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Research Article

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

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ABSTRACT:

Remogliflozin and Teneligliptin was estimated with HPLC in a tablet dosage form, it was processed with Discovery C 18 column of dimensions 150 x 4.6 mm and 5µm. ammonium acetate and Acetonitrile was used as MP in it with a ratio of 65:35. Wavelength was identified at 210 nm. Rt of Remogliflozin and Teneligliptin was found at 2.206 and 2.646 min. %RSD of the drugs found at 0.6 % and 0.6%, with that the regression of both was seen at y = 52765x + 1014.4 and y = 73315x + 624.91 respectively, and the mean recovery was 99.70% and 99.93%. All the other paraments were validated and were observed within the limits.

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

INTRODUCTION

Teneligliptin and remogliflozin are oral antidiabetic agents used in the management of type 2 diabetes mellitus (T2DM). Teneligliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances incretin hormone activity, leading to increased insulin secretion and decreased glucagon release, thereby improving glycemic control. Remogliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor that reduces renal glucose reabsorption, promoting urinary glucose excretion and lowering blood glucose levels.[1]

The combination of teneligliptin and remogliflozin has been explored for its potential synergistic effects in improving glycemic control in patients inadequately managed with metformin alone. A randomized, double-blind, active-controlled study demonstrated that the fixed-dose combination (FDC) of remogliflozin etabonate (100 mg) and teneligliptin hydrobromide hydrate (10 mg) administered twice daily, in addition to metformin, resulted in significant reductions in glycated hemoglobin (HbA1c) levels compared to teneligliptin monotherapy. This combination also offered the benefit of weight reduction and was well-tolerated, with no serious adverse events reported during the study period.[2]

Analytical methods have been developed to simultaneously quantify teneligliptin and remogliflozin in pharmaceutical formulations. For instance, a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method has been established for the simultaneous estimation of these drugs in tablet dosage forms, ensuring quality control and regulatory compliance. [3] Remogliflozin received approval in India in April 2019 as a novel SGLT2 inhibitor for the treatment of T2DM. Its clinical profile includes effective

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glycemic control with a favorable safety and tolerability profile.[4] The fixed-dose combination of remogliflozin and teneligliptin offers a promising therapeutic option for patients with T2DM, providing complementary mechanisms of action that target different aspects of glucose regulation. This combination therapy may enhance patient adherence by reducing pill burden and simplifying treatment regimens. [5]

Background: Remogliflozin etabonate is chemically known as ethyl [(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[6] and Teneligliptin 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 [7]

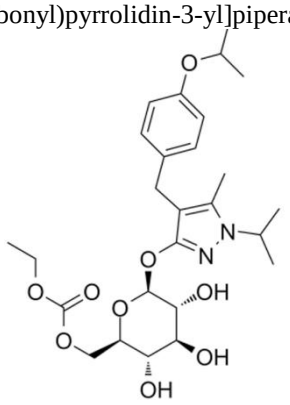
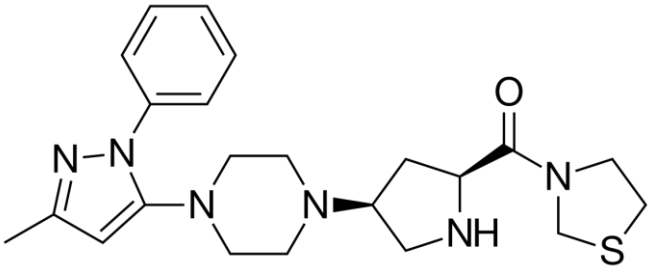



Figure 1 and 2 structure of Remogliflozin and Teneligliptin

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.[8-25] 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 ful fill 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	Ammonium Acetate and Methanol (65:35v/v)
Flow rate	1 ml/min
Column	Discovery C18 Column, 5 µm, 4.6 x 150 mm
Detector wave length	241 nm
Column temperature	30°C
Injection volume	10µL
Run time	5.0 min
Buffer	0.1N Ammonium acetate

Preparation of Standard stock solutions: Remogliflozin 100mg and Teneligliptin 10 mg was weigh and added in 250 ml vf. And then it was filled till the neck of the vf and then drug is dissolved by sonicating for 10 min and when the drug dissolves the dil is added in the vf till the mark (Remogliflozin- 400µg/ml, Teneligliptin- 40µg/ml), then from it 1 ml of stock sol was spiked and was add in a 10 ml vf and made up till mark with diluent. (Remogliflozin- 40µg/ml, Teneligliptin- 4µg/ml)

Preparation of Sample stock solutions: Remogliflozin and Teneligliptin marketed formulation 10 tablets were taken and weight. Their average weight was noted and the drug weight equivalent to 1 tablet was taken and added in 500 ml vf, later 100 ml of diluent was added in it and kept for sonication for the drug to dissolve for 15 min. after that HPLC filter was taken to filter out the volume after makeup of diluent. (Remogliflozin- 200µg/ml, Teneligliptin- 20µg/ml), from it 1 ml of stock sol was spiked and was add in a 10 ml vf and made up till mark with diluent. (Remogliflozin- 40µg/ml, Teneligliptin- 4µg/ml),

System suitability parameters: Teneligliptin (4 ppm) and Remogliflozin (40 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	2.206	4812	1.31	2.631	9895	1.21	3.5
2	2.207	4806	1.31	2.632	10012	1.21	3.6
3	2.208	4798	1.31	2.633	10065	1.2	3.5
4	2.21	4845	1.31	2.644	10120	1.2	3.6
5	2.211	4810	1.3	2.644	10134	1.21	3.6
6	2.212	4839	1.3	2.645	10289	1.23	3.6

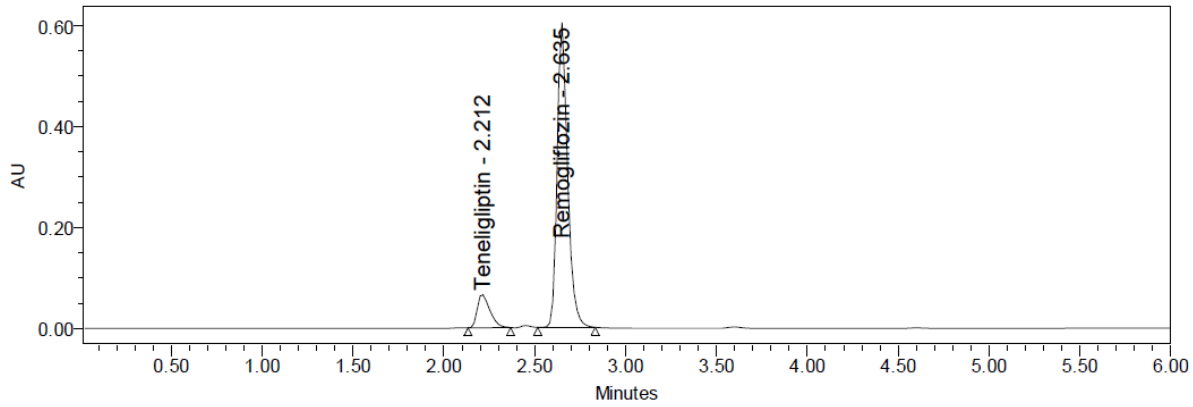
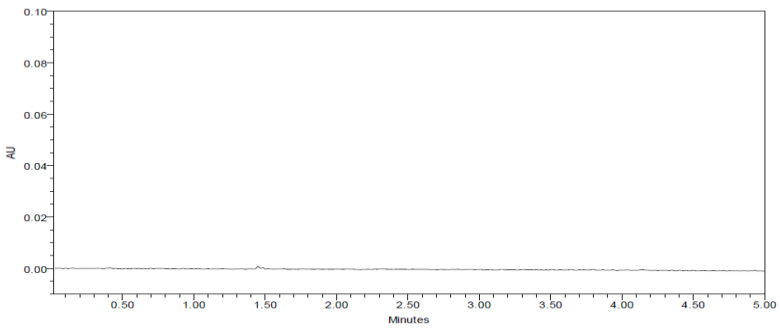


Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Teneligliptin	2.206	296281
Remogliflozin	2.646	2412084



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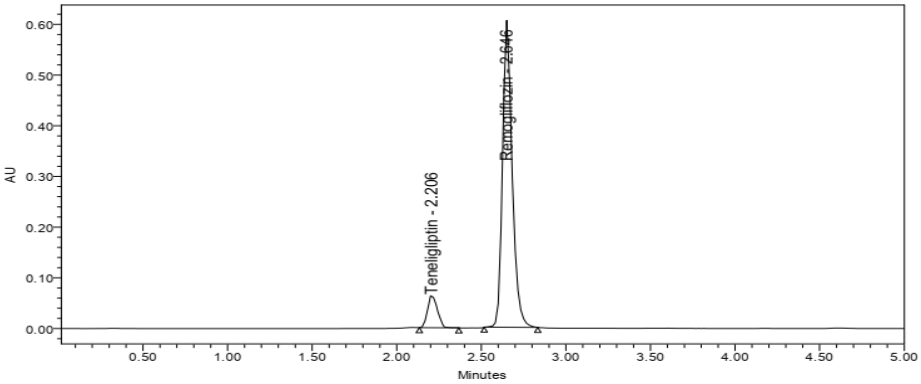

Figure 5: Specificity of Teneligliptin and Remogliflozin

Linearity:

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

Table 4: Calibration data of Teneligliptin and Remogliflozin

Teneligliptin		Remogliflozin	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
1	73645	10	496070
2	144829	20	1058022
3	225906	30	1605591
4	294864	40	2169635
5	364151	50	2620744
6	440587	60	3137792

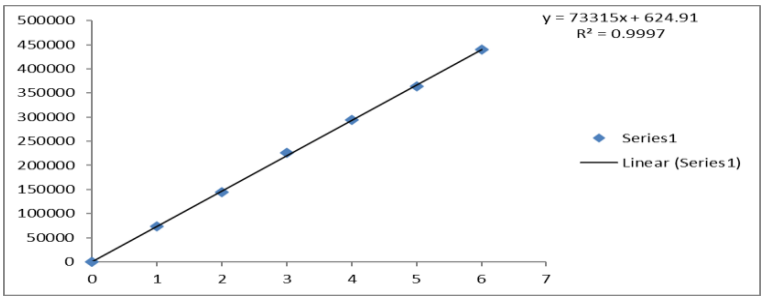

Figure 6: Calibration curve of Teneligliptin

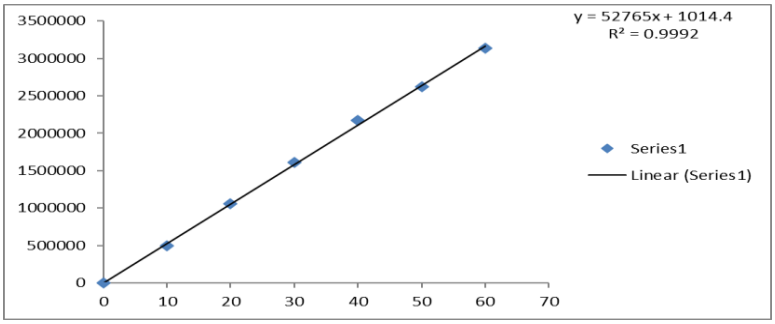

Figure 7: Calibration curve of Remogliflozin

Table 5: regression data

Parameter	Teneligliptin	Remogliflozin
Conc range (µg/mL)	1-6	10-60
Regression Equation	y = 73315x + 624.91	y = 52765x + 1014.4
Co-relation	0.999	0.999

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	2.02	100.88	20	19.93	99.66
	2	2.00	99.86	20	20.03	100.14
	2	2.02	101.00	20	20.07	100.36
100%	4	3.98	99.61	40	39.81	99.52
	4	4.01	100.20	40	39.78	99.45
	4	3.97	99.14	40	39.91	99.76
150%	6	6.00	100.07	60	59.59	99.32
	6	5.94	98.97	60	59.83	99.71
	6	5.98	99.61	60	59.64	99.39
% recovery	99.93%			99.70%		

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.	291159	2470430
2.	290988	2488573
3.	294125	2469358
4.	295411	2475521
5.	295584	2481579
6.	298513	2478246
Mean	**294297**	**2477285**
S.D	**2880.9**	**7207.2**
%RSD	**1.0%**	**0.3%**

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.

Table 8: method Precision

S. No	Area of Teneligliptin	Area of Remogliflozin
1.	291905	2465900
2.	292627	2486136
3.	292087	2497369
4.	294381	2468241
5.	296605	2493395
6.	293512	2458786
Mean	**293520**	**2478305**
S.D	**1773.0**	**16053.8**
%RSD	**0.6%**	**0.6%**

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 (60A:40B), mobile phase plus (70A:30B), 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	0.5	0.6
Flow rate (+) 1.1ml/min	0.6	0.2
Mobile phase (-) 60A:40B	0.3	0.9
Mobile phase (+) 70A:30B	0.8	0.7
Temperature (-) 27°C	0.1	0.3
Temperature (+) 33°C	0.4	0.5

Sensitivity:

Table 10: sensitivity of Teneligliptin and Remogliflozin

Molecule	LOD	LOQ
Teneligliptin	0.01	0.03
Remogliflozin	0.08	0.24

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

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	278541	94.46	5.54	2339142	94.23	5.77
Base	280125	94.99	5.01	2356891	94.95	5.05
Peroxide	279143	94.66	5.34	2298653	92.60	7.40
Thermal	286546	97.17	2.83	2412578	97.19	2.81
UV	291546	98.87	1.13	2432585	98.00	2.00
Water	293451	99.51	0.49	2461243	99.15	0.85

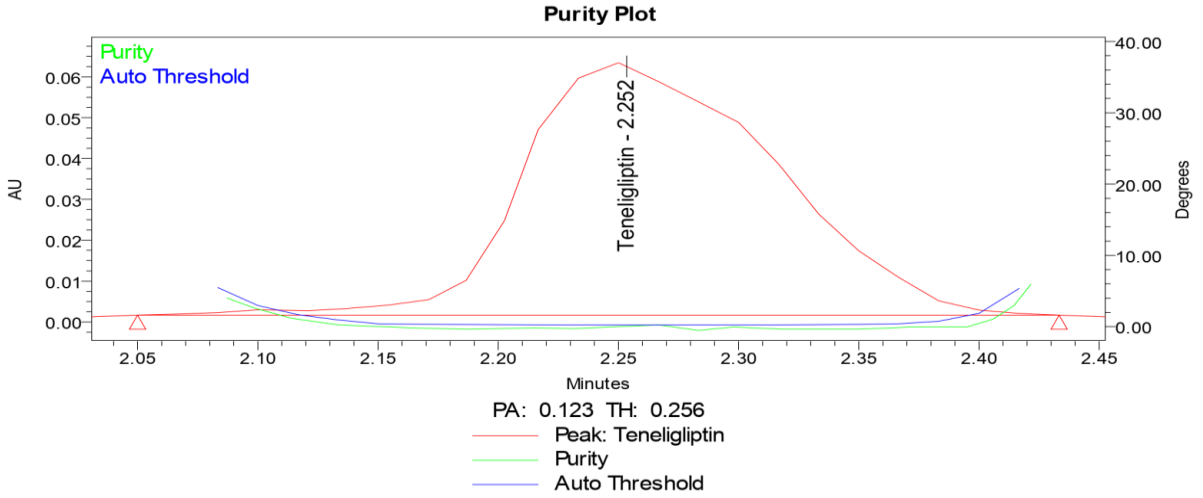

Figure 8: Purity plots for Acid Condition for Teneligliptin

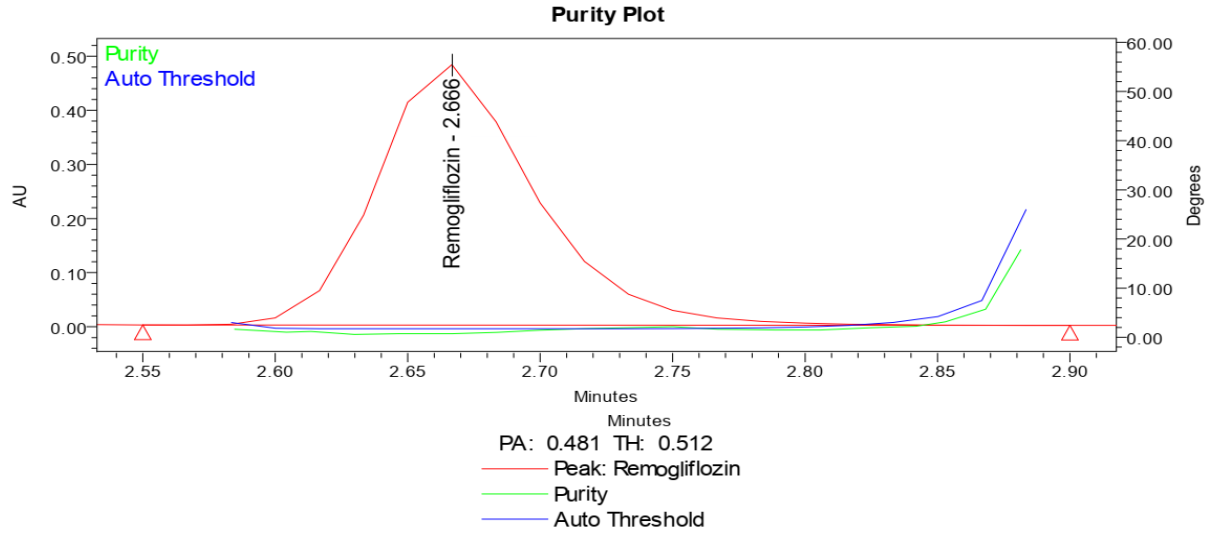

Figure 9: Purity plots for Acid Condition for Remogliflozin

Assay: Zita plus -A Tablet, bearing the label claim Teneligliptin 100mg, Remogliflozin 100mg. Assay was performed with the above formulation. Average % Assay for Teneligliptin and Remogliflozin obtained was 99.54% and 99.84% respectively.

Table 13: assay data

	Teneligliptin			Remogliflozin		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	291159	291905	**98.99**	2470430	2465900	**99.34**
2	290988	292627	**99.23**	2488573	2486136	**100.16**
3	294125	292087	**99.05**	2469358	2497369	**100.61**
4	295411	294381	**99.83**	2475521	2468241	**99.44**
5	295584	296605	**100.58**	2481579	2493395	**100.45**
6	298513	293512	**99.53**	2478246	2458786	**99.05**
Avg	294297	293520	**99.54**	2477285	2478305	**99.84**
Stdev	2880.9	1773.0	**0.60**	7207.2	16053.8	**0.647**
%RSD	1.0	0.6	**0.6**	0.3	0.6	**0.6**

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10: formula

CONCLUSION:

The findings of the study will be very helpful in evaluating the quality of reasonably priced drugs that contain Remogliflozin and Teneligliptin. 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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