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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TENEGLIPTIN AND REMOGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:
Simultaneous estimation of the Remogliflozin and Teneligliptin in pharmaceutical dosage form. Chromatogram was run through Discovery C18 250 x 4.6 mm, 5m. Mobile phase containing Buffer Ammonium acetate: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 0.9 ml/min.. Temperature was maintained at 30°C. Optimized wavelength selected was 229 nm. Remogliflozin and Teneligliptin were eluted at 2.139 min and 2.176 min respectively. %RSD of the Remogliflozin and Teneligliptin were and found to be 0.6 and 0.7 respectively. %Recovery was obtained as 99.50% and 99.50% for Remogliflozin and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Remogliflozin and Teneligliptin were 0.11, 0.33 and 0.005, 0.014 respectively. Regression equation of Remogliflozin is y = 52813x + 14718, and y = 69817x + 586.95 of Teneligliptin.

Key Words: Remogliflozin and Teneligliptin, Rp Hplc, Validation.

INTRODUCTION

Type 2 diabetes is a condition that happens because of a problem in the way the body regulates and uses sugar as a fuel. That sugar also is called glucose. This long-term condition results in too much sugar circulating in the blood. Eventually, high blood sugar levels can lead to disorders of the circulatory, nervous and immune systems. In type 2 diabetes, there are primarily two problems. The pancreas does not produce enough insulin a hormone that regulates the movement of sugar into the cells. And cells respond poorly to insulin and take in less sugar.

Type 2 diabetes used to be known as adult-onset diabetes, but both type 1 and type 2 diabetes can begin during childhood and adulthood. Type 2 is more common in older adults. But the increase in the number of children with obesity has led to more cases of type 2 diabetes in younger people.

Symptoms of type 2 diabetes often develop slowly. In fact, you can be living with type 2 diabetes for years and not know it. When symptoms are present, they may include: 1. Increased thirst 2. Frequent urination 3. Increased hunger 4. Unintended weight loss 5. Fatigue.[1]

Type 2 Diabetes is a chronic endocrine condition characterized by elevated blood glucose levels with micro and macrovascular complications 2,3,4. As a result, when compared to monotherapy, treatment with a combination of oral hypoglycemic medications with different mechanisms of action is widely favored for improving glycemic control 5,6. The combination of dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin (Figure 1A, VLG) and teneligliptin (Figure 1B, TNG) with the sodium-glucose cotrasportase-2 (SGLT-2) inhibitor, remogliflozin etabonate, (Figure 1C, RGE) has just been approved by the Food and Drug Administration for the treatment of diabetes mellitus type 7,8. DPP-4 inhibitors increase the secretion of insulin by inhibiting the enzyme DPP-4 responsible for degradation of incretins in the blood, thereby decreasing the blood glucose level by lowering the blood glucagon level, and improving pancreatic cell function 8,10. DDP-4 inhibitors also lower HbA1c levels without causing hypoglycemia and weight gain 11,12,13. Further, teneligliptin can be taken in patients with renal failure without dose adjustment 14,15. Kissei Pharmaceutical found remogliflozin. Remogliflozin is now being made by BHV Pharma, which is a wholly-owned company of Avolynt and is working with Glenmark Pharmaceuticals 16.

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Here we have used Remogliflozin and Teneligliptin.

Remogliflozin Etabonate chemically known as 4-(4-Isopropoxybenzyl)-1-isopropyl-5-methyl-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D-glucopyranoside [17] Teneligliptin known as 2S, 2'S, 4S, 4'S)-((methylenebis(3-methyl-1-phenyl-1H-pyrazole-4, 5-diyl))bis(piperazine-4, 1-diyl))bis(pyrrolidine-4,[18] is a combination of two antidiabetic medications: Remogliflozin Etabonate and Teneligliptin. Remogliflozin Etabonate works by removing excess sugar from your body through urine. Teneligliptin works by increasing the release of insulin from the pancreas and decreasing the hormones that raise blood sugar levels. This reduces the fasting and post-meal sugar levels.

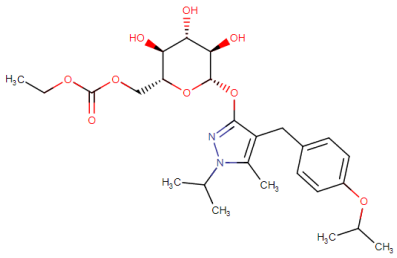

Figure 1: Structure of Remogliflozin

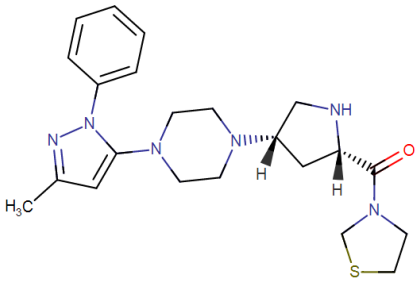

Figure 2: Structure of Teneligliptin

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Remogliflozin, Teneligliptin, and their medicinal dose form using RP-HPLC [19- 24] must be validated and developed as per ICH guidelines

MATERIALS AND METHODS: Spectrum pharma Research Solution with Remogliflozin and Teneligliptin pure drugs (API) gift samples and Combination Remogliflozin and Teneligliptin tablets (Zita plus- R). The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to fulfill ICH standards, we need to design and test an HPLC technique that can detect Teneligliptin and Remogliflozin in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and Ammonium acetate (40:60 v/v)
Flow rate	0.9 ml/min
Column	Discovery C18 (4.6 x 150mm, 5µm)
Detector wave length	229 nm
Column temperature	30°C
Injection volume	10mL
Run time	5.0 min
Buffer	Ammonium acetate

API Preparation:

Preparation of Standard stock solutions: Accurately weighed 25mg of Remogliflozin, 2.5mg of Teneligliptin and transferred to 50ml flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (500µg/ml of Remogliflozin and 50µg/ml Teneligliptin)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml of Remogliflozin and 5µg/ml of Teneligliptin)

Formulation Preparation:

Preparation of Sample stock solutions: 10 tablets were taken and calculated each tablet average tablet and equivalent to 100 mg and 10mg Was taken Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark and was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF) (1000µg/ml of Remogliflozin and 100µg/ml of Teneligliptin).

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (50µg/ml of Remogliflozin and 5µg/ml of Teneligliptin).

System suitability parameters: Remogliflozin (50 ppm) and Teneligliptin (5 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S no	**Remogliflozin**				**Teneligliptin**				
Inj	**RT**	**area**	**Plate Count**	**Tailing**	**RT**	**area**	**Plate Count**	**Tailing**	**RS**
1	2.139	2657455	4296	1.52	2.712	350616	4466	1.22	3.9
2	2.140	2636806	4266	1.52	2.714	349234	4489	1.22	3.9
3	2.141	2671578	4395	1.50	2.715	354804	4627	1.22	4.0
4	2.150	2663686	4675	1.47	2.719	355533	4540	1.24	3.7
5	2.150	2651481	4677	1.47	2.719	351147	4516	1.23	3.7
6	2.151	2627519	4957	1.46	2.722	352364	4599	1.23	3.8
Mean		**2651421**				**352283**			
Std dev		**16601.5**				**2461.3**			
RSD		**0.6**				**0.7**			

The % RSD for the peak areas of Remogliflozin and Teneligliptin obtained from six replicate injections of standard solution was within the limit.

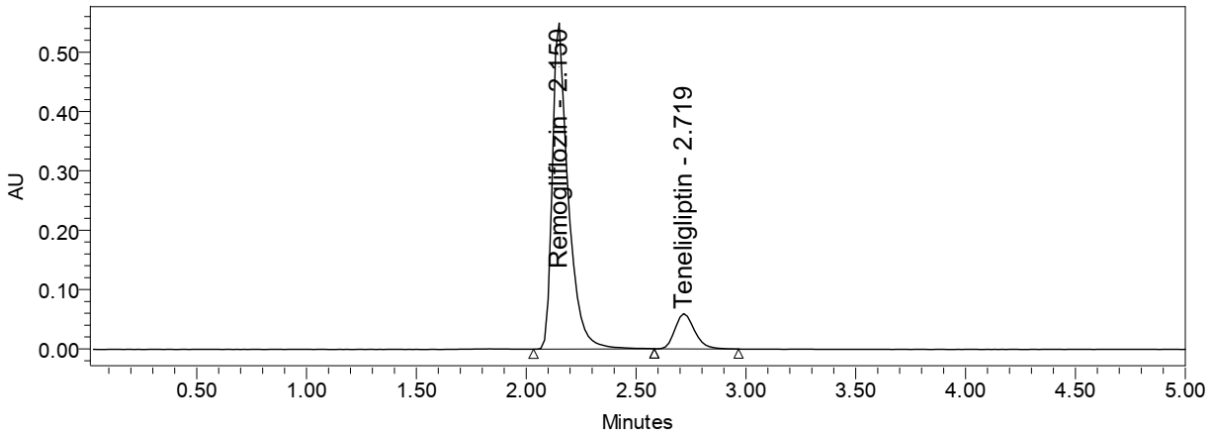

Figure 3: System suitability Chromatogram

Specificity: Checking of the interference in the optimized method. In addition, no interference was observed so, it is specific.

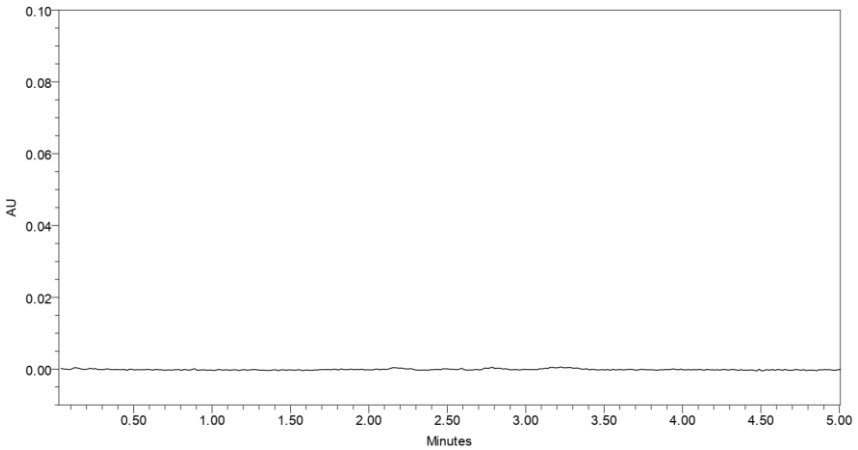

Figure.4 Specificity of Remogliflozin and Teneligliptin

Linearity:

Calibration data is given in table and regression data in table and calibration curve in figure.

Table 3: Calibration data of Remogliflozin and Teneligliptin

Remogliflozin		Teneligliptin	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
12.5	659174	1.25	87853
25	1378773	2.5	177179
37.5	1983648	3.75	261859
50	2666381	5	351090
62.5	3320289	6.25	431099
75	3958291	7.5	527736

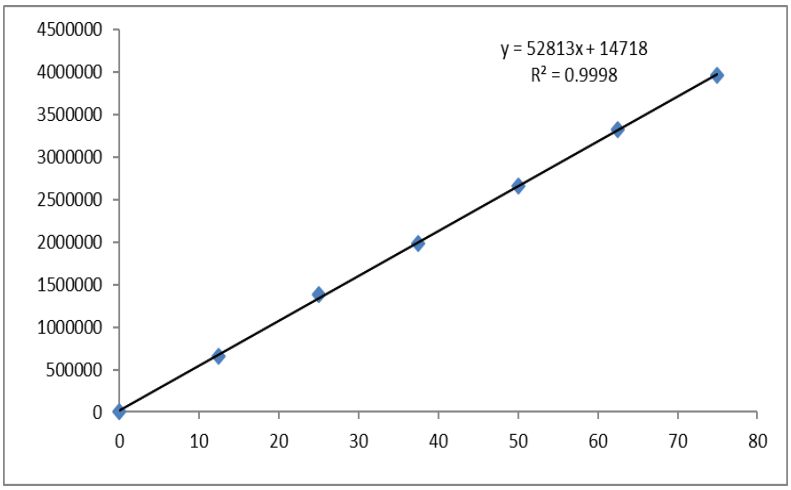

Figure.5 Calibration curve of Remogliflozin

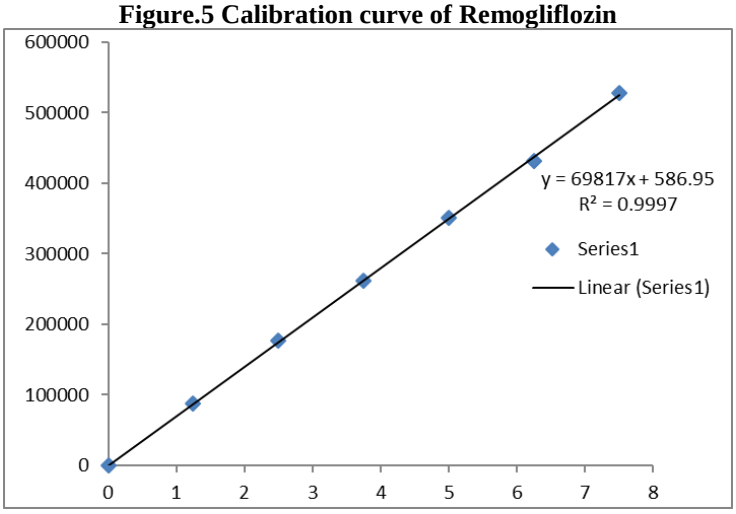

Figure.6 Calibration curve of Teneligliptin

Table 4: Regression data

Parameter	Remogliflozin	Teneligliptin
Conc range (µg/mL)	12.5-75	1.25-7.5
Regression Equation	y = 52813x + 14718	y = 69817x + 586.95.
Co-relation	0.999	0.999

Accuracy:
Recovery data shown in table

Table 5: Recovery data of Remogliflozin and Teneligliptin

	Remogliflozin			Teneligliptin		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	25	24.702	98.81	2.5	2.491	99.65
		24.760	99.04		2.478	99.10
		24.861	99.44		2.474	98.94
100%	50	49.918	99.84	5	4.990	99.81
		49.906	99.81		4.960	99.19
		49.907	99.81		4.999	99.98
150%	75	74.515	99.35	7.5	7.480	99.74
		74.739	99.65		7.472	99.62
		74.782	99.71		7.462	99.50
% recovery	99.50			99.50		

Method Precision: The precision of the method was determined by analyzing a sample of Remogliflozin and Teneligliptin and shown in table.

Table 6: Method Precision

S. No	Area of Remogliflozin	Area of Teneligliptin
1.	2633914	351234
2.	2618734	349540
3.	2635432	350244
4.	2649549	351213
5.	2651514	351390
6.	2619532	345116
Mean	**2634779**	**349790**
S.D	**14066.3**	**2399.4**
%RSD	**0.5**	**0.7**

From the above results, the % RSD of method precision study was within the limit for Remogliflozin and Teneligliptin.

Robustness: Robustness conditions like Flow rate, mobile phase and temperature was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 7: Robustness data for Remogliflozin and Teneligliptin.

Parameter	Optimized condition	Used condition	Remogliflozin	Teneligliptin
			Obtained %RSD	
Flow rate (±0.1ml/min)	1ml/min	0.9ml/min	0.1	0.2
		1.1 ml/min	0.9	0.5
MP (5%v/v)	60:40	55:45	0.6	0.6
		65:35	0.2	0.4
Column temp. (±3^{0}c)	30^{0}c	27 ^{0}C	0.2	0.5
		33 ^{0}C	0.5	0.5

Sensitivity:

Table 8: Sensitivity of Remogliflozin and Teneligliptin

Molecule	LOD	LOQ
Remogliflozin	0.11 µg/ml	0.33 µg/ml
Teneligliptin	0.005 µg/ml	0.014 µg/ml

Force Degradation Studies: table shows degradation conditions and table 10 shows the obtained degraded data and chromatogram in figure.

Table 9: Degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	60 mins
Base	2N NAOH	60^{0}c	60 mins
Oxidation	20% H_2O_2	60^{0}c	60 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	60 mins

Table 10: Degradation data

Conc of degradation study	Remogliflozin		Teneligliptin	
	% drug Undegraded	% drug degraded	% drug Undegraded	% drug degraded
2N HCl, 60 min	96.34	3.66	97.09	2.91
2N NaOH, 60min	96.20	3.80	96.52	3.48
Oxidative, 60 min	97.68	2.32	97.85	2.15
Thermal, 1 hr	98.73	1.27	98.27	1.73
Photo, 6 hr	98.81	1.19	99.33	0.67
Neutral, 1 hr	99.84	0.16	99.56	0.44

Acid degradation chromatogram

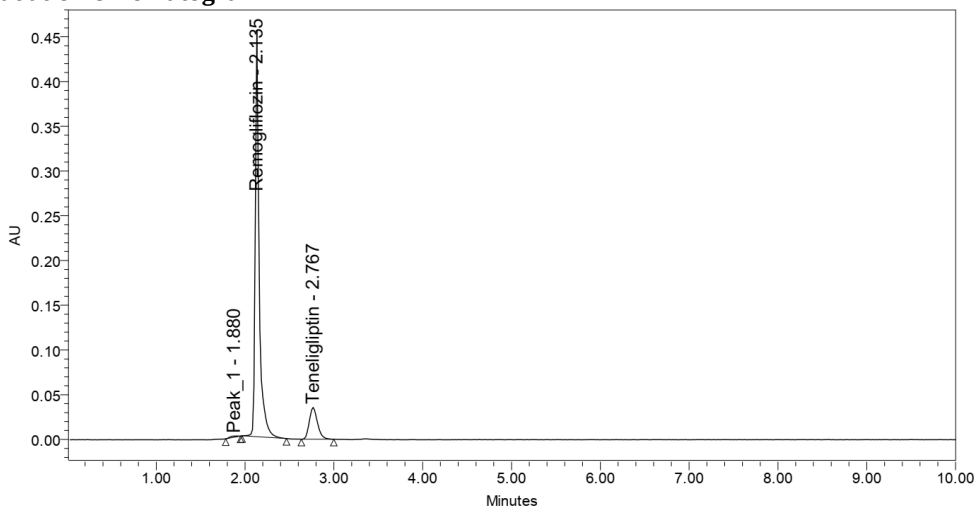

Figure.7 Acid

Base degradation chromatogram

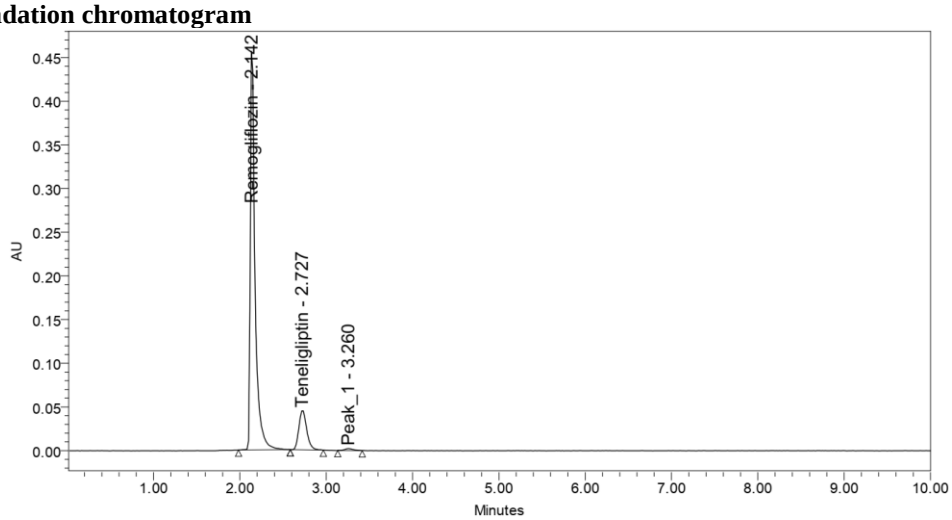

Figure.8 Base

Peroxide degradation chromatogram

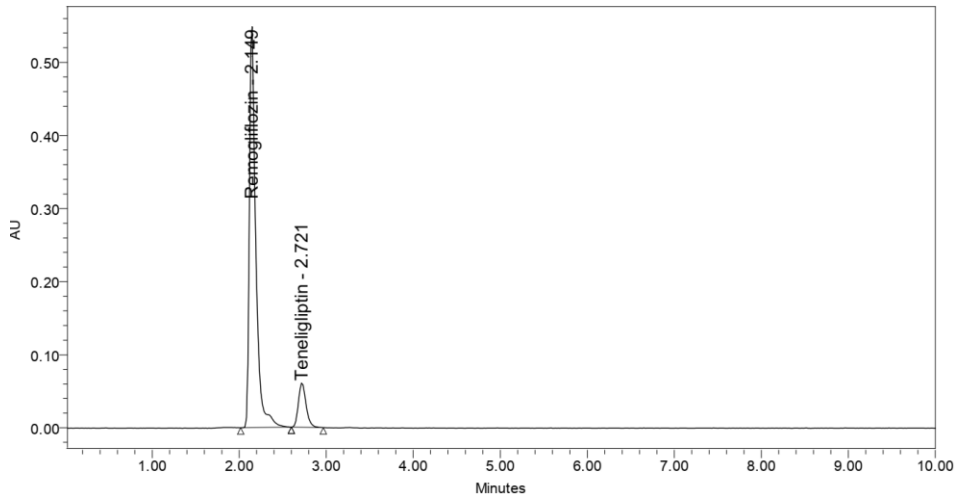

Figure.9 Peroxide

Thermal degradation chromatogram

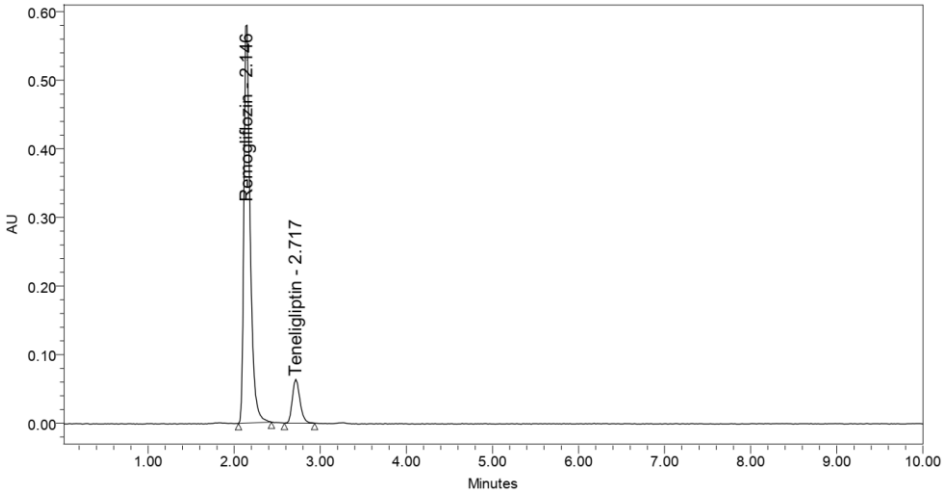

Figure.10 Thermal

UV degradation chromatogram

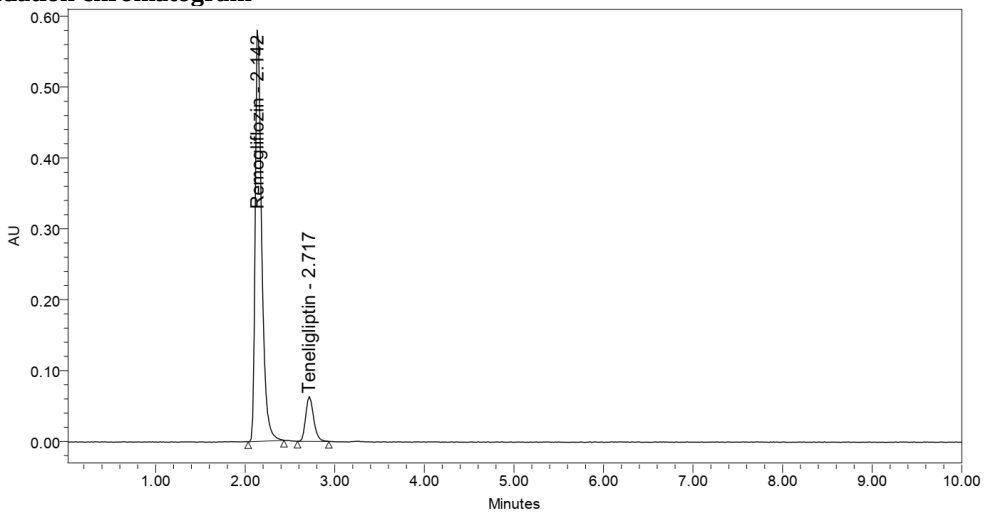

Figure.11 UV

Water degradation chromatogram

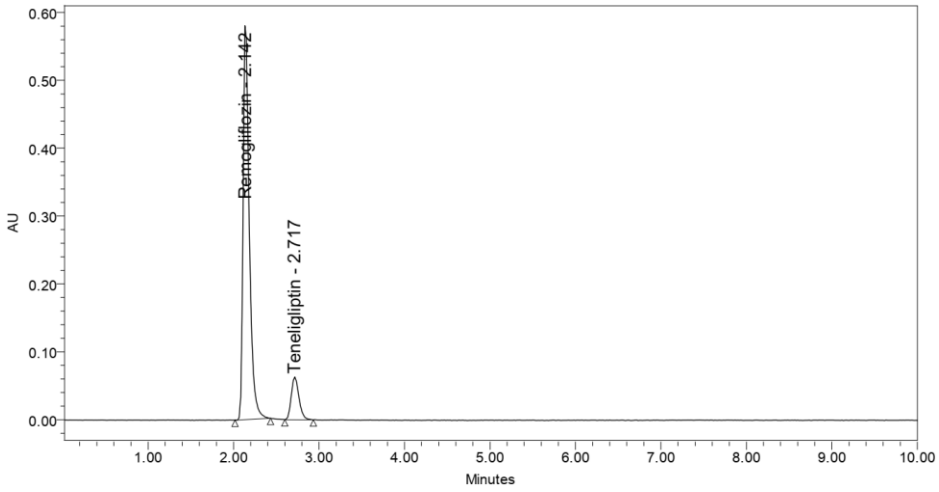

Figure.12 Water

Assay: Zeta Plus R, bearing the label claim Remogliflozin 100mg, Teneligliptin 10mg. Assay was performed with the above formulation. Average % Assay for Remogliflozin and Teneligliptin obtained was 99.27% and 99.19% respectively.

Table 13: Assay data

	Remogliflozin			Teneligliptin		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	2657455	2633914	99.24	350616	351234	99.60
2	2636806	2618734	98.67	349234	349540	99.12
3	2671578	2635432	99.30	354804	350244	99.32
4	2663686	2649549	99.83	355533	351213	99.60
5	2651481	2651514	99.90	351147	351390	99.65
6	2627519	2619532	98.70	352364	345116	97.87
Avg	**2651421**	**2634779**	**99.27**	**352283**	**349790**	**99.19**
Stdev	**16601.5**	**14066.3**	**0.5**	**2461.3**	**2399.4**	**0.68**
%RSD	**0.6**	**0.5**	**0.5**	**0.7**	**0.7**	**0.7**

CONCLUSION:

The study's findings will be very helpful in evaluating the quality of reasonably priced drugs that contain Teneligliptin and Remogliflozin. This could be as a result of the study's straightforward sample preparation method, which required little mobile phase and a brief analytical period. The results of evaluating two medications combined in a single dosage demonstrated that the recently created analysis technique was almost entirely successful.

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