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Remogliflozin etabonate and teneligliptin simultaneous estimation in pharmaceutical dosage form using a stability indicating HPLC - DAD procedure

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ABSTRACT: The objective of the present effort is developing a fully green-assessed stability-indicating reverse phase liquid chromatography for the assessable measurement regarding Remogliflozin etabonate (RGE) & Teneligliptin (TEN) in drug substance and tablets and fully validated as per ICH criteria. Waters Atlantis T3 (150mm x 4.6, 3.5µm) column, 30 volumes acetonitrile along with 70 volumes ammonium formate (pH-3.0) mobile phase pumped at a flow rate of 1.0ml/min were used for the chromatographic separation using PDA exposure at 236 nm wavelength. Remogliflozin etabonate, Teneligliptin were separated at 2.730, 4.468 minutes of retention times respectively. ICH guidelines Q2 R1 was employed to validate the current procedure. Accuracy measurements were acceptable to intra-day and inter-day measurements. For RGE & TGN, respectively, the recommended technique was linear in the concentration ranges of 25–150µg/ml and 2.5–15 µg/ml. y=29777x+67797 and y= 30689 x +22270 respectively were the observed regression equations for RGE and TEN. The limits of detection (LOD) and quantification (LOQ) for Remogliflozin etabonate & Teneligliptin were respectively 0.3 (µg/ml), 1 (µg/ml) and 0.03(µg/ml), 0.1(µg/ml). The recovery percentage of the method was found to remain in between 98% and 100%. Q1A R2 and Q1B guidelines were followed for conducting stability-indicating studies. The technique can also be utilized for quality control and repetitive laboratories evaluation aimed at the simultaneous assessment of RGE & TEN in the drug substance and pharmaceutical dosage form.

Keywords: RP-HPLC; Remogliflozin etabonate; Teneligliptin; Validation; Forced degradation studies; ICH guidelines.

1. INTRODUCTION

An altered glucose, lipid, and protein metabolism characterizes diabetes mellitus, a complex metabolic illness. Most irregularities associated with diabetes represent a serious health issue in contemporary society [1-2]. Chronic metabolic disease diabetes is characterized by high blood glucose levels. The most prevalent type of diabetes is type 2, which often affects adults, and arises when the body stops producing enough insulin or becomes resistant to it [3-4]. RGE is a novel sodium-glucose co-transporter type 2 (SGLT2) compound with the chemical formula 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] methyl carbonate [5]. The molecular structure of RGE is shown in Figure 1. Teneligliptin is chemically [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3yl)-piperazin-1yl]-pyrrolidin-2-yl] (1,3-thiazolidin-3-yl)-methanone], a novel oral dipeptidyl peptidase-4 (DPP-4) inhibitor that is prescribed in the cure of T2DM [6-7]. The molecular structure of TEN is shown Figure 1.

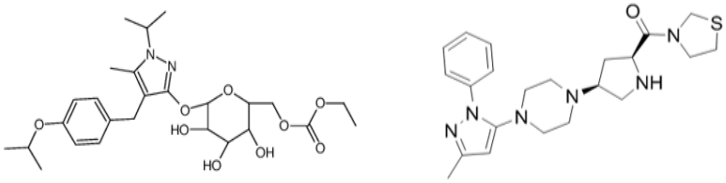

Figure 1. Molecular arrangement of (a) Remogliflozin etabonate [5] (b) Teneligliptin [7]

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A review of the literature showed few analytical techniques for estimating RGE and TEN separately, in combination or in addition to other anti-diabetic medication. According to review of the literature, reverse phase liquid chromatography [8–11] were discovered to remain the most popular procedures utilized for the determination of certain medications.

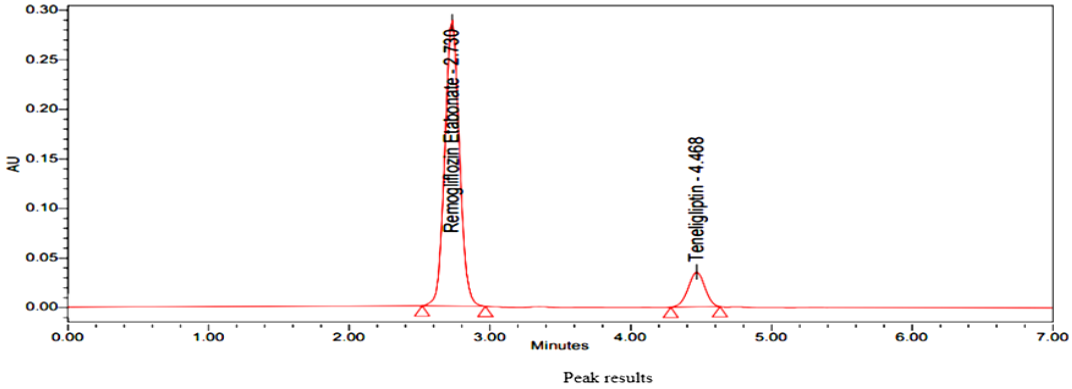
The analysis of Pharmaceutical compounds requires stability testing in a wide range of conditions. These investigations provide the chance to predict the most likely degradation products and to ensure the drugs inherent stability features. So, there was an increase in the need for developing an analytical procedure that could reliably separate and quantify degradation products in pharmaceuticals [12-13].

The present work specifies that the proposed stability indicating assay method by RP-HPLC was enviroment friendly, green, simple, accurate, precise, and specific. Hence it can be utilized for quality control and routine laboratory evaluation for the simultaneous assessment of RGE & TEN in the drug substance and tablet dosage formwith a good impact on the environment, lesser hazardous reagents, and a minimal risk of toxic effects.

2. RESULTS

2.1. Optimized technique

The idyllic isocratic liquid chromatographic conditions for RGE & TEN determination were acquired to remain with Waters Atlantis T3 (150mm x 4.6, 3.5µm) column and 30:70% v/v ratio of acetonitrile: AMF buffer (pH-3.0) thrust with 1.0ml/min flow rate using PDA detection at 236 nm wavelength with 10µl injection volume and 7 min run time at ambient temperature. Remogliflozin etabonate and Teneligliptin were separated at 2.730 and 4.468 min of retention times respectively. The procedure was considered to be optimized as the plate count, tailing factor and peak resolution were observed to remain in the tolerable ranges as represented in Figure 2.



S.No	Name	Area	USP plate count	USP tailing	USP resolution
1	Remogliflozin etabonate	3097743	15173	1.07	-
2	Teneligliptin	431565	7809	0.98	8.27

Figure 2. Optimized chromatogram of RGE & TEN

2.2. Method Validation

2.2.1. Specificity

2.730 min and 4.468 min respectively were observed retention times of Remogliflozin etabonate & Teneligliptin in the proposed technique. Interfering peaks were not observed in blank, placebo and sample at the retention times of the analytes. Henceforth the technique was observed to remain specific as represented in Figure 3.

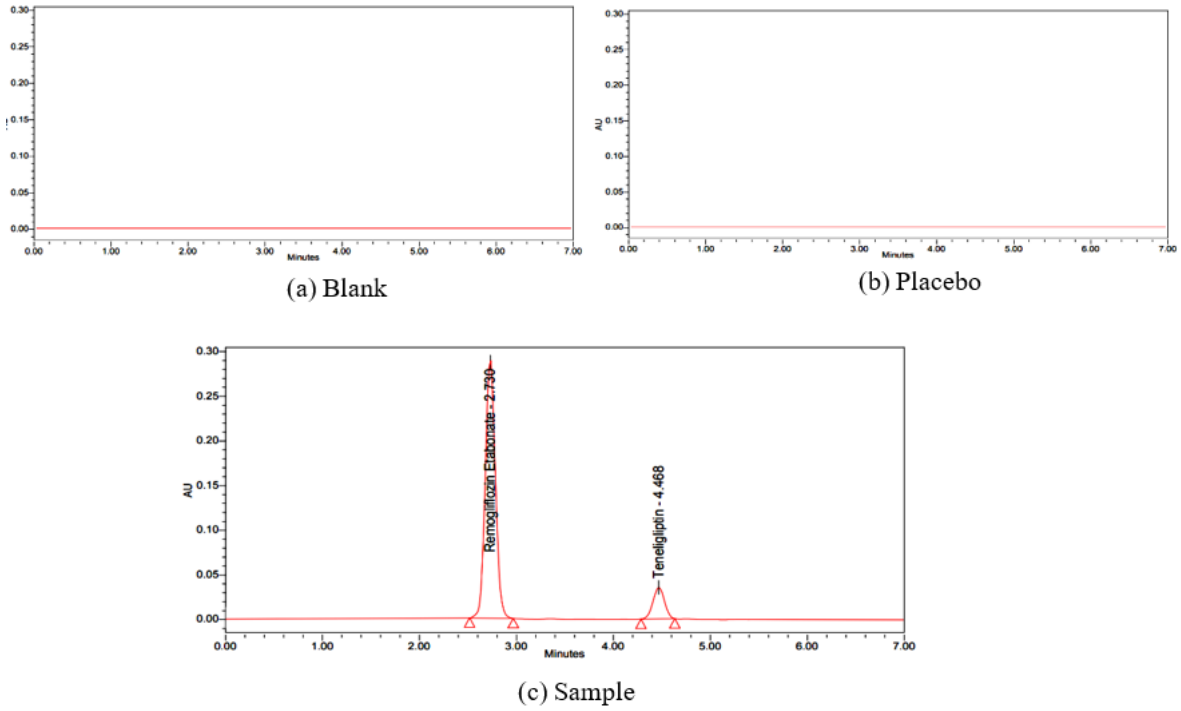


Figure 3. Representative chromatograms for (a) Blank, (b) Placebo, (c) Sample of RGE & TEN

2.2.2. Accuracy

50%, 100% and 150% concentrations were considered for accuracy and the mean percent recovery was 100.3% for both Remogliflozin etabonate & Teneligliptin which was satisfactory and manifest the trueness of the technique. The results are as given in Table 1.

2.2.3. Precision

Repeatability and intermediate precision were considered and the % RSD was measured to be within the range. The results are as given in Table 1.

2.2.4. Linearity

The technique's linearity was recognized by plotting a calibration curve for the individual drug's concentration level and their corresponding peak area. It was perceived on the concentration scale of 25-150µg/ml of Remogliflozin etabonate and 2.5-15µg/ml of Teneligliptin. The coefficient of determination, R^2 was noticed to be not more than 0.999% and therefore the technique is linear. The respective results are given in Table 1. The calibration curves of RGE & TEN are shown in Figure 4.

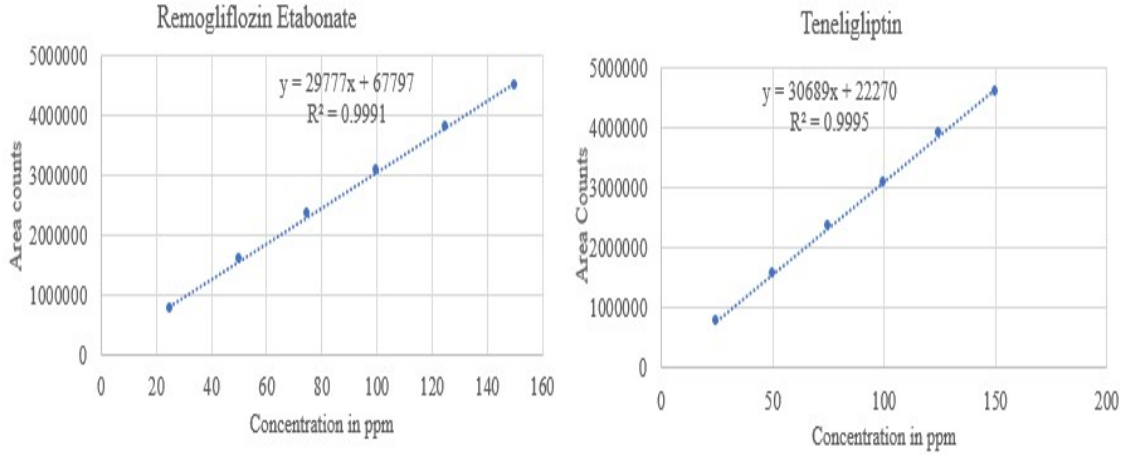


Figure 4. Calibration curve for RGE and TEN

Table 1. Results of the validation parameters

S. No		Parameter	Results	
			RGE	TEN
1	Accuracy	Mean % recovery (%w/w)	100.3	100.3
2	Precision	Repeatability	0.54	0.27
	(% RSD of peak area)	Intermediate precision	1.03	0.66
3	Linearity	Linearity range (µg/ml)	25-150	2-15
		Coefficient of determination (R^2)	0.9991	0.9995
		Regression equation	y = 29777x+67797	y = 30689x +22270
4	Sensitivity	LOD (µg/ml)	0.3	0.03
		LOQ (µg/ml)	1	0.1

2.2.5. Robustness

The technique's robustness was measured by varying the organic phase and the flow rate at the method's proposed wavelength. The values obtained were briefed and remained to be satisfactory signifying the technique remains robust. The results are as given in Table 2.

Table 2. Robustness results for RGE & TEN

Drug Name	Parameter	Condition	Peak area (n=3)			mean	S. D	%RSD
			1	2	3			
RGE	Flow rate change(ml/min)	(-) Flow(0.9ml)	2867452	2998999	2709599	2858683	118309.6	0.041
		(+) Flow(1.1ml)	3106947	3509799	3369789	3328845	204523.2	0.061
	Organic phase change	(-) Org (27:73)	2757416	2706741	2897418	2787192	98764.23	0.035
		(+) Org (33:67)	3361411	3309422	3399872	3356902	45393.29	0.014
TEN	Flow rate change(ml/min)	(-) Flow(0.9ml)	422323	449999	430324	434215.3	14242.44	0.033
		(+) Flow(1.1ml)	449861	435863	458973	448232.3	11640.77	0.026
	Organic phase change	(-) Org (27:73)	412478	439799	429477	427251.3	13795.81	0.032
		(+) Org (33:67)	453373	429999	468976	450782.7	19617.19	0.044

S.D- Standard deviation; %RSD- Relative standard deviation

2.2.6. LOD & LOQ

The observed values of LOD were 0.3, 0.03µg/ml respectively and the LOQ were 1 and 0.1µg/ml respectively for the Remogliflozin etabonate & Teneligliptin.

2.2.7. System suitability

Overall, the parameters of system suitability were within the range and are acceptable as per ICH guidelines and are presented in Table 3.

Table 3. System suitability data for RGE & TEN

Sample	RGE			TEN			
	t_R	N	T_f	t_R	N	T_f	R_s
1	1.298	206	0.98	1.818	653	1.23	1.58
2	1.977	1455	1.3	6.689	4240	1.23	15.04
3	6.225	5168	1.66	6.817	1147	3.57	0.14
4	4.016	3268	1.26	8.781	1106	1.34	8.26
5	2.032	3859	1.11	4.091	4690	1.08	9.58
6	2.73	15173	1.07	4.468	7809	0.98	8.27
Mean	3.046333	4854.833	1.23	5.444	3274.167	1.571667	7.145
SD	1.8096	4886.084	0.242157	2.46989	2814.194	0.987146	5.491964
RSD	0.59	1.006	0.196	0.45	0.85	0.62	0.76

t_R- Retention time, N- No. of theoretical plates, T_f-Tailing factor, R_s- Resolution

2.3. Forced degradation Studies

Stress studies were carried out under various degradation conditions. Amid all observations, the minimum was hydrolytic degradation while the maximum degradation was for peroxide. Results are shown in Table 4 and Figure 5.

Table 4. Forced degradation data for Remogliflozin etabonate & Teneligliptin

Degradation	Remogliflozin etabonate		Teneligliptin	
	Peak Area	%Degradation	Peak Area	%Degradation
Acid	2633017	14.2	275512	88.2
Alkali	2667075	13.1	273849	87.7
Thermal	2650156	13.7	271405	86.9
Peroxide	2579507	16.0	267125	85.6
Reduction	2724461	11.3	279976	89.7
Hydrolysis	3030408	1.3	310154	99.3
Photolytic	3018792	1.7	311289	99.7

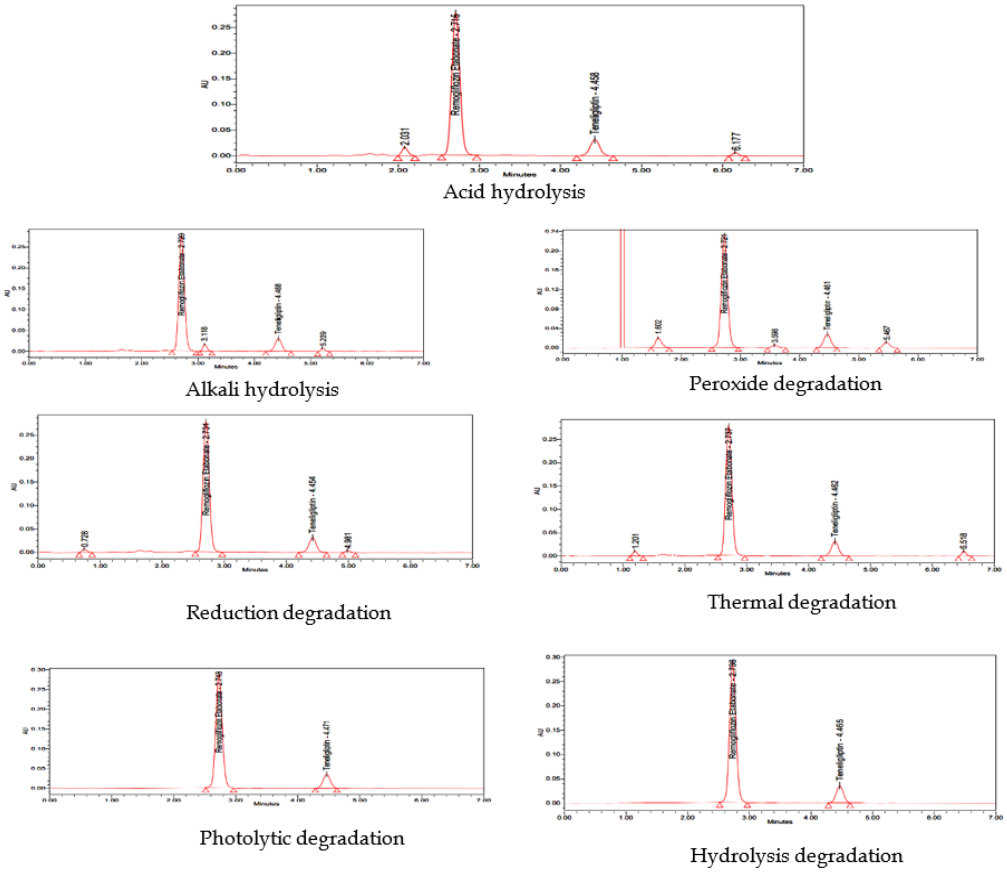


Figure 5. *Forced degradation chromatograms for RGE & TEN*

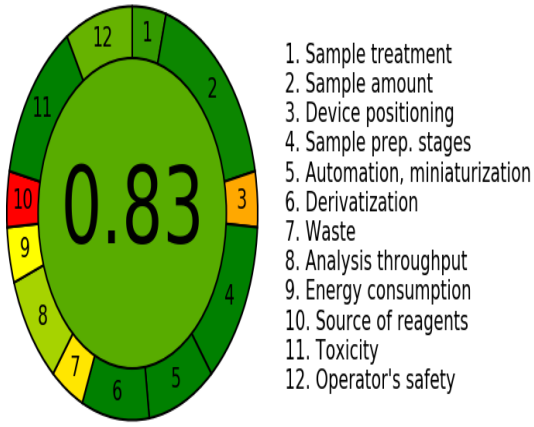
2.4. Assay

100.7% and 100.0% were the calculated mean % assays of RGE and TEN respectively which were satisfactory and in good agreement with the % label claim for RGE and TEN.

2.5. Greenness assessment of the developed procedure

For the evaluation of the environmental impact & greenness assessment of analytical procedure, AGREE® tool was employed. The 12 elements of Green analytical chemistry are the basis for a Pictogram provided by AGREE. The compatibility of proposed analytical procedure with green analytical chemistry concept is graded on a red–yellow–green colour scale, where each principle is represented on a separate subdivision. The central area of the AGREE pictogram has a numerical between 0 and 1 and color which represents the overall rating. Reagent toxicity, waste generation, energy consumption, intensity of labour, degree of automation and intergration were few factors which are calculated based on their impact on environment and operator's safety[14-16]. The eco-friendliness of the approach, was calculated by assigning various weights & scores to the parameters utilized in the proposed method and was reflected with a final

score (0.83). The Greenness assessment of the proposed HPLC method according to the AGREE program is as shown in Figure 6.



Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.9	1
2. Minimal sample size and minimal number of samples are goals.	0.98	4
3. If possible, measurements should be performed in situ.	0.33	1
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	3
5. Automated and miniaturized methods should be selected.	1.0	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.45	1
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.68	2
9. The use of energy should be minimized.	0.5	1
10. Reagents obtained from renewable sources should be preferred.	0.0	1
11. Toxic reagents should be eliminated or replaced.	1.0	3
12. Operator's safety should be increased.	0.8	2

Figure 6. Greenness assessment of the proposed method employing AGREE program [16]

3. DISCUSSION

The technique was perceived to be simple and economical for ideal isocratic chromatographic conditions on Waters Atlantis T3 (150mm x 4.6, 3.5µm) column and mobile phase consisting 30 volumes acetonitrile along with 70 volumes AMF buffer (pH-3.0) pumped at a flow rate of 1.0ml/min for the chromatographic separation using PDA exposure at 236 nm wavelength with 10µl injection volume and 7 min run time at ambient temperature. To separate the medications on LC, different mobile phase combinations were primarily used. Based upon peak parameters, the flow rate and mobile phase ratio were selected. Remogliflozin etabonate & Teneligliptin were separated at 2.730 and 4.468 min of retention times respectively. ICH(Q2B) guidelines were followed to validate the developed technique for estimating RGE & TEN. Pure analyte peak indicates specificity outcome of the technique, while recovery result assessed the procedure's accuracy. Both drugs' recoveries came close to 100%. Data on linearity showed a strong association over the linear concentration ranges of 25–150µg/ml for RGE and 2.5–15 µg/ml for TEN as the coefficient of determination is more than 0.999. y=29777x+67797 and y= 30689 x +22270 respectively were the observed regression equations for RGE and TEN. %RSD values of repeatability and intermediate precision were measured to be ≤ 2.0%. The developed technique was considered to be robust even after altering the flow rates and mobile phase composition as there were no significant changes in the retention times and peak areas of RGE & TEN. The LOD and LOQ for RGE & TEN were observed to be 0.3 µg/ml, 1 µg/ml and 0.03µg/ml, 0.1µg/ml respectively. The forced degradation studies further shows that the drug degradation percentage was constantly found to be within the permitted limits as per Q1A R2 and Q1B ICH guidelines, evidencing the methods' stability-indicating nature. The AGREE tool was utilized for the assessment of the greenness profile of the established method with 0.83 as the final score. As a consequence, this work has a unique novelty and superiority due to its simplicity, cost-saving, and time-saving advantages.

4. CONCLUSION

Considering the results of specificity, accuracy, precision, linearity, robustness and recovery the proposed stability indicating reverse phase liquid chromatography technique is considered to be ideal for qualitative and quantitative determination of Remogliflozin etabonate & Teneligliptin. Additionally, the solvents and mobile phase are economical, simple to compose and resulting in decent resolution. Moreover, neither the degradation products nor any of the excipients interfered with the outcome of the technique. Compared to the previous technique the present technique utilizes more proportions of aqueous phase. The eco-friendliness of the current method was evaluated utilizing AGREE calculator. Therefore, the Green technique can be employed for systematic analysis in repetitive laboratories evaluation aimed at the simultaneous assessment of RGE & TEN in the drug substance and pharmaceutical dosage form for quality control purposes.

5. MATERIALS AND METHODS

5.1. Instrumentation

Alliance Waters HPLC coupled with e2695 pump, Empower2 software, A Eutech pH metre, a weight scale manufactured by Sartouris Pvt.Ltd., Chennai, a Shimadzu UV-1700 UV-Vis-spectrophotometer, and a Nichrome ultrasonicator, model UCA 701 were used in the study.

5.2. Chemicals & reagents

Pure medications: Remogliflozin etabonate & Teneligliptin pure drugs were procured from Glenmark Pharmaceuticals Ltd, Sikkim.
Formulation: Zita® plus-R tablets manufactured by Glenmark were utilized for assay.
Chemicals & reagents: HPLC grade Acetonitrile, methanol, orthophosphoric acid and ammonium formate (AMF) were procured from Rankem chemicals. Study was performed using Milli Q water.

5.3. Composition of solutions

5.3.1. Buffer:
6.3g of AMF was accurately weighed and dissolved in 1 litre Milli-Q water. Orthophosphoric acid was utilized in adjusting pH to 3.0 and passed through a 0.45µ nylon filter.
5.3.2 Mobile Phase (Diluent):
30 volumes acetonitrile along with 70 volumes AMF buffer (pH-3.0) were mixed to be utilized as mobile phase. 0.45µ membrane filter was utilized for the filtration of mobile phase.
5.3.3. Standard solution:
Remogliflozin etabonate (100 milligram) & Teneligliptin (10mg) were accurately measured then taken in a 100ml volumetric flask. Contents of the flask were sonicated along with diluent and the volume was made up with the same (Stock solution). Further, diluent along with 5 ml of the stock solution was sonicated in a 50 ml volumetric flask. Diluent was utilized to top off the solution (100µg/ml Remogliflozin etabonate, 10µg/ml Teneligliptin).
5.3.4. Sample Solution:
Twenty Zita® plus-R tablets were weighed and individual tablet weight was calculated. 238mg tablet powder representing a corresponding weight of 100mg Remogliflozin etabonate and 10mg Teneligliptin was taken precisely and moved to a 100ml volumetric flask. Contents of flask were sonicated along with diluent for 30 min and then centrifuged for 30min**.** Diluent was utilized to top off the solution and was filtered by 0.45µ injection filter (Sample Stock)**.** 5ml of sample stock was taken into 50ml volumetric flask and the solution was top off with diluent. (RGE 100µg/ml & TEN 10µg/ml).
5.3.5. Placebo:
Composition of placebo (150mg) - lactose (135mg), magnesium stearate, talc, starch (5mg each). It was weighed equivalent to sample weight and was further processed as sample preparation.
5.3.6. Forced degradation reagents:
1N HCl: 8.5 ml of hydrochloric acid diluted to 100 ml, with milli-Q water.
1N NaOH: 0.4gm of NaOH dissolved in 100 ml of milli-Q water.
3% H_2O_2: 1ml of 30% hydrogen peroxide was added with 9 ml milli-Q water.
10% w/v $NaHSO_4$: 1g of $NaHSO_4$ dissolved in 10 ml milli-Q water.

5.4. Determination of wavelength (λ_{max})

The wavelength of maximum absorption of the Remogliflozin etabonate & Teneligliptin solution in diluent was scanned utilizing a photodiode detector in 200-400 nm wavelength range with diluent as blank. An isobestic point at 236nm was observed in the PDA spectrum as shown in Figure 7.

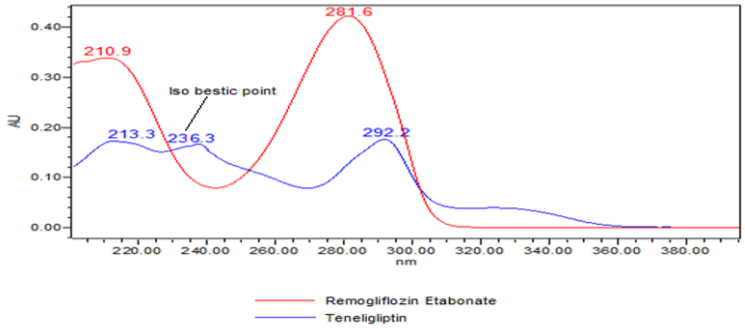


Figure 7. PDA spectrum of RGE & TGN

5.5. Optimization of chromatographic conditions

To attain acceptable plate count, tolerable tailing factor and satisfactory resolution peaks, trials were executed during the development of analytical procedure by altering the mobile phase composition with various buffers and buffer pH.

5.6. Method Validation

Ensuing ICH Q2R1, the progressed technique parameters *viz.*, system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ) were validated [17].

5.6.1. Specificity

Capability to determine the analyte in the existence of anticipated contaminants and degradants by any nosiness noted at RGE and TEN retention times. Assessed by evaluating the outcomes of blank, placebo and sample.

5.6.2. Accuracy

Degree of agreement amid the actual value and the discovered value is stated as accuracy, also known as trueness. It was calculated as a percentage recovery by adding three different concentrations of recognised standards to samples that had already undergone analysis.

5.6.3. Precision

Precision is the degree to which a set of measurements taken under predetermined conditions using repeated samples of the different homogeneous material agree closely. It is reported as %RSD.

Repeatability: Precision over a brief period with the same operating conditions. Six analyses of the sample solution were utilized for evaluation.

Intermediate precision: The variability within laboratories, such as different analysts, instruments, days etc. Often referred to as method precision. Six different determinations of sample made on six different days were utilized for evaluation.

5.6.4. Linearity

Ability of a technique to analyse and produce findings that are proportionate to the sample's analyte concentration within a certain range. The calibration curve's regression equation, created from six linear standard concentrations was considered to estimate linearity.

5.6.5. Robustness

Regarding deliberate changes in method parameters, it is the method's validity which was carried out by altering the mobile phase composition and flow rate.

5.6.6. LOD & LOQ

Detection and quantification limits were assessed for RGE & TEN at 3:1 and 10:1 S/N ratio respectively, by analysing series of known concentration dilutions.

5.6.7. System suitability

System suitability was performed to confirm that the measuring system and the analytical approach were appropriate for the suggested analysis. Six indistinguishable samples were assessed.

5.7. Forced Degradation Studies

Ensuing ICH Q1A (R2) and Q1B guidelines, forced degradation studies were performed which comprises acid and alkali hydrolysis, thermal, peroxide, reduction, hydrolytic degradations along with photolytic degradation.

5.7.1. Acid Hydrolysis

5ml sample stock was added to a 50ml volumetric flask, followed by 1ml of 1N HCl. The volumetric flask was maintained at 60°C for 1hr before being neutralized with 1N NaOH and diluted to 50ml with diluent.

5.7.2. Alkali Hydrolysis

5ml sample stock was added to a 50ml volumetric flask, followed by 1ml of 1N NaOH. The volumetric flask was maintained at 60°C for 1hour before being neutralized with 1N HCl and diluted to 50ml with diluent.

5.7.3. Thermal degradation

Petri dish added with Remogliflozin etabonate and Teneligliptin sample was placed at 105°C for 24 hours in a hot air oven. Diluent was utilized to dilute this sample.

5.7.4. Peroxide degradation

5ml sample stock was added to a 50ml volumetric flask, followed by 1ml of 3% hydrogen peroxide solution and the volume was top off using diluent. The volumetric flask contents were maintained at 60°C for 1hour and was then left for 15 minutes at room temperature.

5.7.5. Reduction degradation

5ml sample stock was added to a 50ml volumetric flask, followed by 1ml of 10% w/v sodium bisulphate and the volume was built up to the required volume with diluent. The volumetric flask was maintained at 60°C for 1 hour. The volumetric flask was left at room temperature for 15 minutes.

5.7.6. Hydrolytic degradation

5ml of the sample stock solution was added to a 50ml volumetric flask, 1ml of milli Q water was added to a flask and the volume was built up to the required volume with diluent. The volumetric flask was then maintained at 60°C for 1 hour and was left for 15 minutes at room temperature.

5.7.7. Photolytic degradation

The Remogliflozin etabonate and Teneligliptin sample was placed in UV light for 24 hours. Diluent was utilized to dilute this sample.

All the above degradation solutions were filtered using 0.22µ syringe filter, diluted and analysed separately using HPLC technique.

5.8. Assay

Validated HPLC technique was successfully implied to analyse the sample solution under the same chromatographic conditions as linearity. Three separate assays were performed to calculate the mean. The % assay of RGE & TEN were assessed from the calibration curve.

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