World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND REMOGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORMS BY HPLC

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Received: 13-02-2025 / Revised Accepted: 13-02-2025 / Published: 15-02-2025

ABSTRACT:

By using HPLC Remogliflozin and Teneligliptin was estimated by using a HSS C18 column of dimension 2.1 x 50mm, 1.8µm With KH2PO4 as buffer together with Acetonitrile in ratio of 40:60 at a flow of 0.2ml/min. the ideal wavelength was detected at 215 nm. The rt of Teneligliptin and Remogliflozin was found at 1.375 min and 1.736 min. the System precision's RSD got at 1.2 and 0.6%. linearity conc was observed at 1.25-7.5 µg/ml for Teneligliptin and Remogliflozin was 12.5-75 µg/ml. the regression from it obtained was y = 11748x + 329.21and y = 4428.9x + 4002.7 respectively. Our confirmation and observation of all the other factors were determined while staying within the limits that were defined.

Key Words Remogliflozin, Teneligliptin, Rp Hplc, Validation, Method Development.

INTRODUCTION

Two varieties of sodium-glucose cotransporters have been identified and shown to exhibit renal (proximal tubule) glucose reabsorption: SGLT1 (high affinity, low-capacity glucose cotransporter) and SGLT2 (low affinity, high-capacity glucose cotransporter)**[1].** Remogliflozin is an SGLT2 inhibitor. SGLT2 inhibitors decrease glucose reabsorption in the kidneys, resulting in higher glucose excretion in urine. This method lowers blood glucose levels and is especially beneficial for those with type 2 diabetes. SGLT2 inhibitors reduce glucose reabsorption, leading to weight loss and maybe lower blood pressure **[2,3,4]**. Teneligliptin is a DPP-4 inhibitor. DPP-4 inhibitors are another type of medication used to treat type 2 diabetes. They function by blocking the enzyme DPP-4, which degrades incretin hormones (GLP-1 and GIP). These hormones help regulate insulin release and glucose levels. Teneligliptin inhibits DPP-4, which increases the levels of these incretin hormones, promoting insulin release and lowering blood glucose levels**[6,7]**. Both Remogliflozin and Teneligliptin can be used as part of a complete treatment strategy for type 2 diabetes, usually in conjunction with other antidiabetic drugs, dietary changes, and exercise**[8].**

Background: Teneligliptin has been investigated for the treatment of Type 2 Diabetes Mellitus. It is known as 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4-[(3S,5S)-5-(1,3-thiazolidine-3-carbonyl)pyrrolidin-3-yl]piperazine chemically[9], whereas Remogliflozin is used to lower blood sugars in patients with type 2 diabetes its known asethyl[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-{[5-methyl-1-(propan-2-yl)-4-{[4-(propan-2-yloxy)phenyl]methyl}-1H-pyrazol-3-yl]oxy}oxan-2-yl]methyl carbonate.**[10]**

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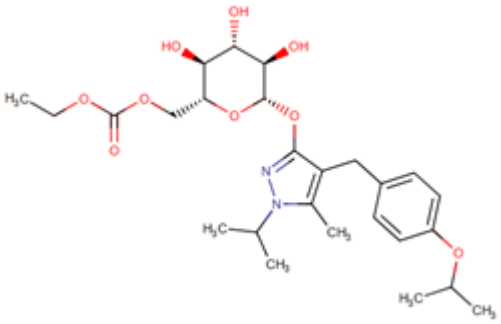
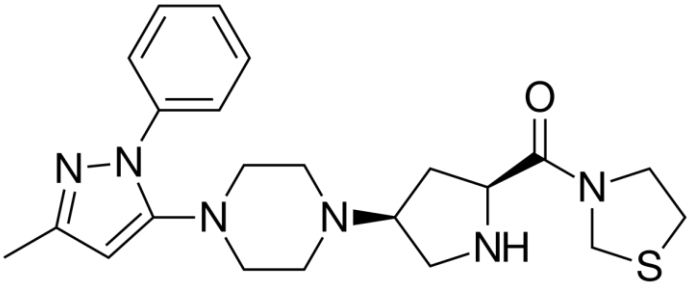
How to Cite this Article: A. Plasencia Rose Maglin, ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND REMOGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORMS BY HPLC. World J Pharm Sci 2025; 13(01): 133-144; https://doi.org/10.54037/WJPS.2022.100905



[A]



[B]

Figure 1 and 2 structure of [A] Remogliflozin and [B]Teneligliptin

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Teneligliptin, Remogliflozin, and their medicinal dose form using RP-HPLC.[11-16] must be validated and developed as per ICH guidelines.

Materials and Methods: Spectrum Pharma Research Solution offers gift samples of pure medications (API) of Teneligliptin and Remogliflozin as well as combination tablets (Zeta Plus-R) of these two medications that are purchased from the local market. Rankem, an Indian supplier, provided the chemicals and buffers used in this estimation.

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 Remogliflozin and Teneligliptin in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	**0.01N** KH2PO4: Acetonitrile(40:60)
Flow rate	0.9 ml/min
Column	HSS C18 Column, 1.8 µm, 2.1 x 50 mm
Detector wave length	215 nm
Column temperature	30°C
Injection volume	1µL
Run time	3.0 min

Preparation of Standard stock solutions: Accurately weighed 25mg of Remogliflozin, 2.5mg of Teneligliptin and transferred to 50ml volumetric flask. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution 1. (500µg/ml of Remogliflozin and 50µg/ml of Teneligliptin). Then 1ml from 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).

Preparation of Sample stock solutions: 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, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters. (1000µg/ml of Remogliflozin and 100µg/ml of Teneligliptin). 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: Teneligliptin (5 ppm) and Remogliflozin (50 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	Teneligliptin			Remogliflozin			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	1.334	2919	1.22	1.728	2792	1.23	3.1
2	1.334	2916	1.19	1.728	2778	1.24	3.1
3	1.334	2928	1.21	1.729	2789	1.22	3.1
4	1.335	2929	1.26	1.73	2924	1.21	3
5	1.335	2930	1.24	1.73	2803	1.24	3.1
6	1.336	2931	1.23	1.73	2779	1.24	3.1

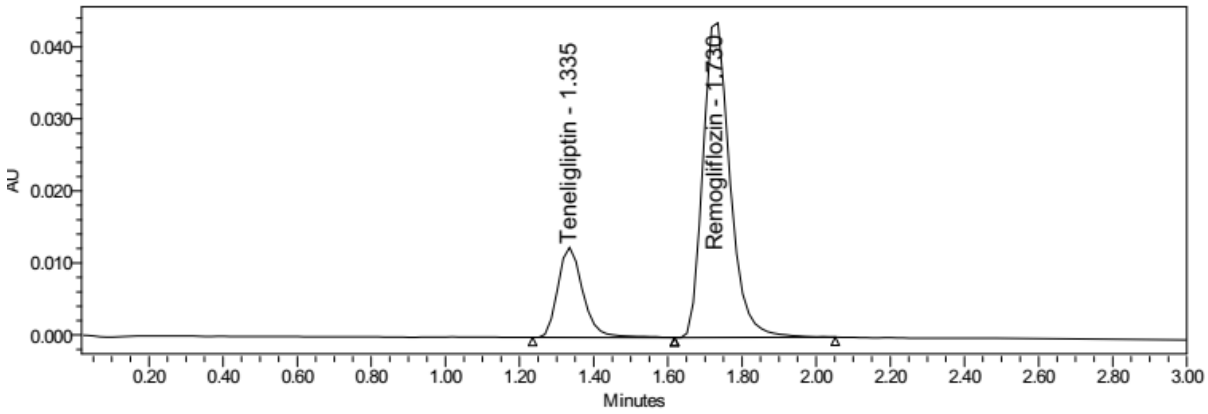


Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Teneligliptin	1.375	59231
Remogliflozin	1.738	223669

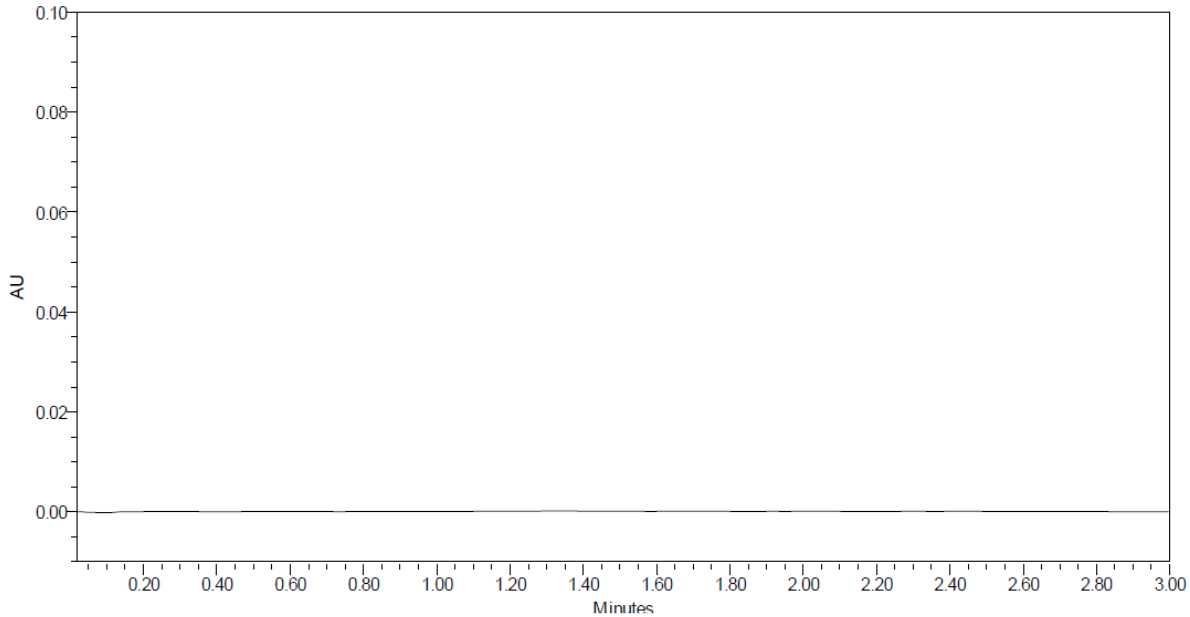


Figure.4 Blank

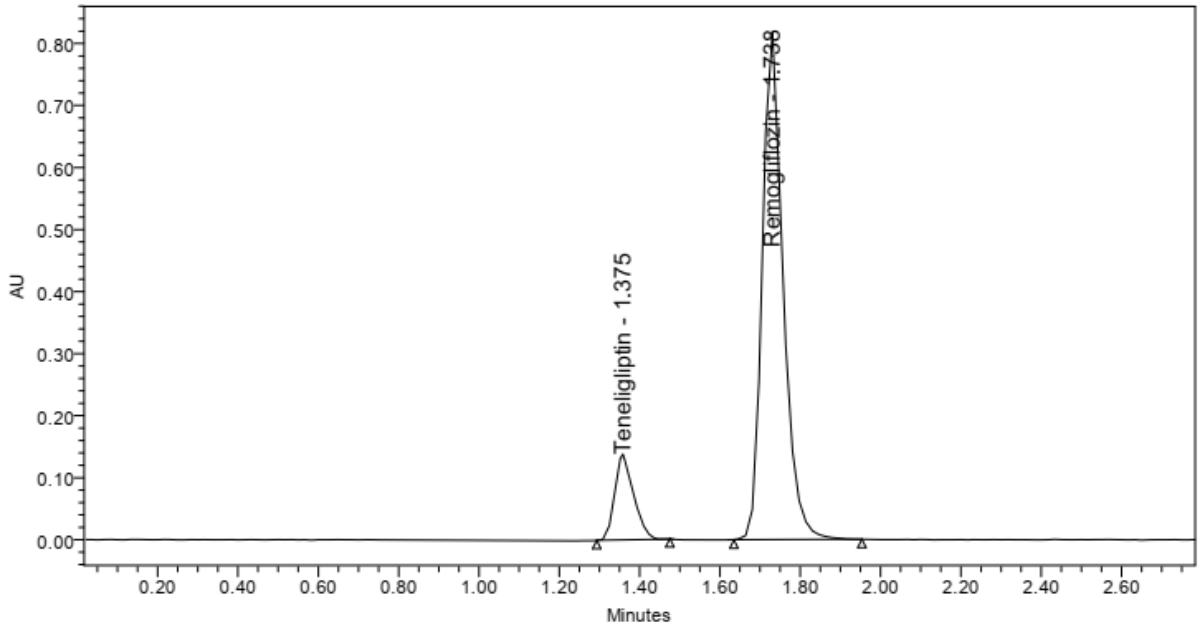


Figure 5: Specificity of Teneligliptin and Remogliflozin

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 6, 7

Table 4: Calibration data of Teneligliptin and Remogliflozin

	Teneligliptin		Remogliflozin	
	Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
1	0	0	0	0
2	1.25	15388	12.5	59435
3	2.5	29264	25	119538
4	3.75	44676	37.5	170510
5	5	59666	50	225487
6	6.25	73532	62.5	283863
7	7.5	88163	75	331766
Concentration range (µg/mL)	1.25-7.5		12.5-75	
Regression Equation	y = 11748x + 329.21		y = 4428.9x + 4002.7	
Co-relation	0.9998		0.9992	
LOD	0.04		0.25	
LOQ	0.13		0.75	

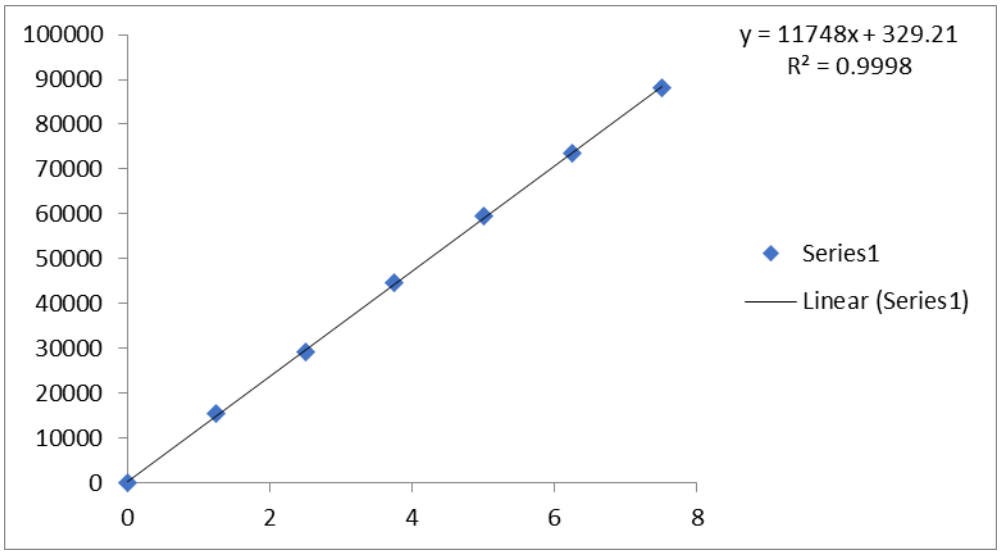


Figure 6 Calibration curve of Teneligliptin

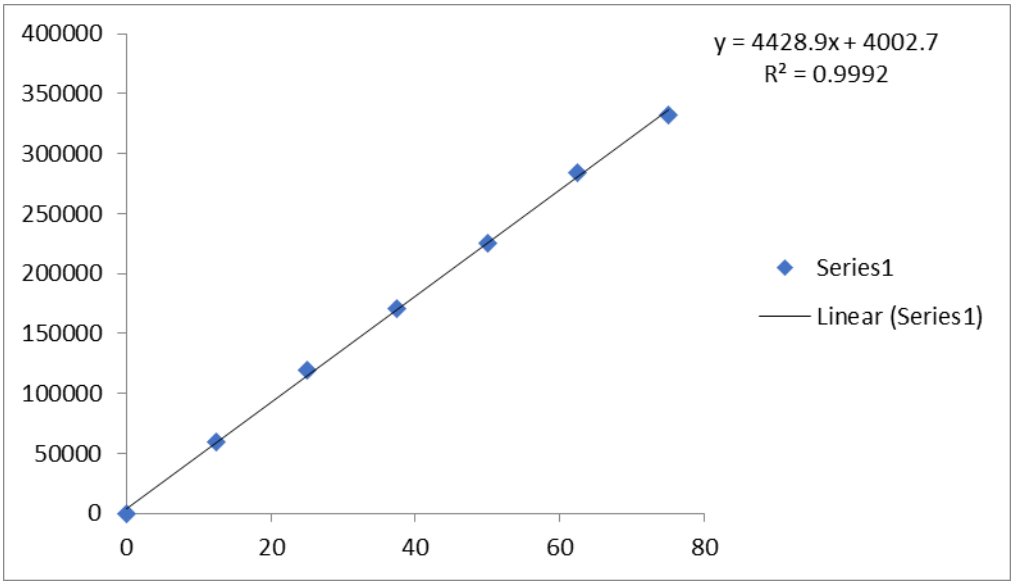


Figure 7 Calibration curve of Remogliflozin

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Teneligliptin and Remogliflozin

	Teneligliptin			Remogliflozin		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	2.5	2.51	100.51	25	24.6	98.3
	2.5	2.48	99.13	25	24.9	99.4
	2.5	2.51	100.44	25	24.8	99.4
100%	5	4.93	98.58	50	49.8	99.6
	5	4.99	99.77	50	49.9	99.8
	5	5.07	101.32	50	49.9	99.8
150%	7.5	7.46	99.48	75	75.2	100.3
	7.5	7.43	99.11	75	73.8	98.4
	7.5	7.49	99.90	75	74.7	99.5
% recovery	**99.80**			**99.40**		

System precision was performed and the data was shown in table 8

Table 7: System precision of Teneligliptin and Remogliflozin

S. No	Area of Teneligliptin	Area of Remogliflozin
1.	60270	222373
2.	58310	223099
3.	59231	224094
4.	59966	221303
5.	59055	223669
6.	59978	224792
Mean	59468	223222
S.D	737.4	1252.5
%RSD	1.2	0.6

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

Method Precision: The precision of the method was determined by analyzing a sample of Teneligliptin and Remogliflozin and shown in table 8.

Table 8: method Precision

S. No	Area of Teneligliptin	Area of Remogliflozin
1.	59315	221860
2.	59216	222359
3.	59187	222138
4.	59899	221838
5.	59560	225756
6.	59236	222412
Mean	59402	222727
S.D	278.5	1503.2
%RSD	0.5	0.7

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

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55A:45B), mobile phase plus (65A:35B), temperature minus (27°C) and temperature plus(33°C) 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 9: Robustness data for Teneligliptin and Remogliflozin.

Condition	%RSD of Teneligliptin	%RSD of Remogliflozin
Flow rate (-) 0.9ml/min	0.3	0.7
Flow rate (+) 1.1ml/min	0.3	0.1
Mobile phase (-) 55A:45B	0.8	0.7
Mobile phase (+) 65A:35B	0.2	1.2
Temperature (-) 27°C	0.4	0.1
Temperature (+) 33°C	0.6	0.4

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

Table 12: degradation data

Type of degradation	Teneligliptin			Remogliflozin		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	206356	93.60	6.40	55717	92.35	7.65
Base	208205	90.85	9.15	54084	93.18	6.82
Peroxide	217912	97.60	2.40	58102	97.52	2.48
Thermal	219140	97.90	2.10	58277	98.07	1.93
Uv	219502	98.32	1.68	58526	98.24	1.76
Water	221757	99.28	0.72	59102	99.24	0.76

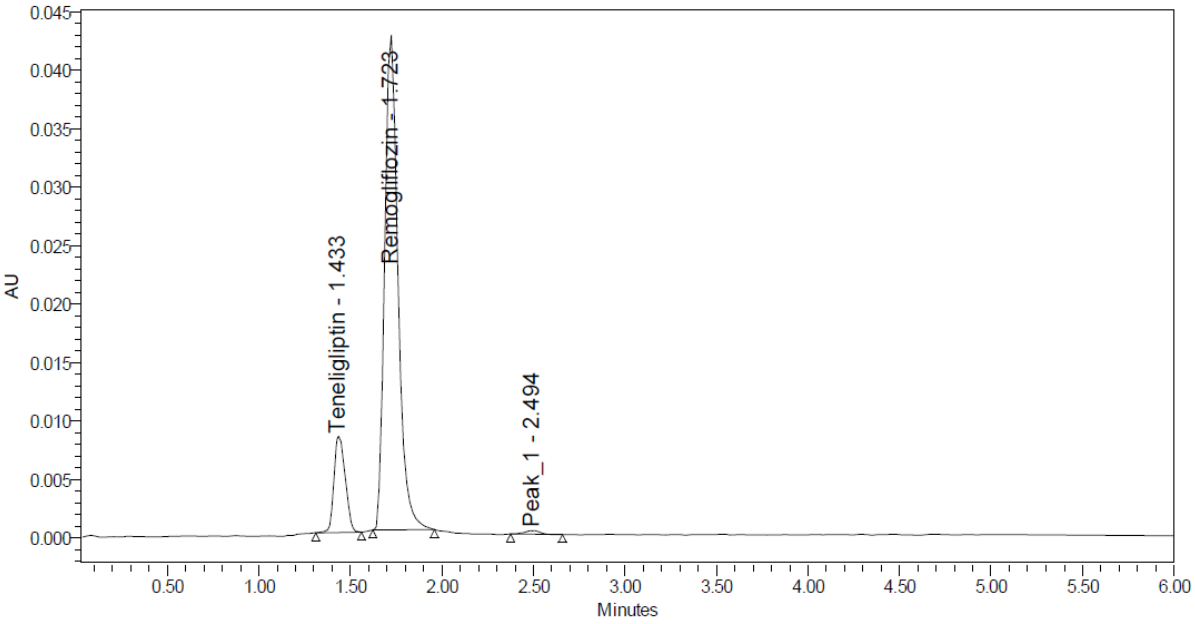


Figure 8: Acid Condition for Teneligliptin and Remogliflozin

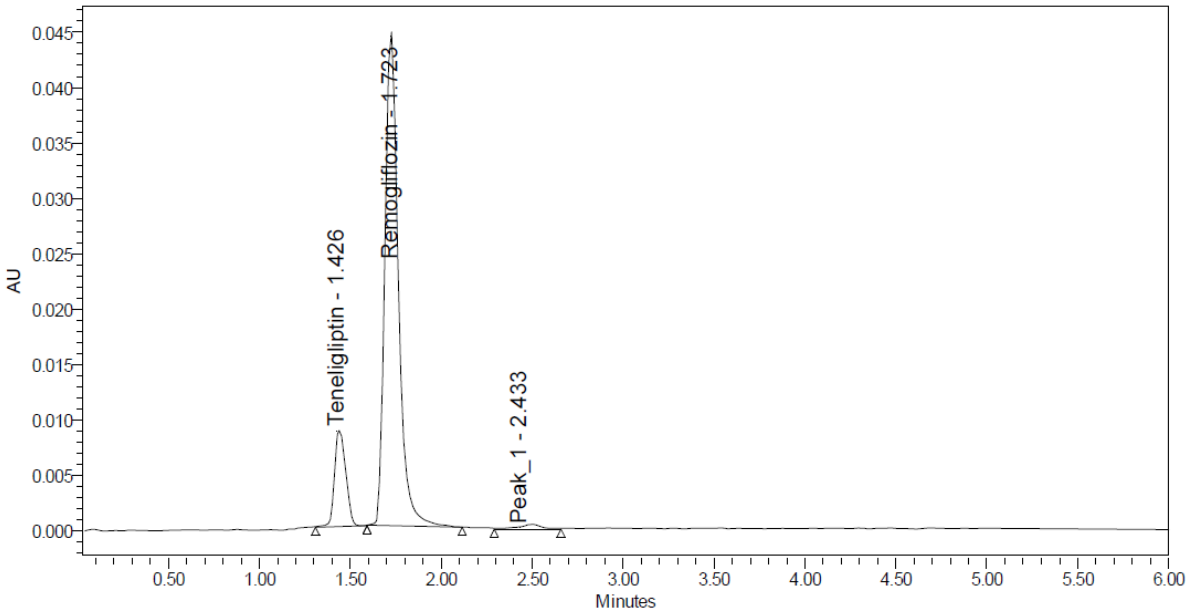


Figure 9: Base Condition for Teneligliptin and Remogliflozin

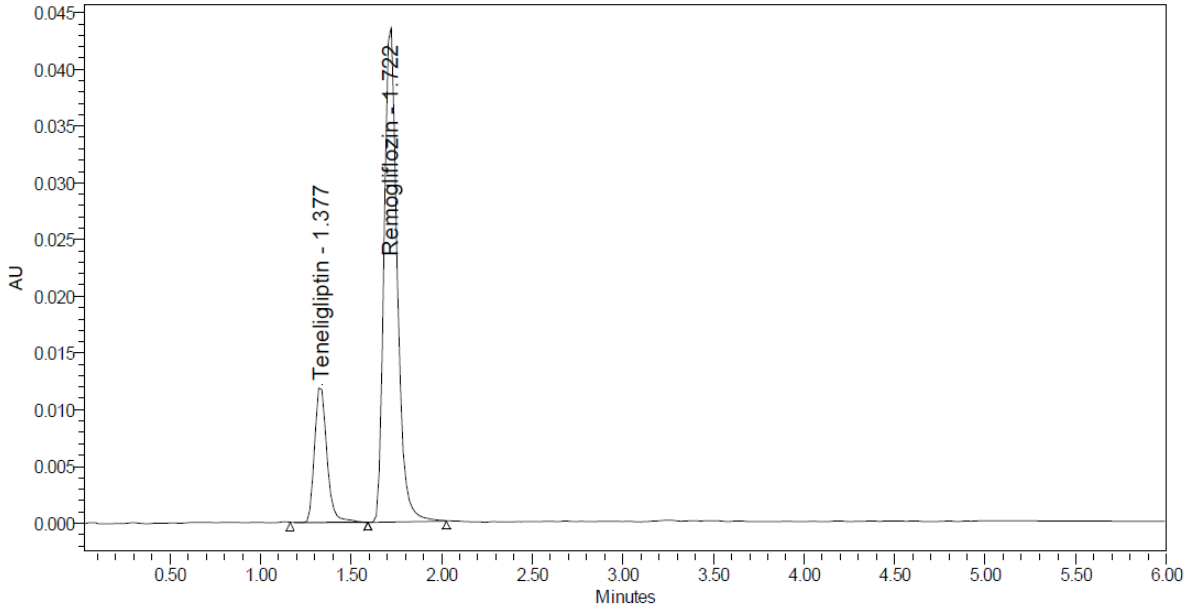


Figure 10: Peroxide Condition for Teneligliptin and Remogliflozin

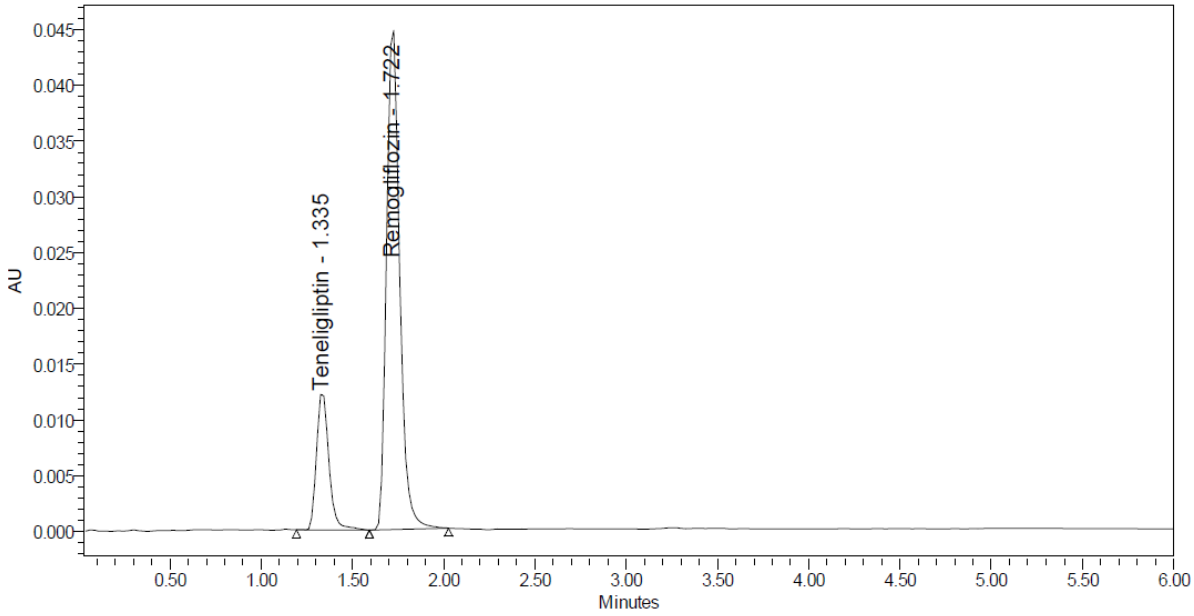


Figure 11: Thermal Condition for Teneligliptin and Remogliflozin

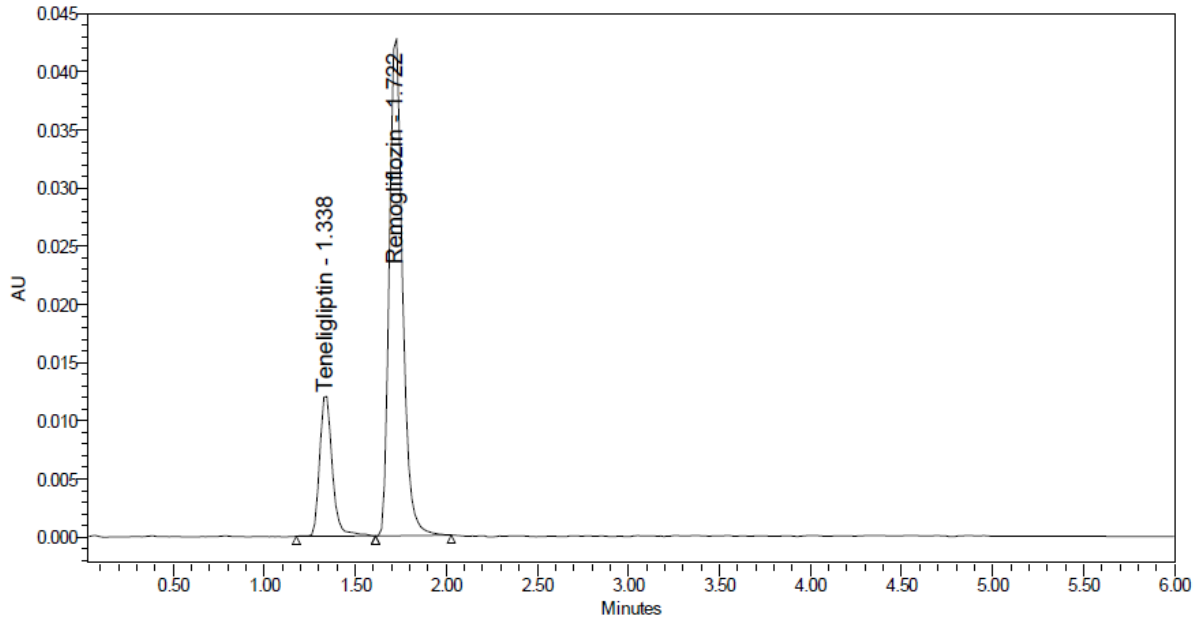


Figure 12: UV Condition for Teneligliptin and Remogliflozin

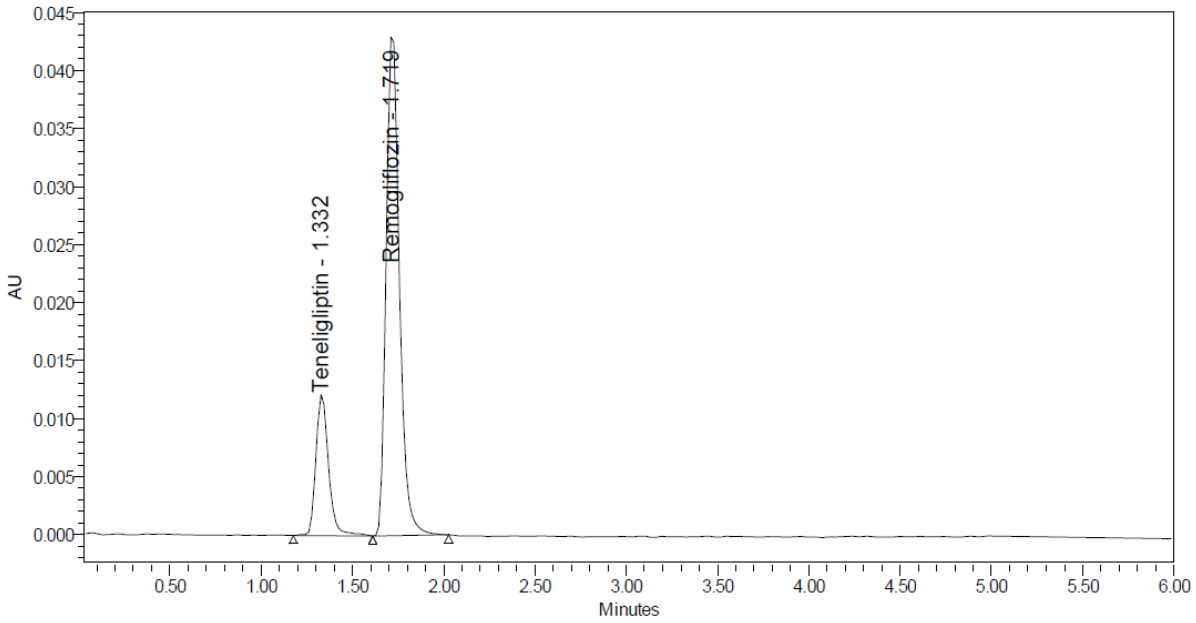


Figure 13: Water Condition for Teneligliptin and Remogliflozin

Assay: Zita plus -R Tablet, bearing the label claim Teneligliptin 300mg, Remogliflozin 300mg. Assay was performed with the above formulation. Average % Assay for Teneligliptin and Remogliflozin obtained was 99.79% and 99.68% respectively.

Table 13: assay data

	Teneligliptin			Remogliflozin		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	60270	59315	99.64	222373	221860	99.29
2	58310	59216	99.48	223099	222359	99.51
3	59231	59187	99.43	224094	222138	99.42
4	59966	59899	100.62	221303	221838	99.28
5	59055	59560	100.05	223669	225756	101.03
6	59978	59236	99.51	224792	222412	99.54
Avg	59468	59402	99.79	223222	222727	99.68
Stdev	737.4	278.5	0.47	1252.5	1503.2	0.67
%RSD	1.2	0.5	0.47	0.6	0.7	0.7

Conclusion:

The study's findings will be extremely valuable in evaluating the quality of low-cost drugs including teneligliptin and Remogliflozin. This could be attributed to the study's straightforward sample preparation process, which required a quick analytical time and a minimal mobile phase. The examination of two medications in a single dosage revealed that the newly established analysis method was almost entirely successful.

ACKNOWLEDGEMENT:

The authors are thankful to Spectrum Pharma Research Solutions, Hyderabad, Telangana, India.

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