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RP-HPLC Method Validation for The Simultaneous Estimation of Remogliflozin and Teneligliptin in Bulk and Pharmaceutical Dosage Form

Kasturi Jahnavi, M Sudhakar, C Parthiban, Dornala Chaitanya Dixit*
Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Dulapally, Kompally, Secunderabad, Hyderabad, Telangana, India.

****Corresponding Author***
E-Mail Id: chaithudixit@gmail.com

ABSTRACT

For the simultaneous estimation of remogliflozin and teneligliptin in API and pharmaceutical dosage form, a simple precise accurate and durable reverse phase RP-HPLC has been validated. This approach uses a simple isocratic mobile phase of Acetonitrile: KH2 taken in the ratio (35:65) and Aglient column and Aglient HPLC chromatographic separation and PDA detection. The average retention time of remogliflozin and teneligliptin were found to be 2.263 min and 2.994 min which indicated the method can be used for the routine analysis. The linearity of remogliflozin and teneligliptin was discovered to be linear with r^2 of 0.999 for all medications, indication that the approach can yield good sensitivity. The precision acceptance is that the % RSD should not exceed 2.0%. The % RSD of the remogliflozin and teneligliptin were and found to be 0.5 and 0.6 respectively thereby indicating the method is precise.

Key words: *Method development, method validation, remogliflozin, teneligliptin, RP-HPLC.*

INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients [1]. The quality of the analytical data depends on the quality of the methods employed in generation of the data. Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities [2-3].

Remogliflozin etabonate has been used in trials studying the treatment and basic science of Type 2 Diabetes Mellitus and Diabetes Mellitus, Type 2. Remogliflozin inhibits the sodium-glucose transport proteins (SGLT), which are responsible for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine (**Dosage forms:** Tablet, **Brand names:** Remozen V) [4,5].

Teneligliptin has been investigated for the treatment of Type 2 Diabetes Mellitus. Teneligliptin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor. SGLT2 co-transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycemia, assists weight loss, and reduces blood pressure [6-9].

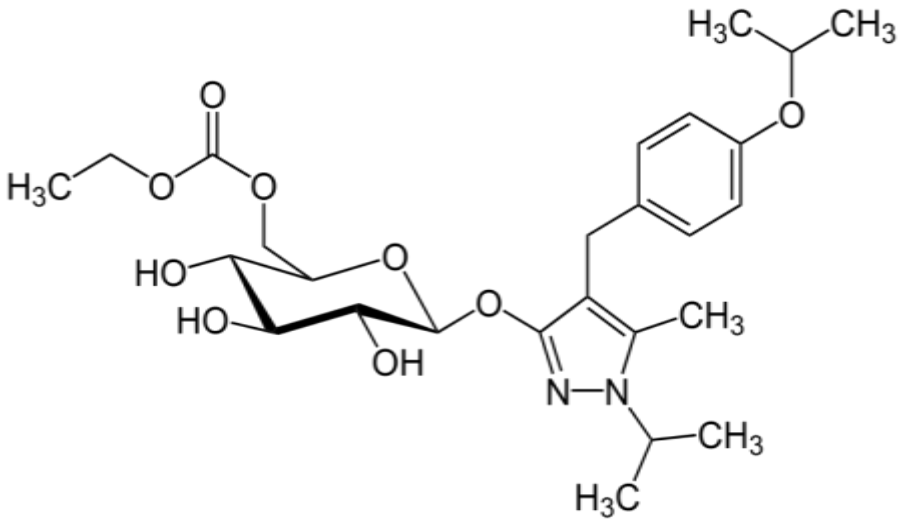

Fig. 1: Structure of Remogliflozin

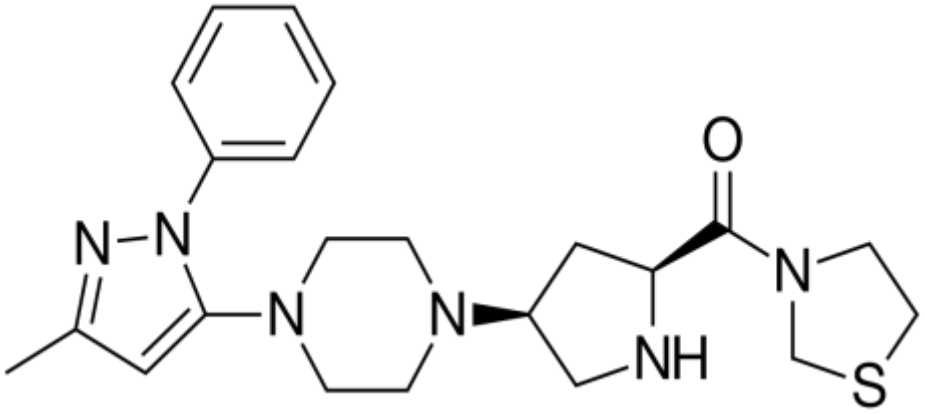

Fig. 2: Structure of Teneligliptin

According to literature survey there are some methods reported for the simultaneous estimation of these drugs, some methods for estimation of these individual drugs or with other drugs. UV -Spectroscopy method (**Dosage form**: Tablet, Extended release, **Brand name**: Jardiance) [10-11].

Used for treating type 2 diabetes in certain patients. It is used along with diet and exercise. Teneligliptin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor. It works by decreasing the amount of sugar the body absorbs and increasing the amount of sugar that leaves the body in the urine [12-14]. On the basics of the review of literature, no official method has been indicating simultaneous estimation of remogliflozin and teneligliptin by RP-HPLC in pharmaceutical dosage form. The main purpose of this study is to develop simple, accurate relatively sensitive and rapid RP-HPLC technique for estimation of remogliflozin and teneligliptin in bulk and pharmaceutical dosage form. a validated method applied for remogliflozin and teneligliptin estimation as per the ICH guidelines.

MATERIALS AND METHODS

Sun Pharma, Hyderabad has provided the remogliflozin and teneligliptin pure API drugs. Rankem, India provided all of the chemicals and buffers utilized in this work. Combination

remogliflozin and teneligliptin tablets (Zita PLUS-R) received from local market, Hyderabad, Telangana, India.

Instrumentation and Chromatographic Conditions

AGILENT HPLC, model G4-286b-HPLC system with photo diode array detector was used for the development and method validation with an automated sample injector. Agilent (150mm 4.5mm 3.5mm) column was used for the separation. The mobile phase used was KH2: Acetonitrile (65:35). having flow rate of 1ml/min, detected wavelength of 240nm, column temperature of 30°C, injection volume was set at 10µL and run time was 6min. The data acquired was at 240nm and the output signal was monitored and integrated using Empower2 Software.

Preparations of Solutions

Diluent

The diluent used was Acetonitrile and Water in the ratio of (50:50) as per solubility of drugs.

Preparation of Stock Solutions [15-16]

Accurately weighed 25mg of remogliflozin, 2.5mg of teneligliptin and transferred to 50ml and 50ml volumetric flasks separately. 3/4 Th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and from these stock solutions take 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50 µg/ml of remogliflozin and 5µg/ml of teneligliptin).

Preparation of Sample 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 HPLC filters and 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).

Preparation of Buffer [17]

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36g of potassium dihydrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5.4 with dil. formic acid.

0.1% Formic acid buffer: 1ml of conc. formic acid was diluted to 1000ml with water.

Method Development [18, 19]

The method validation of HPLC was carried out for the simultaneous estimation of remogliflozin and teneligliptin drug substance as per ICH guidelines to demonstrate that the method is proposed for routine analysis.

RESUTS AND DISCUSSION

Remogliflozin and teneligliptin were eluted at 2.263min and 2.994 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

System Suitability Data

System suitability: The system suitability was performed for each validation parameters by injecting standard solutions containing (50µg/ml of remogliflozin and 5µg/ml of teneligliptin). The % RSD for the area of six standard injections results should not be more than 2%. System suitability parameters are shown in figure 3 and values and mentioned in Table 1.

Specificity Data

Specificity (Selectivity): 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. Representative chromatogram is shown in Figure 4 and the experimental data is shown in Table 2.

Table 1: System Suitability Parameters for Remogliflozin and Teneligliptin

S. No.	Teneligliptin			Remogliflozin			
Injection	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.237	7555	1.20	2.950	11892	1.23	6.5
2	2.244	7803	1.17	2.954	12415	1.26	6.7
3	2.257	7636	1.16	2.989	11468	1.33	6.7
4	2.257	8116	1.16	2.990	12118	1.28	6.6
5	2.258	6584	1.25	2.994	12455	1.29	6.6
6	2.260	7925	1.17	2.997	11839	1.24	6.6

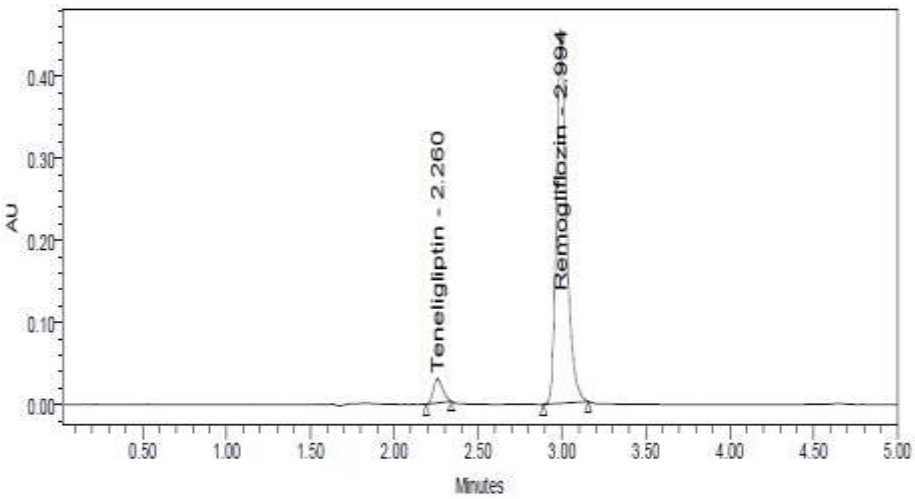

Fig. 3: System suitability chromatogram of remogliflozin and teneligliptin

Table 2: Specificity data of remogliflozin and teneligliptin

Sample name	Retention time (mins)
Remogliflozin	2.263
Teneligliptin	2.994

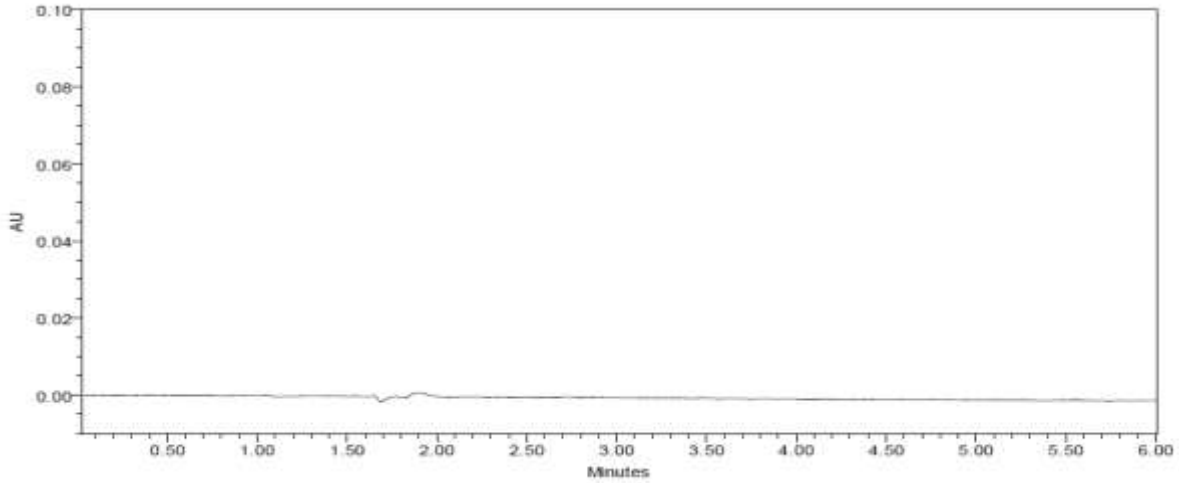

Fig. 4: Blank chromatogram of remogliflozin and teneligliptin

Table 3: Linearity of Remogliflozin and Teneligliptin

Remogliflozin		Teneligliptin	
Conc. (µg/mL)	**Peak area**	**Conc. (µg/mL)**	**Peak area**
0	0	0	0
12.5	497501	1.25	35433
25	995001	2.5	67916
37.5	1508074	3.75	104299
50	1995210	5	135732
62.5	2482220	6.25	172134
75	2885003	7.5	206097

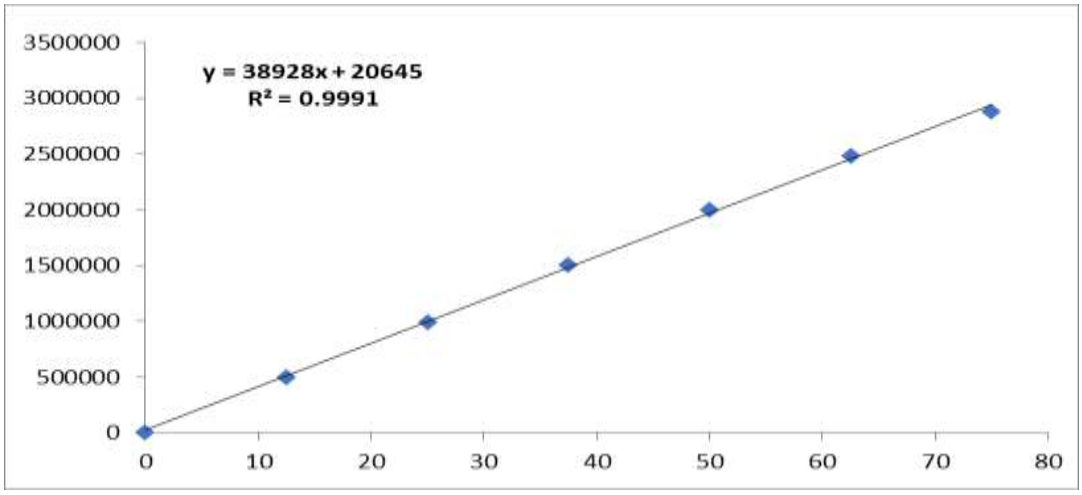

Fig. 5: Calibration of remogliflozin

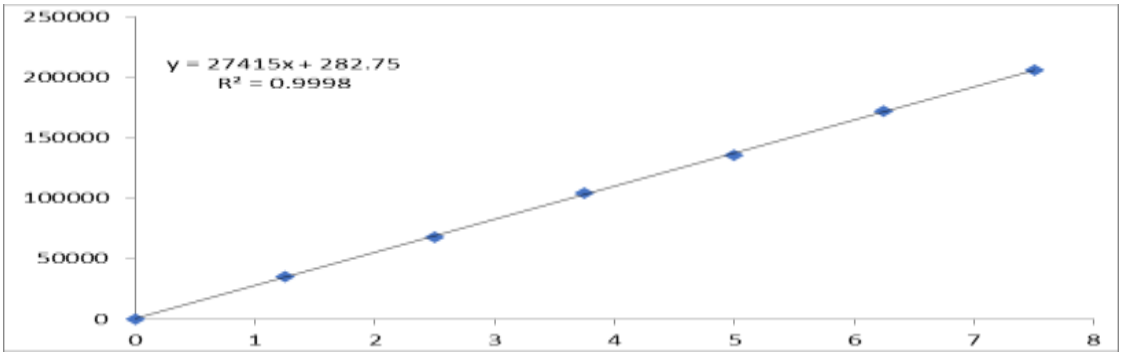

Fig. 6: Calibration curve of teneligliptin

Six linear concentrations of remogliflozin (12.5-75µg/ml) and teneligliptin (1.25-7.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for remogliflozin was y = 38928x + 20645. and of teneligliptin was y = 27415x + 282.75. Correlation coefficient obtained was 0.999 for the two drugs.

Table 4: Accuracy table of Remogliflozin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50	25	24.94	99.76	99.63
	25	24.87	99.49	
	25	24.75	99.01	
100	50	50.17	100.35	
	50	49.98	99.95	
	50	49.87	99.74	
150	75	74.89	99.85	
	75	74.46	99.28	
	75	74.44	99.25	

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.63% and 100.23% for remogliflozin and teneligliptin respectively.

Table 5: Accuracy Table of Teneligliptin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50	2.5	2.51	100.53	100.23
	2.5	2.51	100.59	
	2.5	2.52	100.99	
100	5	4.96	99.17	
	5	5.04	100.84	
	5	5.00	100.08	
150	7.5	7.57	100.97	
	7.5	7.46	99.46	
	7.5	7.46	99.47	

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.6% respectively for remogliflozin and Teneligliptin. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 6: System Precision Table of Remogliflozin and Teneligliptin

S. No.	Area of remogliflozin	Area of teneligliptin
1.	1907093	111848
2.	1912489	110435
3.	1907366	111567
4.	1903190	110470
5.	1900303	110284
6.	1884684	111647
Mean	**1902521**	**111042**
S.D	**9667.1**	**715.7**
%RSD	**0.5**	**0.6**

The % RSD for the peak areas of drospirenone and estetrol obtained from six replicate injections of standard solutions which was within range of limit (<2%).

Table 7: Sensitivity table of remogliflozin and teneligliptin

Molecule	LOD	LOQ
Remogliflozin	0.42	1.26
Teneligliptin	0.02	0.05

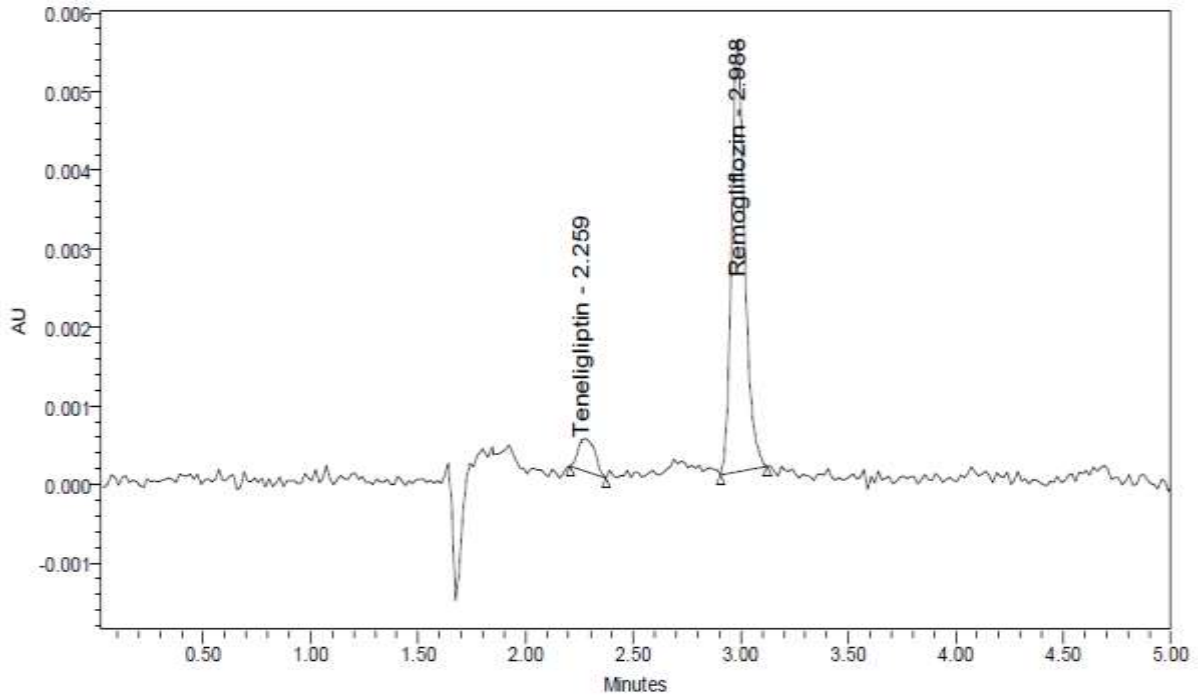

Fig. 7: LOD chromatogram of standard

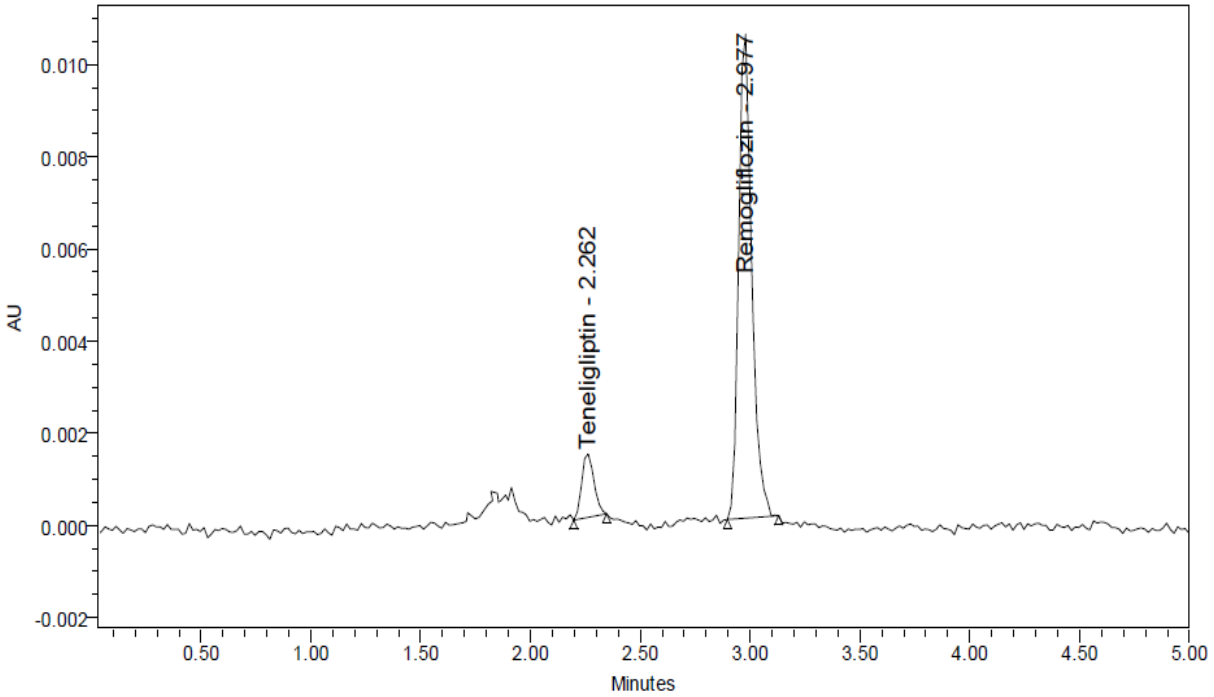

Fig. 8: LOQ chromatogram of standard

Table 8: Robustness data for Remogliflozin and Teneligliptin

S. No.	Condition	% RSD of Remogliflozin	% RSD of Teneligliptin
1	Flow rate (-) 0.9ml/min	0.4	0.1
2	Flow rate (+) 1.1ml/min	0.9	0.6
3	Mobile phase (-) 55B:45A	0.9	0.9
4	Mobile phase (+) 70B:30A	1	0.4
5	Temperature (-) 27°C	0.3	0.2
6	Temperature (+) 33°C	0.4	0.2

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (70B:30A), 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: Assay table of Remogliflozin and Teneligliptin

Drug name	Label claim	%Assay	Brand name
Remogliflozin	100mg	100.54%	Remozen V
Teneligliptin	10mg	100.27%	Jardiance

Zita 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 100.54% and 100.27% respectively.

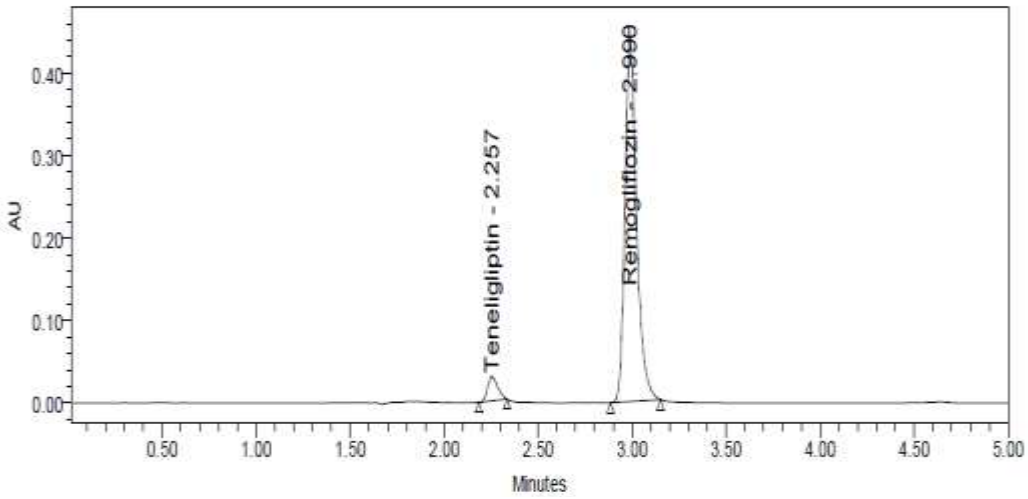

Fig. 9: Chromatogram of working standard solution

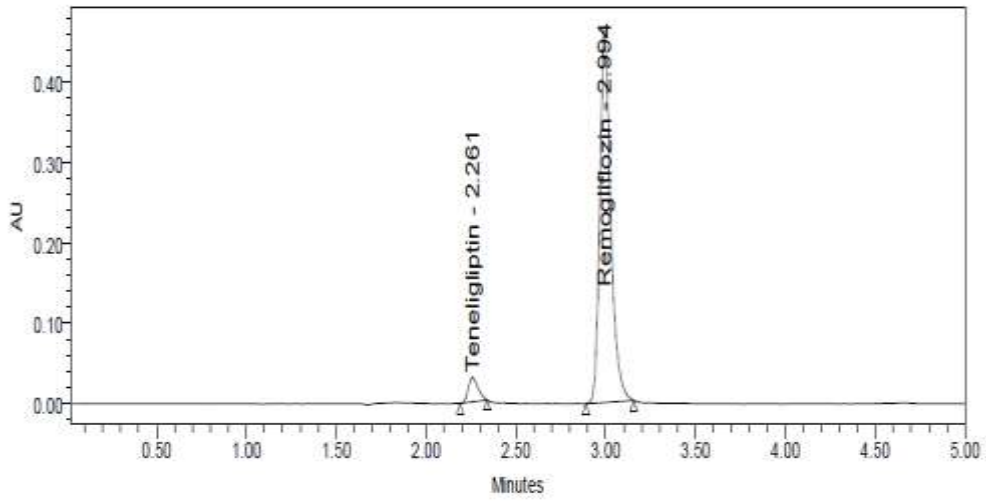

Fig. 10: Chromatogram of working sample solution

A simple, accurate, precise method was developed for the simultaneous estimation of the remogliflozin and teneligliptin in tablet dosage form. Retention time of remogliflozin and teneligliptin were found to be 2.263 min and 2.994 min. % RSD of the remogliflozin and Teneligliptin were and found to be 0.5 and 0.6 respectively. %Recovery was obtained as 99.63% and 100.23% for remogliflozin and teneligliptin respectively. LOD, LOQ values obtained from regression equations of remogliflozin and teneligliptin were 0.26, 0.78 and 0.03, 0.09 respectively. Regression equation of remogliflozin is y = 38928x + 20645. And y = 27415x + 282.75 of teneligliptin. 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.

CONCLUSION

A new stability indicating RP-HPLC technique was developed and validated for the simultaneous stimulations of remogliflozin and teneligliptin in the pharmaceutical dosage form. The develop method was precise, higher resolutions, shorter retentions with various degradants and economical. Hence, this method can be used for in process evaluation in pharmaceutical firms and quality control of these in drug testing.

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ETHICAL APPROVALS
This study does not involve experiments on animals or human subjects.

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