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SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN ETABONATE AND TENELIGLIPTIN HYDROBROMIDE HYDRATE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT
A simple, precise, accurate method was developed for the simultaneous estimation of Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate in tablet dosage form by RP-HPLC method. The separation was achieved by Force scientific C_{18} (250mm x 4.6mm, 5µm) column and Methanol: Phosphate buffer (pH 3) in the ratio of (70:30) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 235 nm. Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate had respective retention times of 16.1 minutes and 3.4 minutes. The approach's linearity, accuracy, precision, and robustness have all been validated. Remogliflozin etabonate 12.5-75 g/ml and Teneligliptin Hydrobromide Hydrate 1.25-7.5 g/ml showed linearity with correlation coefficients of 0.9998 and 0.9984, respectively. For the simultaneous measurement of remogliflozin etabonate and teneligliptin hydrobromide hydrate in their tablet dosage form, the developed approach was proven to be accurate, exact, and quick.

KEYWORDS: *Remogliflozin etabonate, Teneligliptin Hydrobromide Hydrate, ICH guidelines, RP-HPLC.*

INTRODUCTION

Chemically, Remogliflozin Etabonate (REM) is Ethyl[(2R, 3S, 4S, 5R, 6S)-3,4,5-trihydroxy-6-[5-methyl-1-propan-2-yl-4-[(4-propan-2-yloxyphenyl)methyl]pyrazol-3-yl]oxyoxan-2-yl]methylcarbonate(Fig.1).

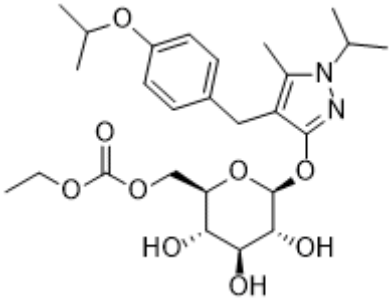

Fig. 1. Structure of Remogliflozin etabonate

Remogliflozin etabonate, an inactive prodrug that becomes active after injection and absorption, operates primarily on the sodium-glucose co-transporter subtype 2 (SGLT2) and is used to treat Diabetes Mellitus Type-2[1].

A brand-new medication called teneligliptin hydrobromide hydrate is used to treat type 2 diabetes mellitus. It is an anti-diabetic medication that belongs to the class of "gliptins," or dipeptidyl peptidase-4 inhibitors. Chemically, it is (1, 3-thiazolidin-3-yl)(2S, 4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]- pyrrolidinylmethanone (Fig.2).

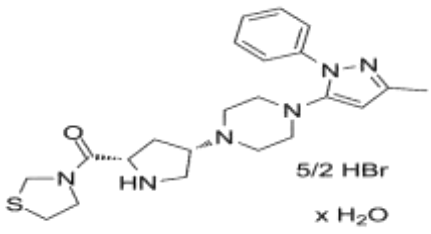

Fig. 2. Structure of Teneligliptin Hydrobromide Hydrate

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Teneligliptin hydrobromide hydrate is teneligliptin's prodrug. It decreases blood glucose levels by inhibiting GLP-1 breakdown by inhibiting dipeptidyl peptidase-4 (DPP-4) and raising the blood concentration of active GLP-1[1].

According to a literature review and patent search on this research topic, there has been no method reported for this combination of medications currently exist. For single medications or in combination with other drugs, some spectrophotometric and chromatographic procedures were available. Thus, a simple, precise, and accurate RP-HPLC approach for simultaneous quantification of these medicines in combined dose form is required. As a result, it was felt that developing and validating a method for it would be of interest.

MATERIALS AND METHODS

Materials

Combination Remogliflozin etabonate and Teneligliptin tablets (Zita plus-R), Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate pure medicines (API), Distilled water, Phosphate buffer, Methanol, Potassium dihydrogen phosphate buffer, Ortho-phosphoric acid. The aforementioned chemicals and solvents were all acquired from Rankem.

Instruments

Ultrasonicator, HPLC device Dionex with Force scientific column C18, UV-VIS detector Shimadzu SPD-20A VP, and Auto sampler combined with Chromeleon Software are some examples of the electronics balance made by Shimadzu. Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate absorbances were measured using a Jasco UV-VIS spectrophotometer with unique bandwidth of 2 mm and 10 mm and matching quartz cells integrated with UV Probe.

Methods

Preparation of standard stock solutions

- **Remogliflozin etabonate**
  Standard stock-1 solution (1000 ppm): Remogliflozin etabonate 100 mg was weighed, transferred to a volumetric flask of 100 ml, and then dissolved in methanol using a sonicator for roughly five minutes. Methanol was added to bring the volume up to the required level, producing a solution with 1000 ppm.
  Standard stock-2 solution (500 ppm): A 100 ml volumetric flask containing 50 ml of standard stock-1 solution was filled to the proper level with methanol to produce a 500 ppm solution.
- **Teneligliptin HBr Hydrate**
  Standard stock-1 solution (1000 ppm): Teneligliptin HBr Hydrate 100 mg was weighed, transferred to a volumetric flask of 100 ml, and then dissolved in methanol using a sonicator for roughly five minutes. Methanol was added to bring the volume up to the required level, producing a solution with 1000 ppm.
  Standard stock-2 solution (50 ppm): A 100 ml volumetric flask containing 5 ml of standard stock-1 solution was filled to the proper level with methanol to produce a solution with 50 ppm.

Preparation of working standard solution for selection of mobile phase:

1 ml of Remogliflozin etabonate standard stock-2 solution and 1 ml of Teneligliptin HBr Hydrate stock-2 solution was transferred in 10 ml volumetric flask. Volume was made up to mark with mobile phase used for trails to give a solution containing 50 ppm of Remogliflozin etabonate and 5 ppm of Teneligliptin HBr Hydrate solution.
The above working standard solution was injected for selection of mobile phase.

Method Development

Method development was done by changing various, mobile phase ratios, buffers etc [2].

Table 1: Optimization of chromatographic conditions

Trial no.	Mobile Phase	Ratio (%v/v)	Remark
1.	Methanol: Water	60:40	Peak splitting & peak tailing obtain
2.	Methanol: Phosphate buffer (pH 3)	60:40	Two peaks obtain but retention time high
3.	Methanol: Phosphate buffer (pH 3)	70:30	Two peaks obtain with sharp peak and better resolution than 2 trial.
4.	Methanol: Phosphate buffer (pH 3)	70:30	To confirm peak of Teneligliptin HBr Hydrate

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RESULTS AND DISCUSSION

Observation: With good resolution, remogliflozin etabonate and teneligliptin hydrobromide hydrate were eluted at 16.17 and 3.43 minutes, respectively. Plate count and tailing factor satisfied all requirements, hence this approach was improved and will be validated (Fig. 3).

System suitability: According to ICH criteria[4], all of the system suitability metrics were acceptable and within the acceptable range. (Table 2 and Fig. 4).

LOD and LOQ: The smallest amount of analyte that can be detected but not always measured is known as the detection limit. The three calibration curves were used to calculate the LOD. The LOD may be determined as (table 3)
LOD = 3.3 × (SD / Slope).
The 3 calibration curves were used to calculate the LOQ. The LOQ could be determined as
LOQ = 10 × (SD/Slope).

Linearity: Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate's linearity of response was estimated by analysing three separate levels of the calibration curve in the ranges of 12.5-75 g/ml and 1.25-7.5 g/ml, respectively. Average areas were listed below, and linearity equations for Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate were respectively y = 19545x + 12018 and y = 31665x + 12086. Both medications' correlation coefficients were discovered to be 0.9998 and 0.9984, respectively. (Table 4 & 5) (Fig. 5,6,7).

Precision

Repeatability: A series of three 100 ml volumetric flasks were filled with aliquots of the standard stock-2 solution of REMO (500 g/ml) and TENE (50 g/ml) in the amounts of 5, 10, and 15 ml, or 50%, 100%, and 150%, respectively. To obtain 25, 50, and 75 g/ml solutions of REMO and 2.5, 5, and 7.5 g/ml solutions of TENE, the volume was adjusted up to the mark with mobile phase. The solutions were then injected into the system under the specified chromatographic conditions, analysed by repeated injection, and the percent RSD was calculated. (Table 6).

Intra-Day Precision: A series of three 100 ml volumetric flasks were filled with aliquots of the standard stock-2 solution of REMO (500 g/ml) and TENE (50 g/ml) in the amounts of 5, 10, and 15 ml, or 50%, 100%, and 150%, respectively. To obtain a 25, 50, 75 g/ml solution of REMO and a 2.5, 5, 7.5 g/ml solution of TENE, the volume was raised to the proper level with mobile phase. On the same day that the solutions were injected into the system under the specified chromatographic conditions, they were analysed, and the percent RSD was computed. (Table 7).

Inter-Day Precision: A series of three 100 ml volumetric flasks were filled with aliquots of the standard stock-2 solution of REMO (500 g/ml) and TENE (50 g/ml) in the amounts of 5, 10, and 15 ml, or 50%, 100%, and 150%, respectively. To obtain a 25, 50, 75 g/ml solution of REMO and a 2.5, 5, 7.5 g/ml solution of TENE, the volume was raised to the proper level with mobile phase. 50%, 100%, and 150% solutions were added to the system under the specified chromatographic conditions, and each sample was then analysed on a different day to determine the percent RSD. (Table 8).

Accuracy: The standard addition procedure was used to prepare accuracy samples at three different levels. For each degree of accuracy, triplicate injections were given, and for the drugs remogliflozin etabonate and teneligliptin hydrobromide hydrate, recovery was achieved in the ranges of 99.22-99.81% and 98.88-99.53%, respectively (Table 9 &10).

Robustness: Robustness conditions including flow rate, mobile phase pH and ratio were changed, and samples were administered in duplicate. The parameters for system suitability were not significantly impacted, and all of the parameters were met. The limit was reached for %RSD (Table 11 & 12).

Assay: Applicability of the proposed method was tested by analysing the commercially available Tablet formulation Zita Plus R
The amount of powder was determined after weighing twenty tablets. Teneligliptin Hydrobromide Hydrate (5 mg) and 50 mg of Remogliflozin Etabonate from the tablet powder were added to a 100 ml volumetric flask. To completely dissolve the medication, the mixture was sonicated for 15 minutes while being combined with methanol. The solution was filtered using Whatman filter paper No. 42, and methanol was added to bring the volume up to the desired level. To get sample solutions of the medication concentrations of Remogliflozin etabonate (50 g/ml) and Teneligliptin Hydrobromide Hydrate (5 g/ml), the original stock solution was further diluted. The sample solution was injected into HPLC in a volume of 20 µl. The peak area for the drug was measured at 235nm and amount of Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate were determined using the related linear regression equations. (Table 13)

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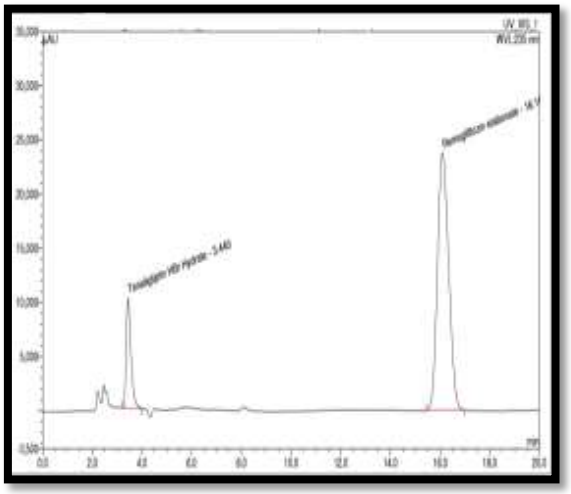

Fig. 3: Optimized chromatogram

Table 2: System suitability parameters

System suitability parameter	Remogliflozin Etabonate (n=6)	Teneligliptin Hydrobromide Hydrate (n=6)
Retention time (min)	16.07	3.43
Resolution (R)	28.41	-
Tailing factor (T)	1.02	1.8
Theoretical plate number (N)	97291	3720

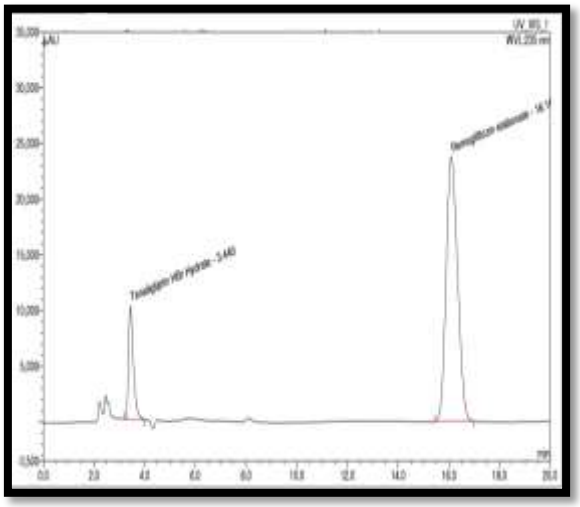

Fig. 4: System suitability chromatogram

Table 3: LOD and LOQ of Remogliflozin etabonate and Teneligliptin hydrobromide hydrate

Drug	LOD (µg/ml) (n=3)	LOQ (µg/ml) (n=3)
Remogliflozin Etabonate	2.97	9.01
Teneligliptin HBr Hydrate	0.34	1.04

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Fig 5: Overlain chromatogram of Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate

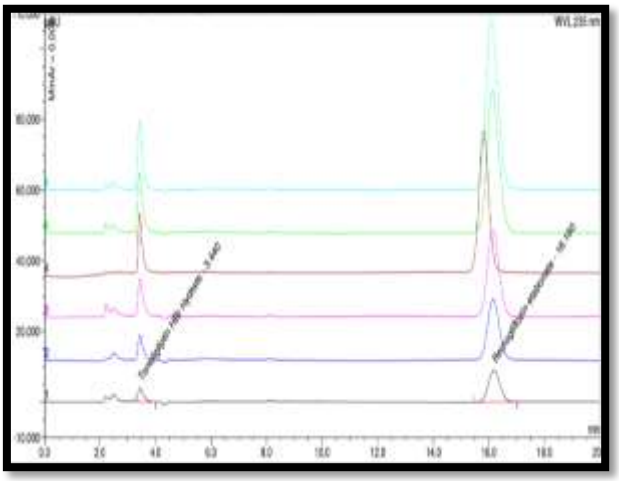

Table 4: Linearity of Remogliflozin etabonate

Conc. (µg/ml)	Mean area ± SD (n=3)	% RSD
12.5	263293.22 ± 329.48	0.12514
25	500599.04 ± 410.51	0.082
37.5	734397.15 ± 354.002	0.0482
50	987755.39 ± 278.98	0.02821
62.5	1233695.99 ± 392.99	0.03186
75	1482960.69 ± 285.69	0.01927

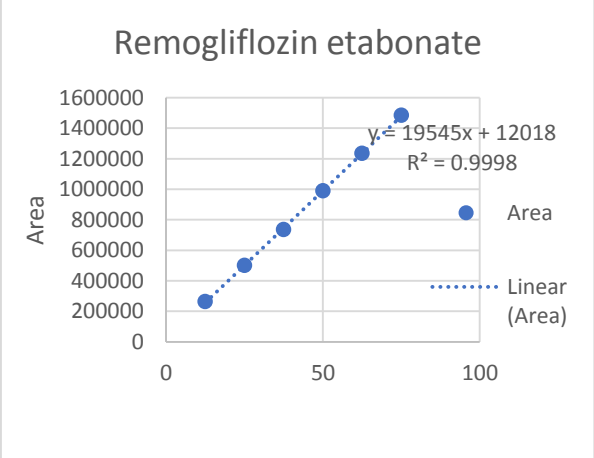

Fig 6: Calibration curve of Remogliflozin etabonate

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Table 5: linearity of Teneligliptin Hydrobromide Hydrate

Conc. (µg/ml)	Mean area ± SD (n=3)	% RSD
1.25	52500.11 ± 267.03	0.50863
2.5	87841.22 ± 465.87	0.53036
3.75	134620.54 ± 406.89	0.30225
5	167960.10 ± 284.81	0.16958
6.25	212977.15 ± 419.36	0.19691
7.5	247817.74 ± 353.72	0.14274

Fig 7: Calibration curve of Teneligliptin Hydrobromide Hydrate

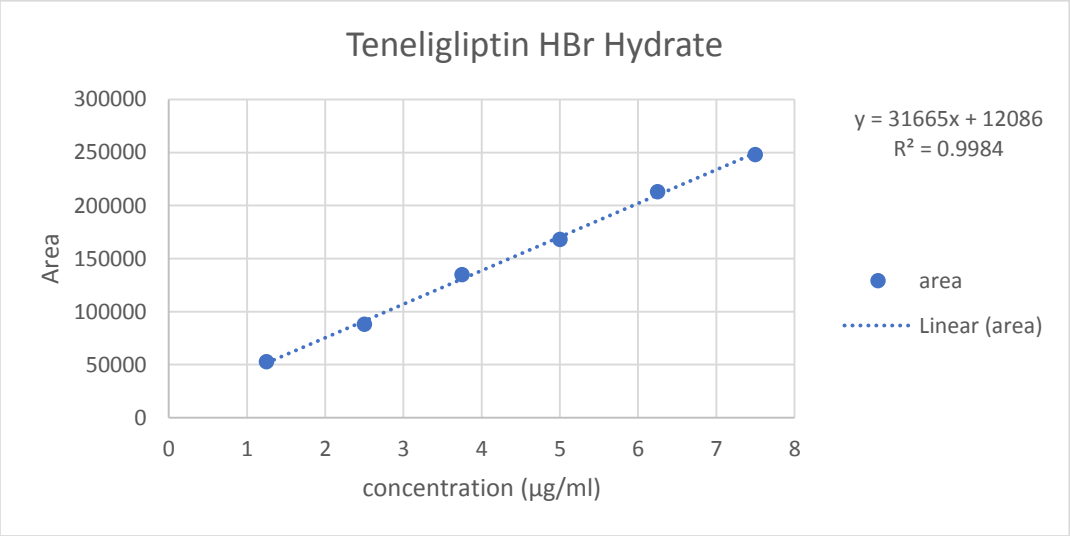

Table 6: Repeatability of Remogliflozin etabonate and Teneligliptin hydrobromide hydrate

Drug	conc.(µg/ml)	Mean area ± SD (n=3)	% RSD
Remogliflozin etabonate	25	500640.4 ± 601.44	0.120135
	50	987788.7 ± 524.17	0.053065
	75	1482927 ± 535.20	0.036091
Teneligliptin HBr Hydrate	2.5	87807.9 ± 520.38	0.592638
	5	167906.8 ± 404.74	0.241056
	7.5	247851.1 ± 602.86	0.243237

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Table 7: Intraday Precision of Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate

Drug	conc.(µg/ml)	Mean area ± SD (n=3)	% RSD
Remogliflozin etabonate	25	500763.5 ± 1187.01	0.237041
	50	987745.4 ± 1290.15	0.130617
	75	1483361 ± 1432.35	0.096561
Teneligliptin HBr Hydrate	2.5	87927.75 ± 1281.03	1.45692
	5	167813 ± 1248.46	0.743957
	7.5	247717 ± 1307.607	0.527862

Table 8: Interday Precision of Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate

Drug	conc.(µg/ml)	Mean area ± SD (n=3)	% RSD
Remogliflozin etabonate	25	500560.2 ± 2492.77	0.497996
	50	987645.4 ± 2443.187	0.247375
	75	1483327 ± 2656.43	0.179086
Teneligliptin HBr Hydrate	2.5	85922.77 ± 1571.85	1.8
	5	167746.8 ± 2349.67	1.4
	7.5	247736.4 ± 2576.612	1.04

Table 9: Accuracy of Remogliflozin etabonate

% Level	Target Conc. (µg/ml)	Standard Spiked Conc. (µg/ml)	Total amount (µg/ml)	Area	Standard amount recovered (µg/ml)	% Recovery	Mean % Recovery ± SD	%RSD
75%	25	12.5	37.5	758867.122	12.25	98.06	99.25 ± 1.50	1.513343
	25	12.5	37.5	760711.146	12.34	98.76		
	25	12.5	37.5	766432.324	12.61	100.94		
100%	25	25	50	993991.236	24.63	98.54	99.22 ± 0.65	0.658185
	25	25	50	997758.436	24.82	99.3		
	25	25	50	1000466.256	24.96	99.84		
125%	25	37.5	62.5	1228478.654	37.16	99.1	99.81 ± 0.70	0.701547
	25	37.5	62.5	1233843.642	37.43	99.83		
	25	37.5	62.5	1238740.546	37.68	100.5		

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Table 10: Accuracy of Teneligliptin Hydrobromide Hydrate

% level	Target Conc. (µg/ml)	Standard Spiked Conc. (µg/ml)	Total amount (µg/ml)	Area	Standard amount recovered (µg/ml)	% Recovery	Mean % Recovery ± SD	%RSD
75%	2.5	1.25	3.75	139247.223	1.23	98.44	99.53 ± 1.24	1.251889
	2.5	1.25	3.75	139682.918	1.24	99.27		
	2.5	1.25	3.75	140536.541	1.26	100.89		
100%	2.5	2.5	5	173809.225	2.45	98.18	99.24 ± 1.64	1.653389
	2.5	2.5	5	174014.188	2.46	98.41		
	2.5	2.5	5	177104.223	2.52	101.13		
125%	2.5	3.75	6.25	219965.125	3.68	98.35	98.88 ± 0.91	0.925448
	2.5	3.75	6.25	219990.564	3.68	98.36		
	2.5	3.75	6.25	222116.324	3.74	99.94		

Table 11: Robustness of Remogliflozin etabonate

Srno.	pH (3)		Flow rate (1ml/min)		Mobile phase (Methanol: Phosphate buffer pH 3) (70:30%v/v)	
	+0.2	- 0.2	+0.2	-0.2	+2 %	-2 %
1	996716.436	970246.452	980658.235	990265.456	981564.234	985325.2
2	995856.654	971325.214	980245.235	990998.235	982185.456	985956.3
3	996556.326	970986.235	981125.214	989856.254	981285.325	984656.3
Mean	996376.472	970852.6337	980676.228	990373.315	981678.3383	985312.6
Stdev	457.2383053	551.6510223	440.2653418	578.5804387	460.7860712	650.0923
%RSD	0.045890115	0.056821293	0.044894057	0.058420439	0.0469386	0.065978

Table 12 Robustness of Teneligliptin Hydrobromide Hydrate

Srno.	pH (3)		Flow rate (1ml/min)		Mobile phase (Methanol: Phosphate buffer pH 3) (70:30%v/v)	
	+0.2	- 0.2	+0.2	-0.2	+2 %	-2 %
1	156659.256	186256.245	160253.254	172356.256	175652.245	169235.3
2	157256.235	186856.245	160854.226	172785.452	175286.235	169786.3
3	156056.231	185986.214	159896.256	172006.562	175956.256	169008.3
Mean	156657.2407	186366.2347	160334.5787	172382.7567	175631.5787	169343.3
Stdev	600.0045385	445.3221085	484.1352149	390.1206506	335.4882384	400.0705
%RSD	0.383004664	0.238949995	0.30195309	0.226310716	0.191018176	0.236248

Table 13: Analysis of Marketed formulation

Formulation (Tablet)	Tablet amount (mg)		Amount found (mg)		% Assay	
	REMO	TENE	REMO	TENE	REMO ± SD (n=3)	TENE ± SD (n=3)
1	100	10	99.73	9.84	99.81333 ± 1.59	98.33333 ± 3.80
2	100	10	101.45	9.45		
3	100	10	98.26	10.21		

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A simple, Accurate, precise method was developed for the simultaneous estimation of the Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate in Tablet dosage form. The RP-HPLC method developed and validated allows a simple and rapid quantitative determination of Remogliflozin etabonate and Teneligliptin Hydrobromide Hydrate in tablet dosage forms. According to ICH recommendations, all validation parameters were confirmed to be within the limitations. The proposed method was found to be simple, accurate and specific for the drugs of interest irrespective of the excipients present with good resolution. The method developed was found to be simple, accurate, precise, rugged, robust. Therefore, the routine analysis of commercial formulations can be successfully applied using the established method.

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