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HPLC method for simultaneous estimation of Remogliflozin and Teneligliptin

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KEYWORDS
Remogliflozin, Teneligliptin, RP-HPLC

Abstract: A simple, Accurate, precise method was developed for the simultaneous estimation of the Remogliflozin and Teneligliptin in tablet dosage form. Chromatogram was run through Std Azilent 150(4.6 x 150mm, 5µm). Mobile phase containing Acetonitrile: Formic acid taken in the ratio 65:35 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method Phosphate buffer and ph is 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 and Teneligliptin were found to be 2.189 min and 2.824 min. %Recovery was obtained as 99.89% and 100.27% for Remogliflozin and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Remogliflozin and Teneligliptin were 0.66, 2.00 and 0.53, 1.61 respectively. Regression equation of Remogliflozin is y = 11909x +42491. And y = 11725x + 1485of 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.

Introduction

When there are no approved procedures available, methods are created for new items. For current (non-pharmacopoeias) goods, alternative techniques are created to cut costs and time while improving precision and durability. Trial runs are made, and the procedure is verified and optimized. When a different approach is suggested to replace the current process, comparative laboratory data with advantages and disadvantages should be made accessible [1-3].

For pharmaceutical analysis, since quality determination is the only means of ensuring the ongoing efficacy and safety of each batch created, proper validation of analytical methods is crucial. The capacity of the analytical procedures, when used under precise conditions and at a predetermined degree of sensitivity, to provide a trustworthy proof of every departure from the goal criteria is what determines the ability to manage this quality [4-5].

It is crucial that the analytical techniques created for estimating purity and impurities be able to effectively separate all components—desired and unwanted—from the formulation matrix without encountering any interference [6-8].

The active components should be precisely measured by a stability-indicating test technique, free from interference by excipients, degradation products, process contaminants, or other possible impurities [9—12].

The newest medication in the SGLT2 inhibitor family to be licensed in India for the treatment of type 2 diabetes is remogliflozin etabonate (RE). 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. Remogliflozin is soluble in water (0.189mg/ml) [13-15]. 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 hyperglycaemia, assists weight loss, and reduces blood pressure. It is very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol and acetonitrile; soluble in 50%

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acetonitrile/water; and practically insoluble in toluene [16-19].

The primary goal of this work is to provide a fast, accurate, sensitive, selective, repeatable, and precise analytical method for the simultaneous measurement of teneligliptin and remogliflozin in pharmaceutical dosage forms.

Based on a study of the literature and a search for patents related to this research issue, no technique has been reported for this combination of drugs as of yet. Certain spectrophotometric and chromatographic techniques were available for use with single pharmaceuticals or in combination with other drugs. Therefore, for the simultaneous measurement of these medications in combination dosage form, an accurate, precise, and straightforward HPLC method is needed. Therefore, it was thought that creating and confirming a technique for it would be interesting.

The objectives of the work are:

- To develop a new stability indicating HPLC method for simultaneous estimation of Remogliflozin and Teneligliptin and to develop the validated method according to ICH guidelines.
- To apply the validated method for the simultaneous estimation of Remogliflozin and Teneligliptin in pharmaceutical formulation.

Material and Methods

Materials:

- Remogliflozin and Teneligliptin pure drugs (API) received from spectrum Pharma labs.
- Combination Remogliflozin and Teneligliptin tablets (Zeta PLUS_R) received from local market.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions: 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 labeled as Standard stock solution 1and 2. (500µg/ml of Remogliflozin and 50µg/ml of 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. (50 µg/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 HPLC filters (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)

Preparation of buffer:

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

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Remogliflozin(50ppm) and Teneligliptin(5ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be 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.

Precision:

Preparation of Standard stock solutions: Accurately weighed 25mg of Remogliflozin, 2.5mg of Teneligliptin and transferred to 50ml and 50ml

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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 labeled as Standard stock solution 1and 2. (500µg/ml of Remogliflozin and 50µg/ml of 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. (50 µg/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 HPLC filters.(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).

Linearity:

Preparation of Standard stock solutions: 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 labeled as Standard stock solution 1and 2. (500µg/ml of Remogliflozin and 50µg/ml of Teneligliptin)

25% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (12.5µg/ml of Remogliflozin, and 1.25µg/ml of Teneligliptin)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Remogliflozin, and 2.5µg/ml of Teneligliptin)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (37.5µg/ml of Remogliflozin, and 3.75µg/ml of Teneligliptin)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Remogliflozin, and 5µg/ml of Teneligliptin)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (62.5µg/ml of Remogliflozin and 6.25µg/ml of Teneligliptin)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Remogliflozin and 7.5µg/ml of Teneligliptin)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 50mg of Remogliflozin, 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 labeled as Standard stock solution 1and 2. (500µg/ml of Remogliflozin and 50µg/ml of Teneligliptin)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

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Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, 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.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Remogliflozin, Teneligliptin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Remogliflozin, Teneligliptin , and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation:

To 1 ml of stock solution of Remogliflozin and Teneligliptin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 50µg/ml&5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock ssolution Remogliflozin and Teneligliptin, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^{0c}. The resultant solution was diluted to obtain 50µg/ml&5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Remogliflozin and Teneligliptin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 100µg/ml&10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 50µg/ml&5µg/ml l solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 1000µg/ml&100µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 50µg/ml&5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

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Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 50µg/ml&5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Results and Discussion

Method development was done by changing various, mobile phase ratios, buffers etc.

Table 1: Chromatographic condition of Trials

	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**
Mobile phase	ACN and OPA (50:50)	Acetonitrile: AmmF (40:60)	ACN: AmmF (45:55)	Acetonitrile: AmmF (55:45)

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Flow rate	**1** ml/min
Column	Ascentis C18 (4.6 x 150mm, 3.2µm)
Detector wave length	210nm
Column temperature	30°C
Injection volume	30µL
Run time	10.0 min

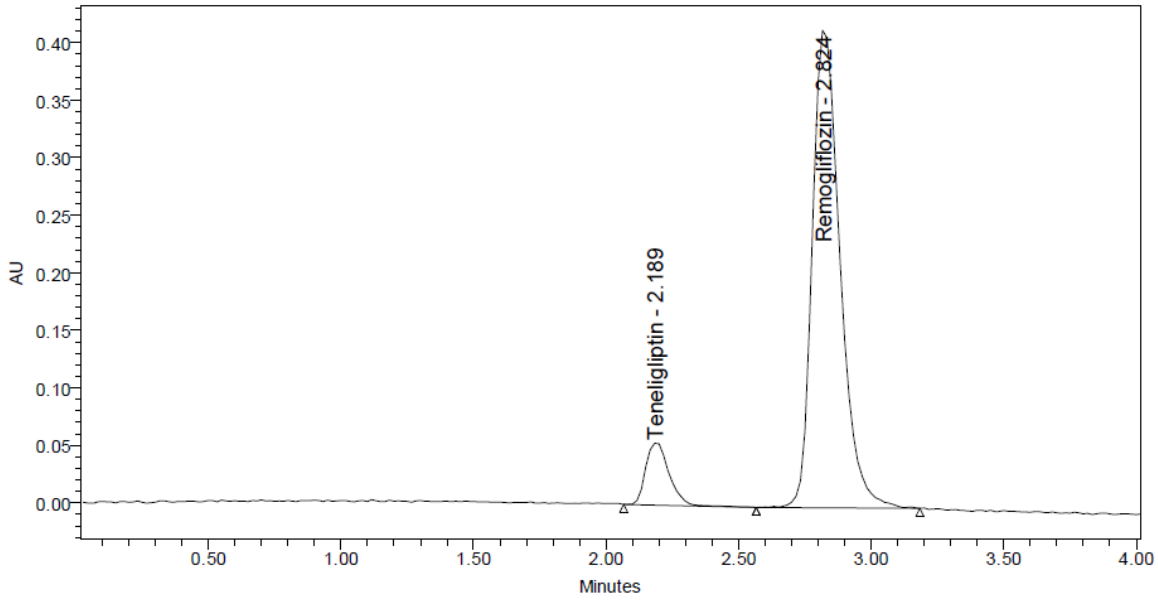

Figure 1: Optimized Chromatogram of Trial 4

Remogliflozin and Teneligliptin were eluted at 2.189 min and 2.824 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.

Table 2: System suitability parameters for Remogliflozin and Teneligliptin

S no	**Teneligliptin**			**Remogliflozin**			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.200	2104	1.53	2.808	3396	1.32	3.1
2	2.200	2375	1.42	2.813	3306	1.31	3.2
3	2.202	2597	1.43	2.820	3543	1.28	3.2
4	2.208	2297	1.55	2.821	3556	1.31	3.1
5	2.209	2130	1.60	2.822	3561	1.28	3.2
6	2.209	2172	1.47	2.823	3428	1.32	3.2

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

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Validation:

Specificity:

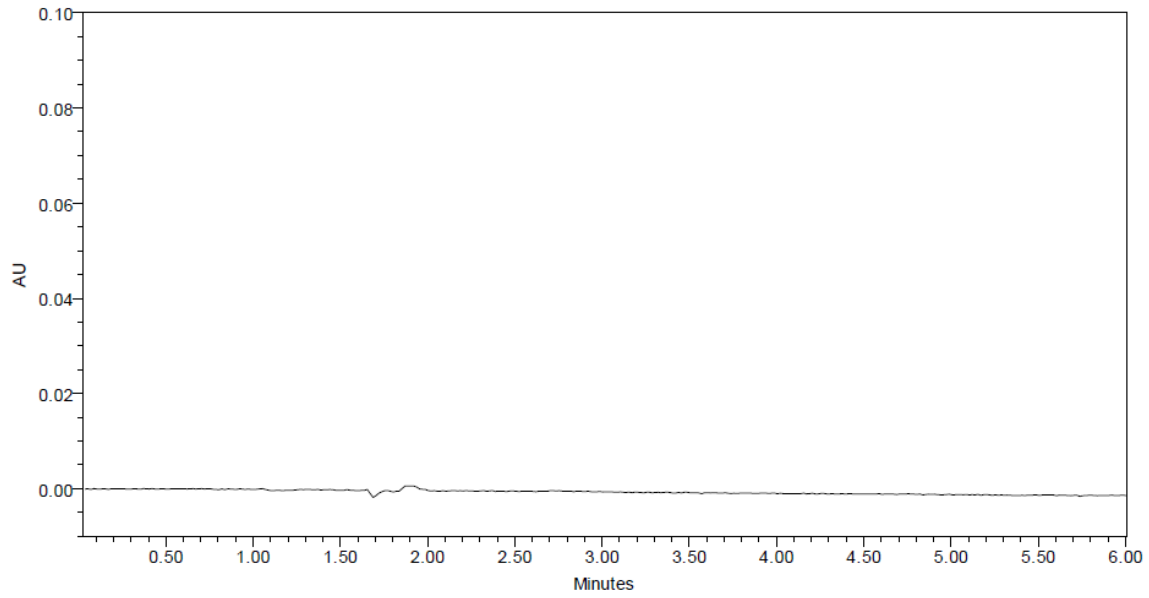

Figure 2: Chromatogram of blank

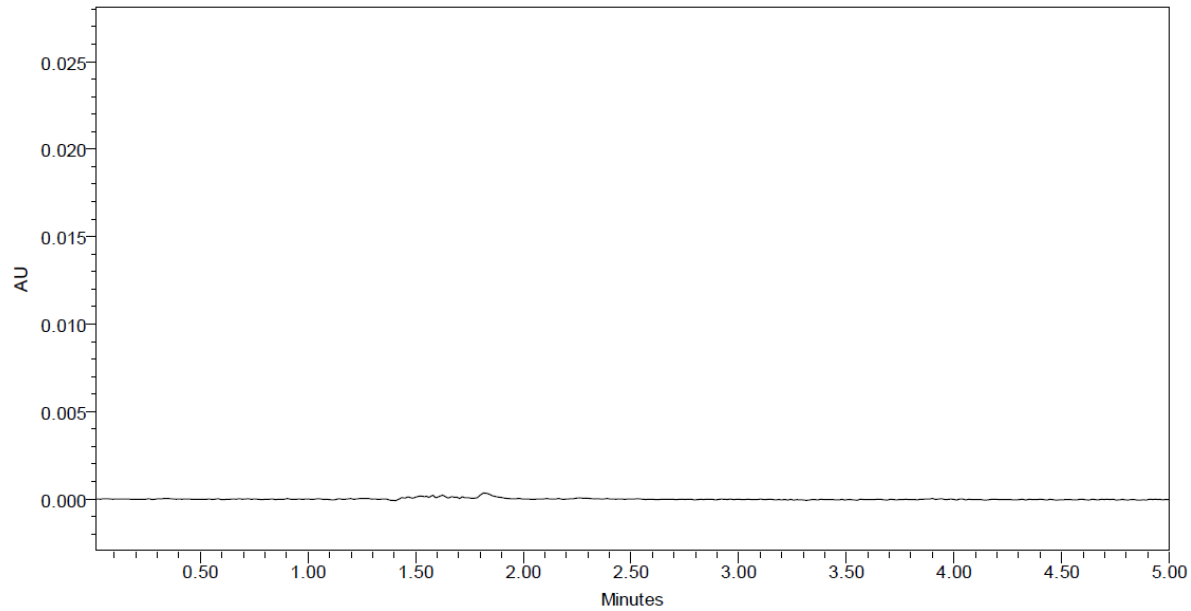

Figure 3: Chromatogram of placebo

Linearity:

Table 3: Linearity table for Remogliflozin and Teneligliptin

Remogliflozin		Teneligliptin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**

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0	0	0	0
12.5	1508524	1.25	144427
25	3007113	2.5	286487
37.5	4595550	3.75	456076
50	6019596	5	598723
62.5	7597729	6.25	727477
75	8830321	7.5	875230

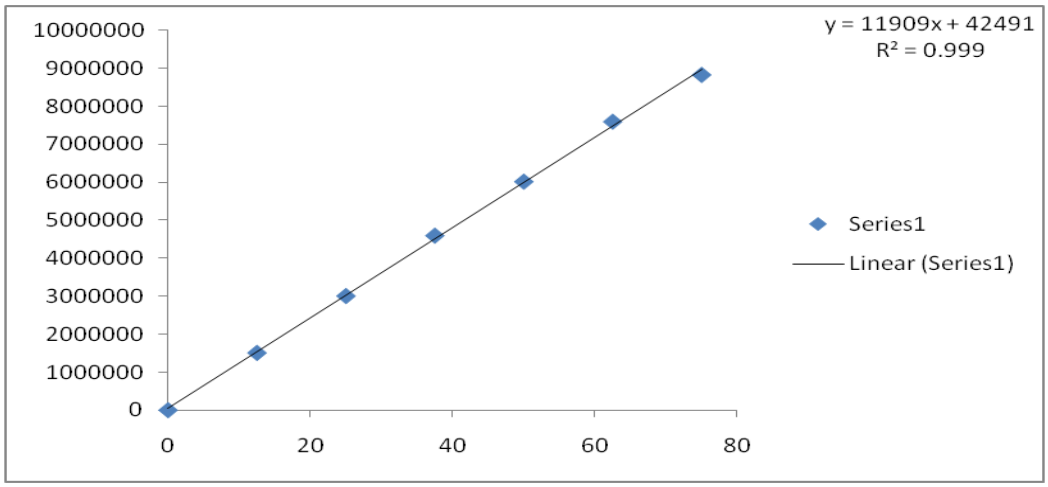

Figure 4: Calibration curve of Remogliflozin

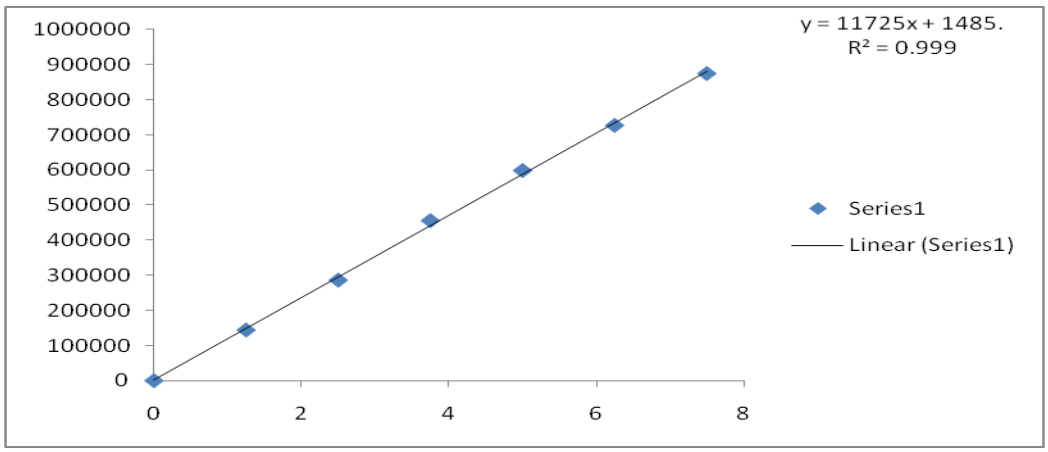

Figure 5: Calibration curve of Teneligliptin

Precision:

System Precision:

Table 4: System precision table of Remogliflozin and Teneligliptin

S. No	Area of Remogliflozin	Area of Teneligliptin
1.	6076482	591569
2.	6062397	589232
3.	5929817	589179
4.	6013524	597717
5.	5932158	598740
6.	6030877	598737

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Mean	6007543	594196
S.D	63349.9	4698.5
%RSD	1.1	0.8

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 1.1% and 0.8% respectively for Remogliflozin and Teneligliptin. As the limit of Precision was less than "2" the system precision was passed in this method.

Method precision:

Table 5: Method precision table of Remogliflozin and Teneligliptin

S. No	Area of Remogliflozin	Area of Teneligliptin
1.	5994939	599250
2.	5920678	592993
3.	5936155	593527
4.	6055962	595874
5.	6052731	592340
6.	6031456	599067
Mean	5998654	595509
S.D	58795.7	3068.8
%RSD	1.0	0.5

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 1.0% and 0.5% respectively for Remogliflozin and Teneligliptin. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_Day Precision):

Table 6: Intermediate precision table of Remogliflozin and Teneligliptin

S. No	Area of Remogliflozin	Area of Teneligliptin
1.	1278543	209353
2.	1241481	205976
3.	1266374	204229
4.	1254466	209243
5.	1247390	206438
6.	1247141	208632
Mean	1255899	207312
S.D	14010.8	2082.8
%RSD	1.1	1.0

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Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 1.0% and 1.1% respectively for Remogliflozin and Teneligliptin . As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy:

Table 7: Accuracy table of Remogliflozin

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	25	25.17372	100.69	99.98%
	25	25.12933	100.52	
	25	24.93111	99.72	
100%	50	49.97906	99.96	
	50	50.28488	100.57	
	50	50.37131	100.74	
150%	75	74.51134	99.35	
	75	74.27733	99.04	
	75	74.42034	99.23	

Table 8: Accuracy table of Teneligliptin

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	2.5	2.502861	100.11	100.27%
	2.5	2.498702	99.95	
	2.5	2.488101	99.52	
100%	5	5.065448	101.31	
	5	4.980123	99.60	
	5	4.99903	99.98	
150%	7.5	7.530802	100.41	
	7.5	7.584402	101.13	
	7.5	7.529037	100.39	

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 99.98% and 100.27% for Remogliflozin and Teneligliptin respectively.

Sensitivity:

Table 9: Sensitivity table of Remogliflozin and Teneligliptin

Molecule	**LOD**	**LOQ**
Remogliflozin	0.66	2.00
Teneligliptin	0.53	1.61

Robustness:

Table 10: Robustness data for Remogliflozin and Teneligliptin .

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S.no	Condition	%RSD of Remogliflozin	%RSD of Teneligliptin
1	Flow rate (-) 0.9ml/min	0.5	0.4
2	Flow rate (+) 1.1ml/min	0.3	0.8
3	Mobile phase (-) 55B:45A	0.9	0.5
4	Mobil;e phase (+) 70B:30A	0.6	0.3
5	Temperature (-) 27°C	0.5	0.8
6	Temperature (+) 33°C	0.6	0.4

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 11: Assay Data of Remogliflozin

S.no	Standard Area	Sample area	% Assay
1	6076482	5994939	99.59
2	6062397	5920678	98.36
3	5929817	5936155	98.61
4	6013524	6055962	100.60
5	5932158	6052731	100.55
6	6030877	6031456	100.20
Avg	6007543	5998654	99.65
Stdev	63349.9	58795.7	0.977
%RSD	1.1	1.0	1.0

Table 12: Assay Data of Teneligliptin

S. no	Standard Area	Sample area	% Assay
1	591569	599250	100.65
2	589232	592993	99.60
3	589179	593527	99.69
4	597717	595874	100.08
5	598740	592340	99.49
6	598737	599067	100.62
Avg	594196	595509	100.02
Stdev	4698.5	3068.8	0.52
%RSD	0.8	0.5	0.5

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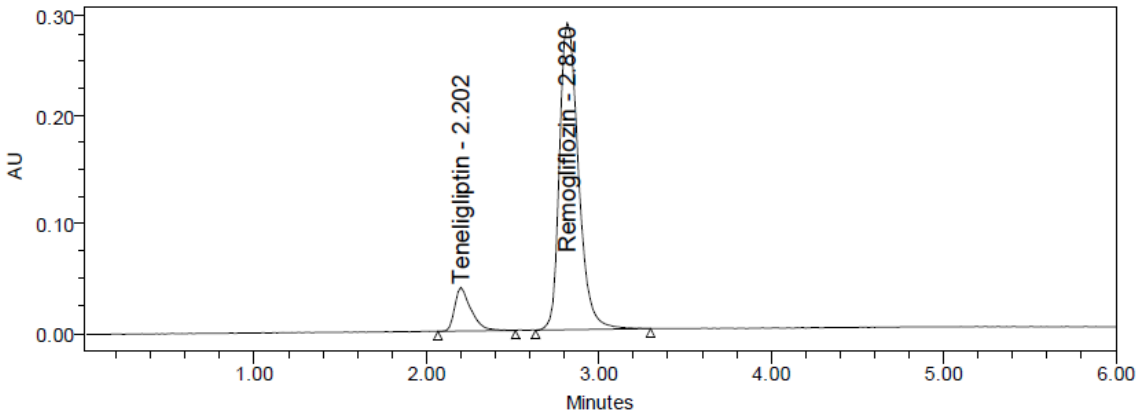

Figure 6: Chromatogram of working standard solution

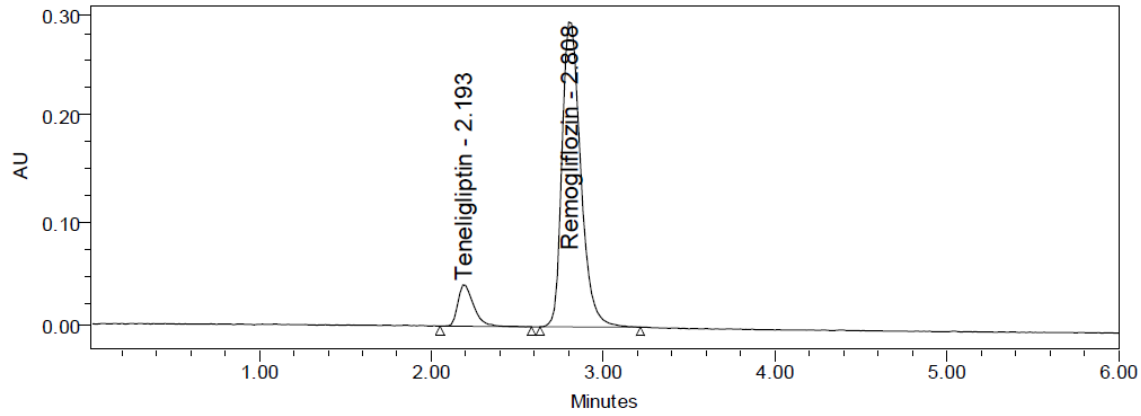

Figure 7: Chromatogram of working sample solution

Table 13: Degradation data

Type of degradation	Remogliflozin			Teneligliptin		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	5583007	92.75	7.25	560836	94.20	5.80
Base	5643366	93.75	6.25	568814	95.54	4.46
Peroxide	5516003	91.63	8.37	560301	94.11	5.89
Thermal	5894232	97.92	2.08	572697	96.19	3.81
Uv	5851435	97.21	2.79	584174	98.12	1.88
Water	5962668	99.05	0.95	592208	99.47	0.53

Conclusion

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 2.189 min and 2.824 min. %RSD of the Remogliflozin and Teneligliptin were and found to be 1.1 and 0.8 respectively. %Recovery was obtained as 99.89% and 100.27% for Remogliflozin and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Remogliflozin and Teneligliptin were 0.66, 2.00 and 0.53, 1.61 respectively. Regression equation of Remogliflozin is y = 11909x +42491. And y = 11725x + 1485 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.

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