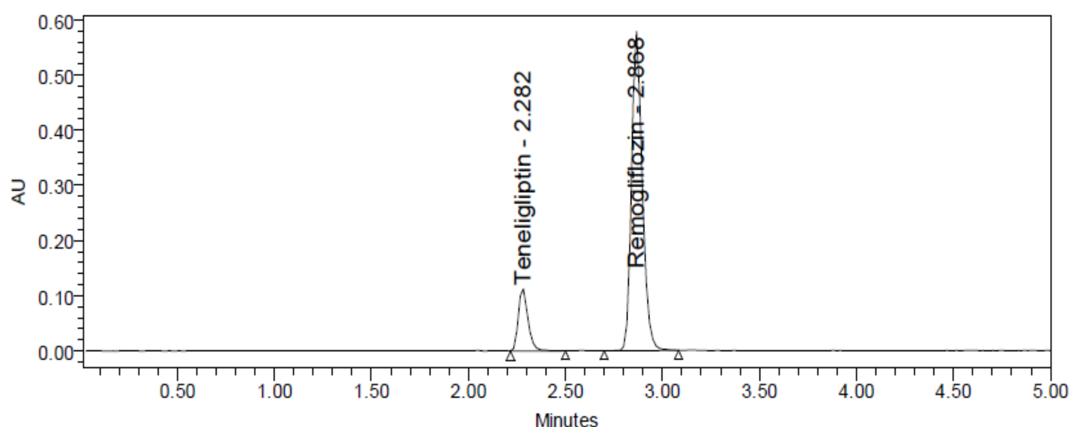

Figure 7 : Chromatogram of working sample solution

The LOD and LOQ values were calculated using slope and SD values, given in the Table 8.

Table 8 : Sensitivity table for TEN and REM.

Drug	LOD	LOQ
TEN	0.03	0.09
RE	0.26	0.78

No remarkable changes in results were noted in the robustness studies and hence the method considered to be robust, results are tabulated in the Table 9.

Table 9 : Robustness data for TEN and REM

S.no	Condition	%RSD of TEN	%RSD of REM
1	Flow rate (-) 0.9 mL/min	0.4	0.8
2	Flow rate (+) 1.1 mL/min	0.3	0.9
3	Mobile phase (-) 55B:45A	0.4	1.1
4	Mobile phase (+) 70B:30A	0.5	0.8
5	Temperature (-) 27°C	0.8	0.8
6	Temperature (+) 33°C	1.6	1.1

Forced degradation study:
The standard solutions were subjected to different forced degradation conditions as mentioned in the procedure. The degradation data of the selected drugs were tabulated in Table 10.

Table 10 : Degradation data of TEN and REM

Degradation	TEN			REM		
	Area	**% Recovered**	**% Degraded**	**Area**	**% Recovered**	**% Degraded**
Acid	195454	95.49	4.51	1193863	96.13	3.87
Base	191959	93.78	6.22	1160784	93.46	6.54
Peroxide	196129	95.82	4.18	1186850	95.56	4.44
Thermal	199600	97.51	2.49	1214408	97.78	2.22
UV	199656	97.54	2.46	1216306	97.93	2.07
Water	202107	98.74	1.26	1221220	98.33	1.67

Under acidic condition, the drugs showed degradation of about 4.51% for TEN and 3.87% for REM. The acid degradation chromatogram is given in (Figure 8). Under alkali condition, the drug showed degradation of about 6.22% for TEN and 6.54% for REM (Figure 9). Under oxidative conditions, the drugs showed degradation of about 4.18% for TEN and 4.44% for REM (Figure 10). Under thermal condition, the drugs showed degradation of about 2.49% for TEN and 2.22% for REM (Figure 11). For photo-stability degradation study, the drugs showed degradation of about 2.46% for TEN and 2.07% for REM (Figure 12). Under neutral conditions, the drugs showed degradation of about 1.26% for TEN and 1.67% for REM (Figure 13). Only under alkali condition, 3 peaks were recorded. Under other degradation conditions, only 2 peaks were recorded.

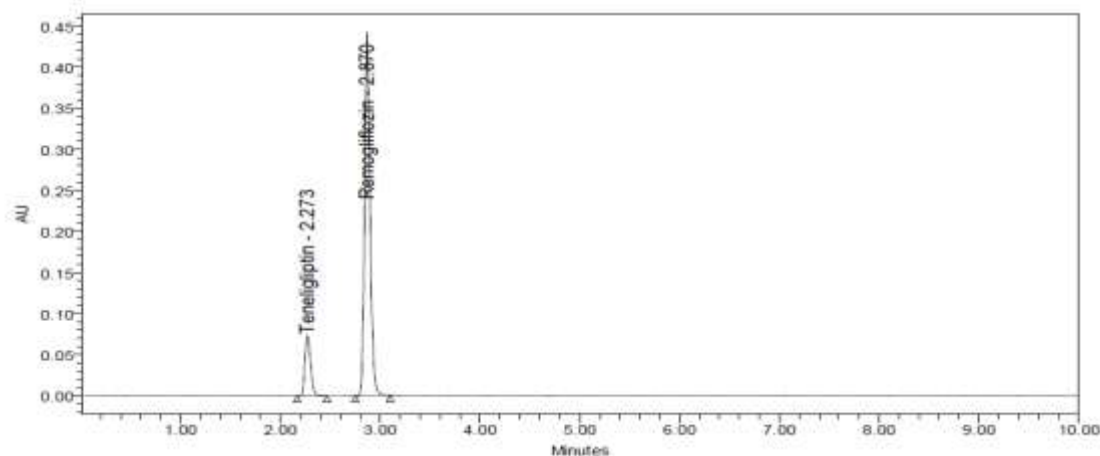

Figure 8 : Acid Degradation Chromatogram

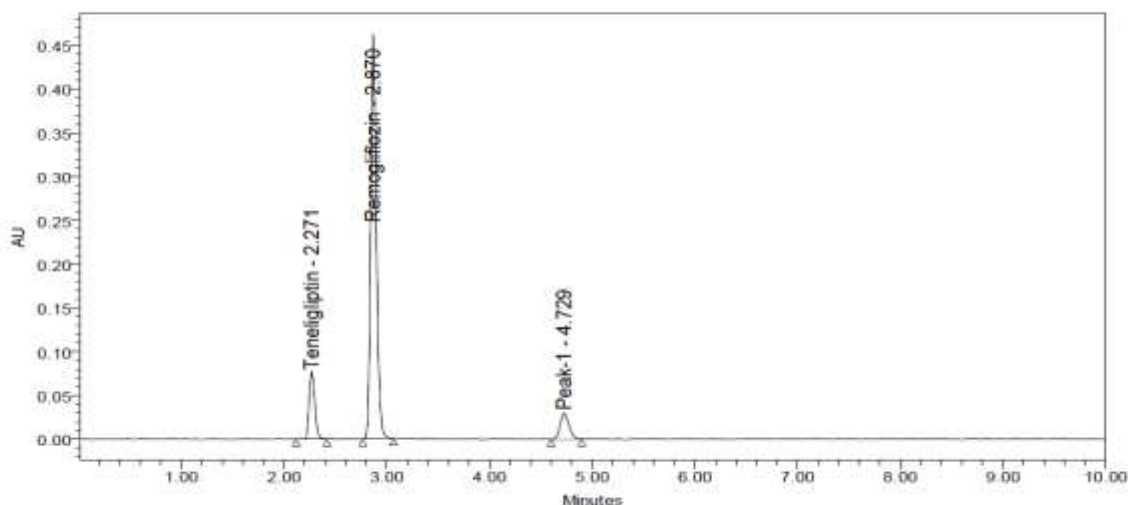

Figure 9 : Base Degradation Chromatogram

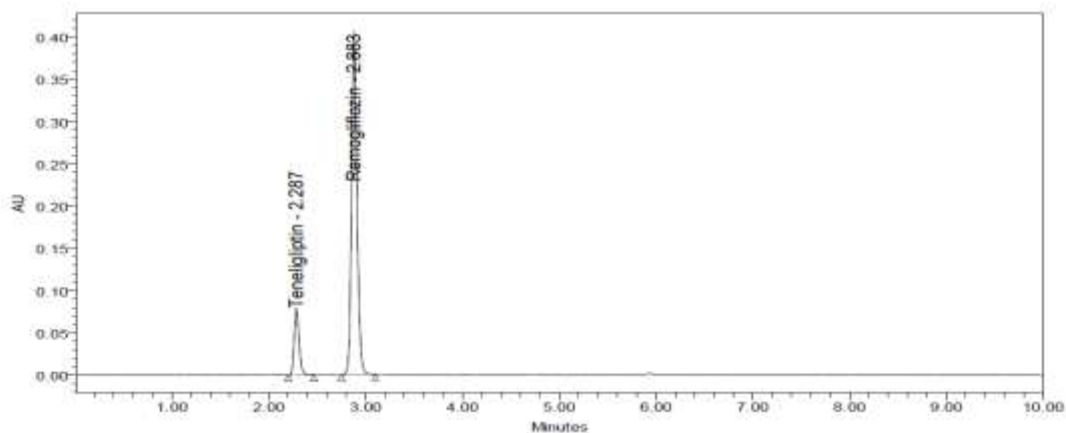

Figure 10 : Peroxide Degradation Chromatogram

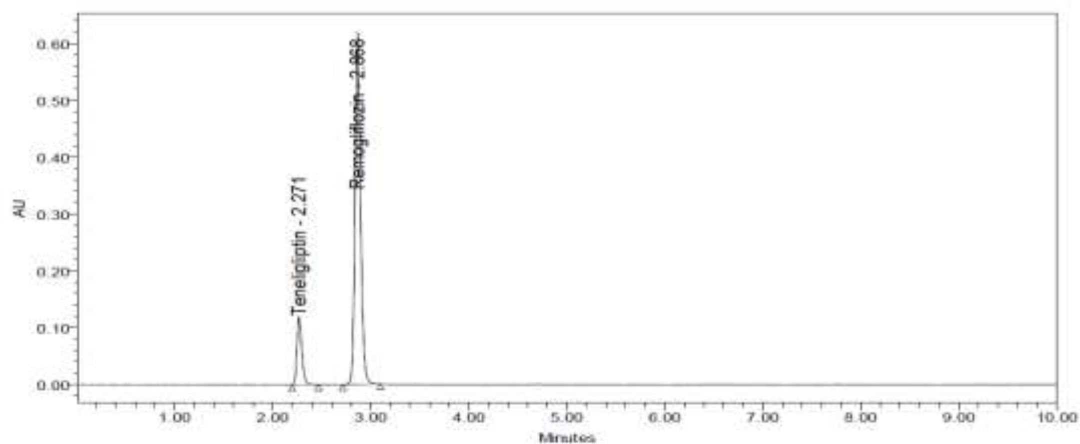

Figure 11 : Thermal Degradation Chromatogram

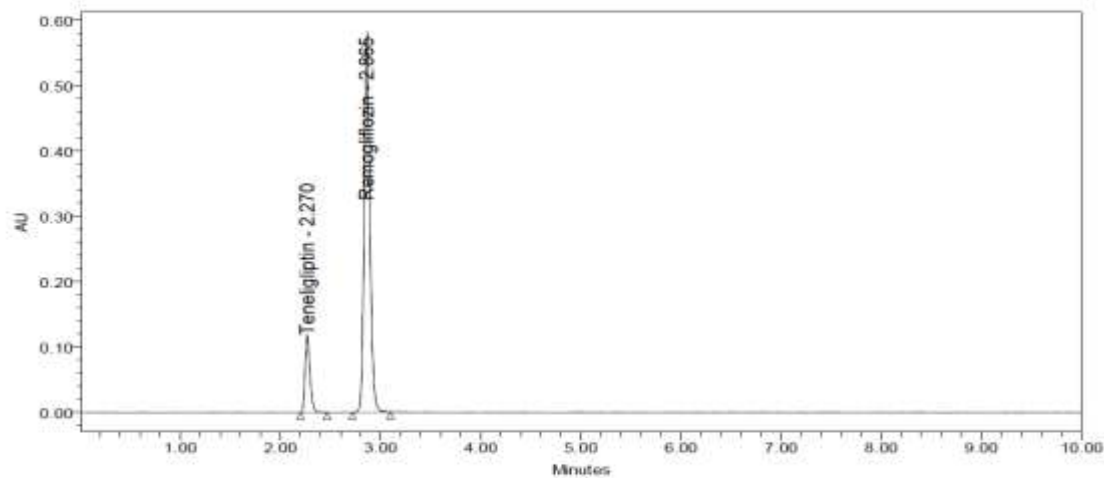

Figure 12 : UV Degradation Chromatogram

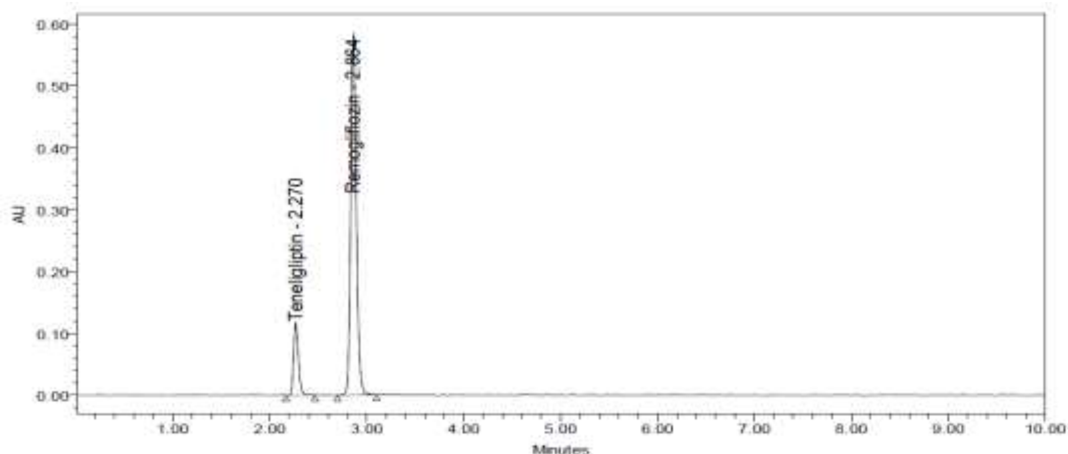

Figure 13 : Water Degradation Chromatogram

4. Discussion

A simple, accurate, precise method was developed for the simultaneous estimation of the Remogliflozin and Teneligliptin in tablet dosage form. Retention time of Remogliflozin etabonate and Teneligliptin were found to be 2.263 min and 2.994 min. %RSD of the Remogliflozin etabonate and Teneligliptin were and found to be 0.9 and 0.4 respectively. %Recovery was obtained as 100.21% and 100.08% for Remogliflozin etabonate and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Remogliflozin etabonate and Teneligliptin were 0.26, 0.78 and 0.03, 0.09 respectively. Regression equation of Remogliflozin etabonate is y = 24560x + 3087 and y = 40746x + 617.4 of Teneligliptin. Forced degradation behaviour of samples were also assessed as per ICH guidelines. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in Industries.

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Conflict of Interest

No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of this article.

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