Quantification Of Remogliflozin And Teneligliptin In Tablets Using A Stability Indicating High Performance Liquid Chromatographic Method

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Abstract: By using HPLC to test both Remogliflozin and Teneligliptin at the same time, a quick and easy-to-understand method was made. This method uses chromatographic separation with a 250 cm x 4.6 mm x 5 m symmetrically packed C18 Grace Column. The mobile phase was made up of Sodium bisulfate and methanol in a ratio of 60:40, with a flow rate of 1 mL/min, a pH of 4.3, and room temperature. 245 nm was used to measure UV.It was decided that the limits on recoveries, linearity, and quantification were all within a range that was acceptable. The results of the evaluation were good enough. The method that was made was clearly good enough for regular analysis of drug formulations. The suggested method was checked to make sure it met the requirements of ICH Q2 (R1).

Keywords: Remogliflozin, Teneligliptin, validation, Development, HPLC, ICH guidelines.

1. INTRODUCTION
1.1Profile of the drugs

Remogliflozin etabonate is a gliflozin-class drug that is used to treat nonalcoholic steatohepatitis and type 2 diabetes. Remogliflozin etabonate has been shown to increase the amount of glucose that mice and people pee out. Early tests showed that diabetics' blood glucose levels got better [1-2]. Researchers have looked at remogliflozin etabonate doses up to 1000 mg [3-4]. Remogliflozin, dapagliflozin, and pioglitazone were all shown to have the same effect on blood sugar (decrease in HbA1c and fasting glucose).The drug remogliflozin is made from remogliflozin etabonate. Remogliflozin stops the sodium-glucose transport proteins (SGLT) in the kidney from bringing glucose back into the blood. By blocking this transporter, glucose is taken out of the blood through pee.RE is the newest type of SGLT2 inhibitor that has just been cleared for treatment in India. Remogliflozin etabonate is a strong and specific SGLT2 inhibitor that is given as a prodrug, has active metabolites inside it, and needs to be taken twice a day [5]. Kissei Pharmaceutical found remogliflozin. Remogliflozin is now being made by BHV Pharma, which is a wholly-owned company of Avolynt and is working with Glenmark Pharmaceuticals [6].

Teneligliptin is a drug that is used to treat people with type II diabetes. It belongs to a group of diabetes medicines called dipeptidyl peptidase4 inhibitors [7]. Under ICH criteria [8-14], we set up a high-performance liquid chromatographic method to show that a drug is stable and how it is made. The molecular structures of Remogliflozin and Teneligliptin are shown in **Figure 1**.
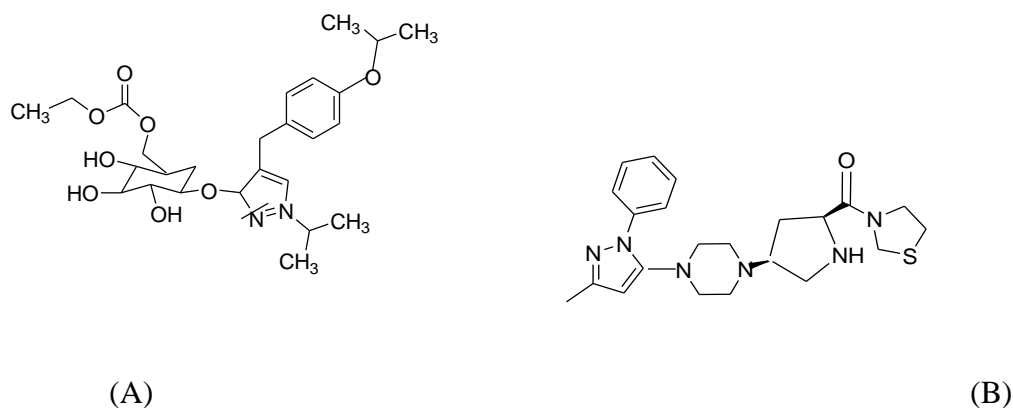
(A)   (B)

Fig. 1: Structural representation of (A) Remogliflozin (B) Teneligliptin

1.2 Literature Survey

In the past few years, there haven't been many reports about using HPLC to measure Remogliflozin and Teneligliptin [15–17]. In this method, we tried to come up with a selective, reliable, and new way to measure Remogliflozin and Teneligliptin using High Performance Liquid Chromatography (HPLC).

1.3 Aim and objectives of the Present Investigation

In the present investigation, we quantify the Remogliflozin and Teneligliptin in Tablets by Stability-Indicating High Performance Liquid Chromatography. This method uses chromatographic separation with a 250 cm x 4.6 mm x 5 m symmetrically packed C18 Grace Column. The mobile phase was made up of Sodium bisulfate and methanol in a ratio of 60:40, with a flow rate of 1 mL/min, a pH of 4.3, and room temperature. 245 nm was used to measure UV.It was decided that the limits on recoveries, linearity, and quantification were all within a range that was acceptable. The results of the evaluation

II.RESEARCH METHODOLOGY
2.1Reagents and Chemicals

Merck Ltd. in Mumbai, India, was where the HPLC-marked Sodium Bisulphate, Methanol, and water were bought. APIs of Remogliflozin (99.9% purity) and Teneligliptin (99.9% purity) were bought from Cipla Pharmaceutical Ltd. in Mumbai, India.

2.2 Instrumentation

A Waters Alliance e2695 HPLC with a quaternary pump, a Photodiode Array detector, and boost 2.0 software was used.

2.3 Analytical Method development

Several trials were conducted to establish a good resolution between Zanubrutinib and its impurities. Various buffers and mobile phases were used to develop the approach. There was no mobile phase capable of effectively separating the active ingredient from the associated compounds. The selected mobile phases improved the resolving power and provided better resolving power between teneligliptin and remogliflozin. The mobile phases are Sodium bisulphate and methanol. The Develosil, Waters, and Grace columns were used in the development trials (**Table 1**), but the Grace C18, 250cmx4.6mm, 5µm column coupled to the PDA detector was used to separate tenegliptin and remogliflozin. The mobile phase flow rate was kept constant at 1.0 mL/min. The components were detected using a UV detector set to 245 nm. Active pharmaceutical ingredients were well separated in optimized chromatographic conditions. All of the findings were within the limits. Method optimized chromatograms are shown in **Fig.2.**

Table.1: Results of Method Optimization.

Trail Number	Mobile phase ratio	Used Column	Observation
Trail-1	Orthophosphoric acid: Methanol (65:35)	Develosil, C18, 250cmx4.6mm, 5μm	Two peaks were detected & peak shape is not good.
Trail-2	Orthophosphoric acid: Methanol (65:35)	Waters, C18, 250cmx4.6mm, 5μm	Two peaks were detected & peak shape is not good.
Trail-3	$NaHSO_4$: Methanol(50:50)	Grace, C18, 250cmx4.6mm, 5μm	Peak shapes are not good
Trail-4	$NaHSO_4$:Methanol(65:35)	Grace, C18, 250cmx4.6mm, 5μm	Peaks are not good
Trail-5	$NaHSO_4$:Methanol(60:40)	Grace, C18, 250cmx4.6mm, 5μm	Two peaks were detected but resolution and RT high.
Trail-6	$NaHSO_4$:Methanol(55:45)	Grace, C18, 250cmx4.6mm, 5μm	Two peaks eluted and all the system suitability parameters are within the limit.
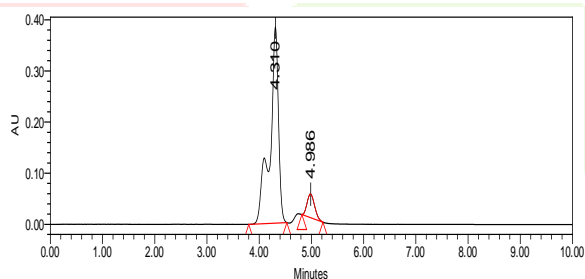
Trail: 1　　　　　　　　　　　　　　　　　　　　　　　　　　**Trail: 2**
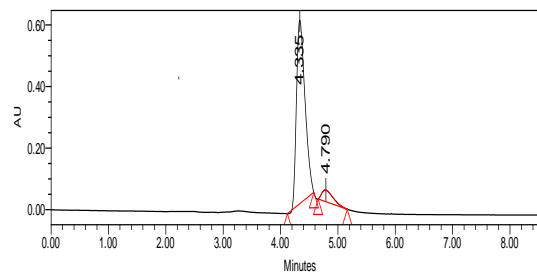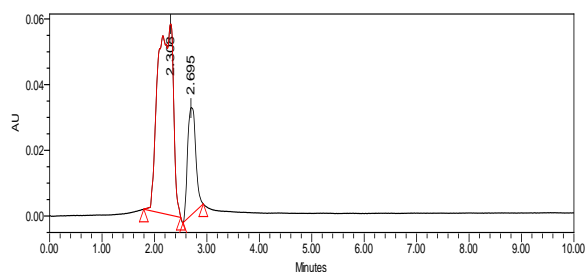
Trail: 3　　　　　　　　　　　　　　　　　　　　　　　　　　**Trail: 4**
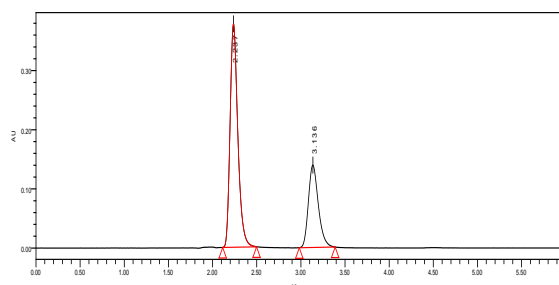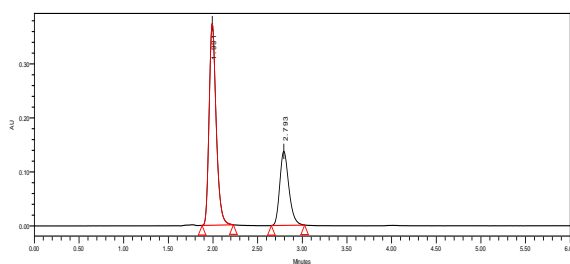
Trail: 5   **Trail: 6**
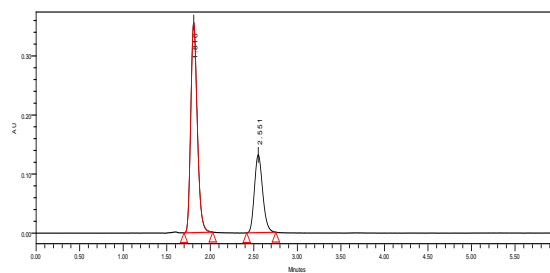
Fig.2: Method optimized chromatograms

2.4 Method Validation

By examining factors including system suitability, linearity, LOD, LOQ, robustness, and accuracy, among others, the HPLC technique was validated, and the results were found to be within the ICH's acceptable range.

2.4.1 System Suitability

To evaluate the efficacy of the system, we utilized USP tailing   and plate count, and relative variation percentage.

2.4.2 Linearity and Accuracy

Linearity was examined by utilising standard solutions of Remogliflozin and Teneligliptin at numerous diluti on levels (50%, 75%, 100%, 125%, and 150%). The accuracy of three distinctdilution levels, 50%, 100%, an d 150%, was investigated. Finally, the percentages of recovery and RSD were computed.

2.4.3 Precision

System Precision: The percent RSD was calculated after six injections of the reference standard mixture of remogliflozin and teneligliptin. Method Accuracy: After injecting sample solutions of remogliflozin and teneligliptin, recovery and RSD were calculated. Intermediate Precision: Teneligliptin and Remogliflozin sample solutions were injected on separate days using various columns. After that, the RSD and recovery percentages were calculated.

2.4.4 Robustness

This approach was investigated by altering the flow by 10% and the organic phase by 10%.

2.4.5 LOD and LOQ

While LOQ refers to the smallest amount of analyte in a sample that can be seen with acceptable precision and accuracy, LOD refers to the smallest amount of analyte in a sample that can be analyzed. Using the developed HPLC approach, the limits of detection and quantification for Remogliflozin and Teneligliptin are established by injecting increasingly smaller volumes of common solutions. The LOD and LOQ are calculated as 3 s/n and 10 s/n, respectively, in accordance with ICH standards. S/N stands for signal-to-noise.

2.4.6 Forced Degradation

The chromatographic peaks formed by forced degradation preparations won't be affected by force degradation. The ICH recommendations Q1 (A) R2 were followed when conducting force degradation learnings. The peak purity of the primary peak form must pass because the deteriorating peaks must be separated from one another; as a result, the resolution between the peaks must be at least 1.0. The application of various types of stress resulted in the degradation of about 20% of the material.

2.4.6.1 Acid Degradation

1000 mg of the sample should be transferred to a 10 ml volumetric flask along with 5 ml of the diluent and sonicated to dissolve. Add 1 ml of 1N HCl after that. Take 15 minutes to wait. The solution should be neutralized with 1 ml of 1N NaOH after 15 minutes, diluted to volume with diluent, filtered, and then added to an HPLC system.

2.4.6.2 Alkali Degradation

In a 10 ml volumetric flask, add 5 ml of diluent and 1000 mg of the material. Sonicate to dissolve. Add 1 ml of 1N NaOH next. 15 minutes should pass. After 15 minutes, neutralize the mixture with 1 ml of 1N HCl, diluent it to volume, filter it, and then inject it into an HPLC system.

2.4.6.3 Peroxide Degradation

1000 mg of the sample should be transferred to a 10 ml volumetric flask along with 5 ml of the diluent and sonicated to dissolve. Add 1 ml of 10% H2O2 solution after that. Take 15 minutes to wait. The sample is filtered, diluted to volume with diluent, and then added to the HPLC system after 15 minutes.

2.4.6.4 Thermal Degradation

Before being evaluated, 1500 mg of the substance were heated for six hours to 105°C. A 10mL volumetric flask containing 1000 mg of specimen was filled, filtered, and then loaded into an HPLC device.

2.4.6.5 Hydrolysis Degradation:

1000 mg of the sample should be transferred to a 10 ml volumetric flask along with 5 ml of the diluent and sonicated to dissolve. Add 3 cc of HPLC water after that. Take 15 minutes to wait. The material is filtered, diluted to volume with diluents, and then added to the HPLC system after 15 minutes.

III.RESULTS AND DISCUSSION

One C18 Grace Column with a flow rate of 1 mL/min was required for the isocratic separation of Remogliflozin and Tenelgliptin, and the column was kept at room temperature. Sodium bisulphate and methanol were combined in the mobile phase at a ratio of 55:45 (v/v). At 245nm, the UV spectrum was seen. A rapid, reliable, and responsive HPLC technique has been developed. When the system's efficiency-influencing factors were fine-tuned, the resulting strategy showed excellent sensitivity and selectivity. There is no HPLC method mentioned in the literature. Designing an HPLC approach for the in vitro quantification of combination medications is therefore intriguing. According to the ICH stability requirements, a number of forced conditions, including heat, alkali, acidic, oxidative, UV, and reductive, have been researched. The process was validated and the results fell within the permitted range as per ICH standards.

3.1 System Suitability

Remogliflozin (100 g/mL) and tenelgliptin (10 g/mL) reference solutions were injected into the HPLC system, and **Figure 3** shows the HPLC chromatogram. The peak areas were used to determine the %RSD, and the findings fell within the acceptable range. The findings of the system's appropriateness are shown in **Table 2.**
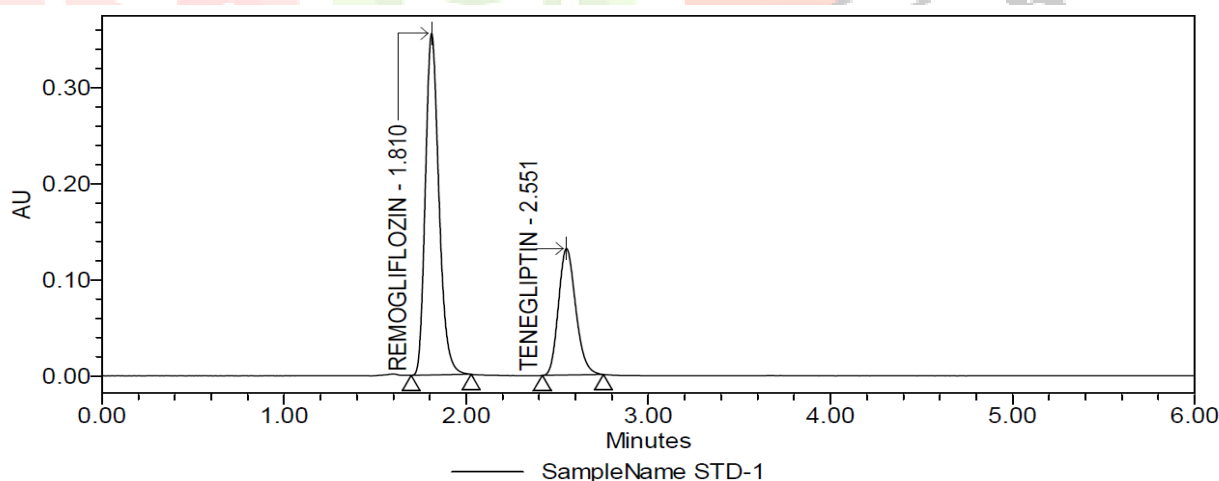

Fig.3: Standard Chromatogram of HPLC

Table.2: Results of System suitability.

| S. No.	System suitability parameter	Acceptance criteria	Drug name	
			Remogliflozin	Teneligliptin
1	%Relative Standard Deviation	NMT 2.0	0.2	0.3
2	USP Tailing	NMT 2.0	1.23	1.18
3	USP plate count	NLT 2000	9019	7593

3.2 Specificity

By differentiating the chromatograms of the blank specimens in **Figure 4**, specificity was used to remove the impacts of all interfering substances from the Remogliflozin and Teneligliptin peak values rather than to assess the sensitivity of the method's test. The method's justification showed that the chosen medications were separated without excipient-related market peaks.
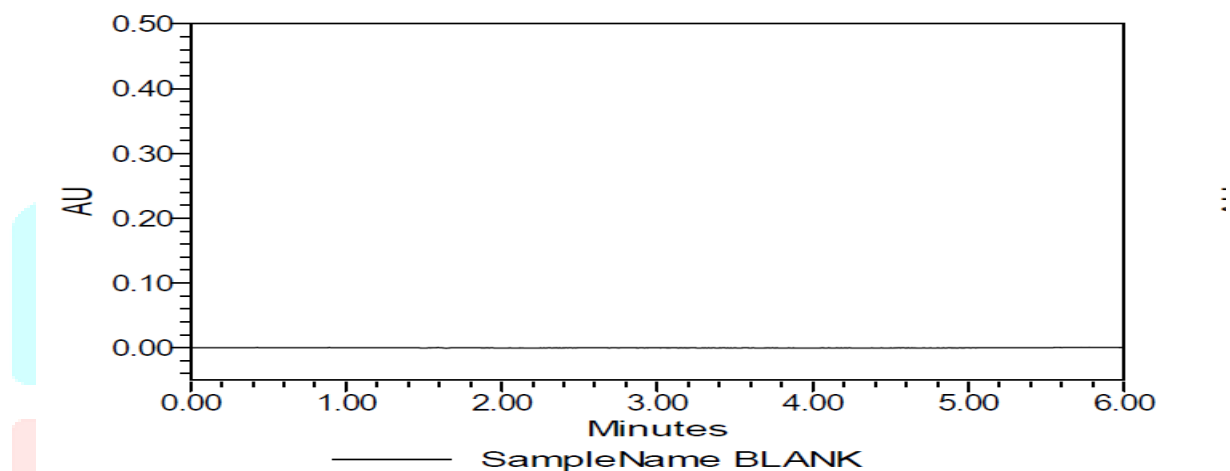

Fig. 4: HPLC blank chromatogram

3.3 Linearity

Remogliflozin and Teneligliptin were prepared in a series of linearity solutions at eight different concentrations, ranging from 50 to 150 g/mL for Remogliflozin and 0.5 to 15 g/mL for Teneligliptin, to show the method's linearity (**Fig. 5**). The calibration curves were linear over the whole range of Remogliflozin and teneligliptin concentrations. **Table 3** had the linearity values. The correlation coefficient values for Remogliflozin and Teneligliptin were 1 and 0.9998 on the calibration curve.

$y = 18254x + 3288$

$R^2 = 1$
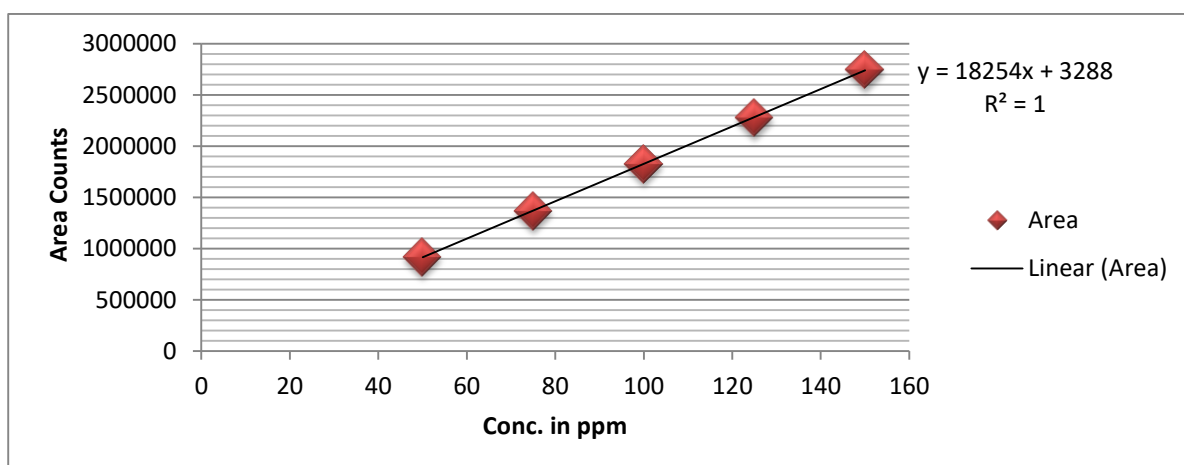
(A)
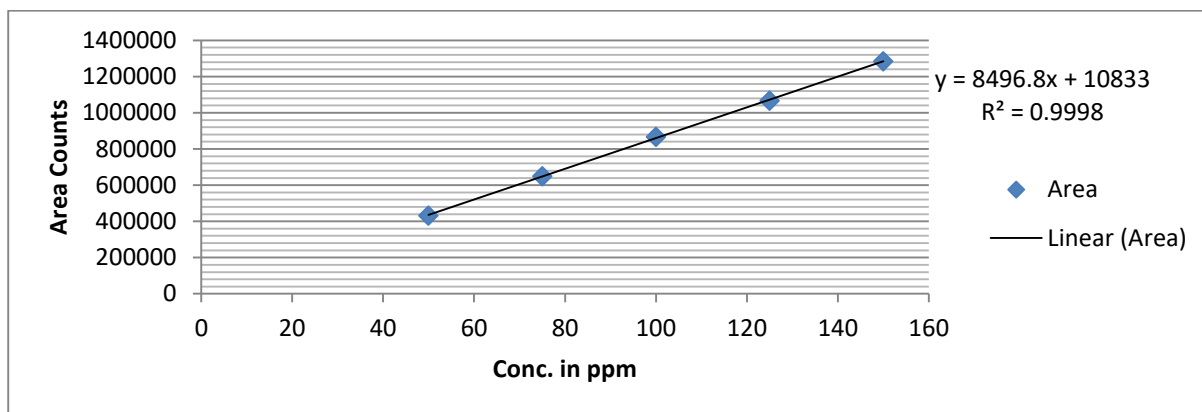
(B)

Figure 5: Linearity plots of (A) Remogliflozin and (B) Teneligliptin

Table.3: HPLC results of Linearity

Linearity	Remogliflozin		Teneligliptin	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-50%	50	920507	5	432129
Linearity-75%	75	1369654	7.5	649997
Linearity-100%	100	1825005	10	867252
Linearity-125%	125	2282545	12.5	1067926
Linearity-150%	150	2745839	15	1285265
Slope	18254		8496.8	
Intercept	3288		10833	
CC	1		0.9998	

3.4 Accuracy

To ascertain the precision, recovery trials were carried out at three different dilution levels (50%, 100%, and 150% dilution). Teneligliptin concentrations of 5, 10, and 15 g/mL and Remogliflozin concentrations of 50, 100, and 150 g/mL, respectively, were created in APIs. Three preparations of each spike level were injected into the test solutions in accordance with the test procedure, and an assay was carried out. Results are shown in **Table 4.** Share recovery rates were seen to range between 98% and 102%.

Table.4: HPLC results of accuracy of Remogliflozin and Teneligliptin

S. No.	Concentration of Remogliflozin (µg/ml)	% of Recovery	Concentration of Teneligliptin(µg/ml)	% of Recovery
1	50	101	5	99
2	100	100	10	100
3	150	100	15	99

3.5 Precision

The approach and intermediate modifications were used to evaluate the precision. Remogliflozin and teneligliptin sample solutions were administered under identical experimental conditions to calculate the intraday studies. By analyzing samples on multiple days and in different columns, the method was able to attain intermediate precision in the same facility. Using this method, the RSD values were found to be less than 2%. At each attached concentration, the chosen medications recovered well (between 98 and 102%), proving the strategy's effectiveness. The results are shown in **Table 5**.

Table.5: Precision results of Remogliflozin and Teneligliptin

S. No.	Area of Remogliflozin	Teneligliptin	% of Assay	% of Assay
1	1823137	867197	99	99
2	1825540	867549	99	100
3	1836131	868041	99	100
4	1823885	868501	99	100
5	1831158	868847	99	100
6	1828931	867642	99	100
Average			99	100
Std. dev			0.27	0.07
% RSD			0.27	0.07

3.6 Limit of detection and Limit of quantification

Using the calibration curve technique, the Limits of Quantification and Detection were independently determined. Using the established HPLC method, the LOD and LOQ of the compounds were determined by continuously injecting a lower accumulation of standard solutions. The LOD values for remogliflozin and teneligliptin were 3.5 g/mL and 10 g/mL, respectively, with s/n values of 3 and 3. The s/n values were 10, 10, and the LOQ values were 3.2 g/mL and 10.2 g/mL.

3.7 Robustness

Process parameters including change in flow (10%) and organic content in the mobile phase (10%) were purposely changed in compliance with ICH requirements. There is no technique immunity to system appropriateness as a result. Table 5 shows that the tactic's robustness was assessed by contrasting the amended parameters for retention time, tailing factor, and content percentage. This was done by looking at the outcomes of the updated parameters for RT, tailing factor, and content percentage using HPLC. The technique was successful, as evidenced by the consistency of the findings obtained after making small, intentional adjustments (**Table. 6**).

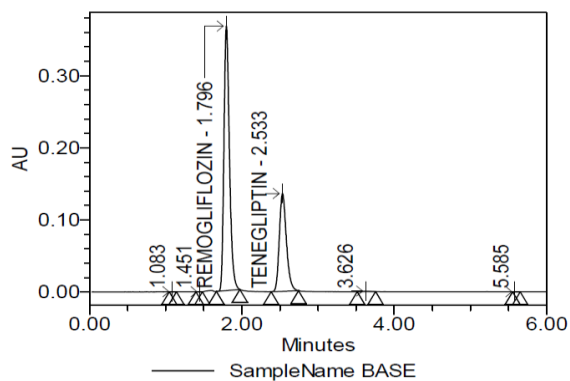**Table.6:** Robustness results of Remogliflozin and Teneligliptin

Parameter change	%RSD of Remogliflozin	%RSD of Teneligliptin
Flow (0.9 ml/min)	0.5	1.6
Flow (1.1 ml/min)	1.9	1.0
Org Phase (45:55)	1.3	1.4
Org Phase (55:45)	1.2	1.3

## 3.8 Forced Degradation studies of Teneligliptin and Remogliflozin

According to the ICH stability recommendations, studies of thermal degradation, basic degradation, acidic degradation, oxidative degradation, photolytic degradation, and reductive forced degradation were carried out (the degradation chromatograms are given in **fig. 6**). The investigations provided information on the circumstances in which the medication is unstable, and suitable safety measures were frequently taken during formulation in order to prevent any instability. The results of Remogliflozin and Teneligliptin degradation are shown   in **Table.7**.
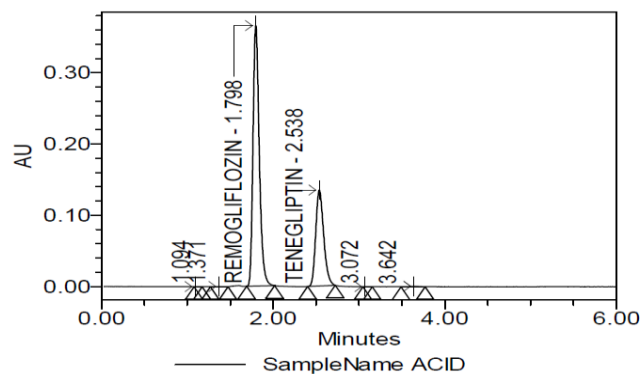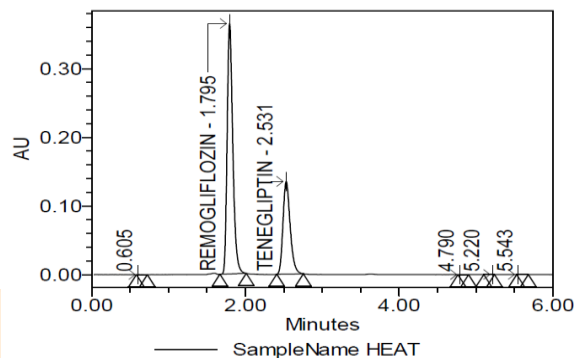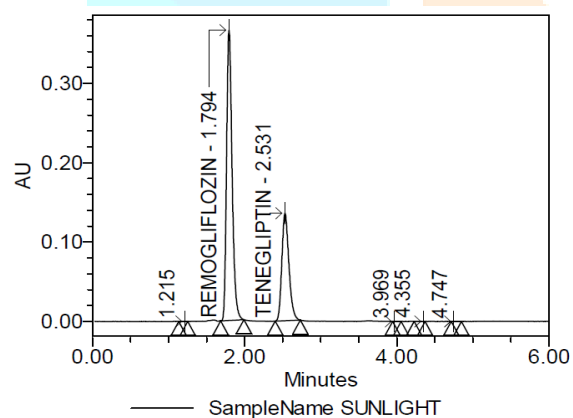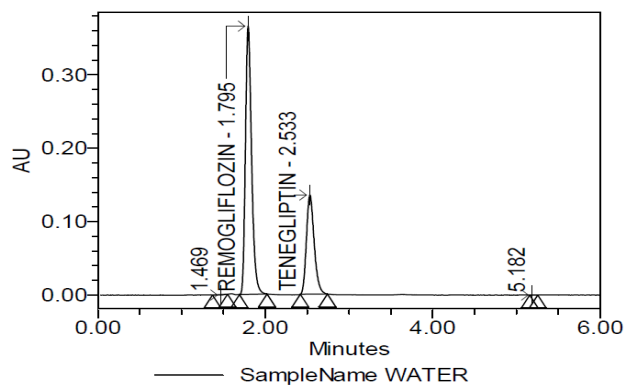
(A)

(B)
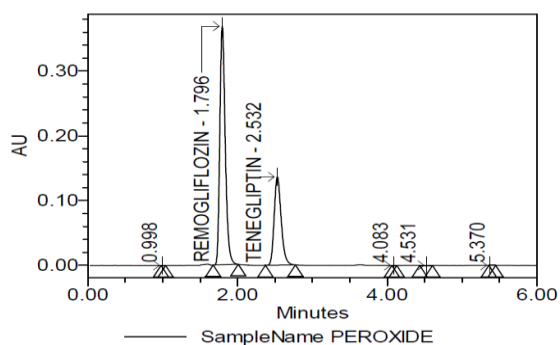
(C)

(D)

(E)

(F)

Fig.6.Chromatograms of Stress Degradation **(A)** Acid **(B)** Alkali **(C)** Peroxide **(D)** Thermal **(E)** Sunlight **(F)** Hydrolysis

Table.7: Forced Degradation results of Remogliflozin and Teneligliptin

Parameter	Remogliflozin Area	% of Assay	Teneligliptin Area	% of Assay
Acid degradation	1661903	89.92	786512	90.23
Alkali degradation	1716044	92.85	813744	93.35
Peroxide degradation	1740194	94.16	819805	94.05
Thermal degradation	1674459	90.60	780124	89.49
Photo degradation	1699587	91.96	799970	91.77
Hydrolysis degradation	1832981	99.18	861992	98.88

IV.CONCLUSIONS

In this study, a high pressure liquid chromatography method for the simultaneous detection of remogliflozin and teneligliptin in bulk and pharmaceutical dose form was developed. It was quick, cheap, sensitive, and easily accessible. Shorter run times, lower costs, assessibility, sensitivity, and reproducibility are all advantages of this approach. Under conditions of hydrolysis, oxidation, photolysis, and heat stress, the effects of the medications' degradation were investigated. The drugs' stability in thermal hydrolysis was shown to be different from their instability in acidic, alkaline, and oxidizing sress.The HPLC approach was subsequently applied to commercially available formulations after being validated in accordance with ICH guidelines.

V. ACKNOWLEDGMENT

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