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Original Article

A New Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Remogliflozin and Teneligliptin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Aim: To estimate Remogliflozin and Teneligliptin simultaneously in bulk and pharmaceutical dosage forms, including forced degradation studies, a simple, Robust, and Stability-Indicating (RP-H-P-L-C) method has been developed and validated. **Materials and Methods:** Discovery (150 mm × 4.6 mm 5 µm) C18 is used as the phase which is stationary for separation, and 0.01N Dibasic Sodium Phosphate Buffer (Na_2HPO_4) at pH 4.6 and acetonitrile in a 65:35 ratio make up the mobile phase. A maximum wavelength of 210 nm, a rate of flow of 1.0 mL/min, and a temp of 30ºC were all maintained. **Results:** Teneligliptin and Remogliflozin were shown to have average retention durations of 2.282 and 2.684 min, respectively. Teneligliptin and Remogliflozin were found to have Relative Standard Deviations (R-S-D) of 0.8 and 0.7, respectively. Teneligliptin and Remogliflozin both had recovery rates of 99.77% and 99.91%, respectively. Teneligliptin and Remogliflozin's regression equations yielded L.O.D and L.O.Q values of 0.04, 0.13, and 0.30, 0.89, respectively. Remogliflozin's regression equation is y=31865x+22823, but Teneligliptin's is y=40034x + 2044.4. Because the RTs were shortened, the run time was also shortened. This suggests that the created approach is simple and economical, which makes it appropriate for regular quality control testing in industries. **Conclusion:** Because the RTs were shortened, the run time was also shortened. This suggests that the created approach is simple and economical, which makes it appropriate for regular quality control testing in industries.

Keywords: Chromatogram, Remogliflozin, RP-H-P-L-C, Standard Deviation, Teneligliptin.

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders marked by chronically elevated blood glucose levels resulting from impaired insulin secretion, insulin action, or both. As insulin is an anabolic hormone, its dysfunction disrupts the metabolism of carbohydrates, fats, and proteins. These abnormalities arise from insulin resistance or inadequate insulin production, leading to poor glucose uptake in tissues such as the liver, muscles, and adipose tissue (Chatterjee *et al*., 2021).

Type 2 diabetes is commonly associated with metabolic syndrome, which includes obesity, insulin resistance, dyslipidaemia, and hypertension-factors that increase cardiovascular risk. Early and proactive management of these conditions has significantly improved diabetes care, aided by the development of novel antihyperglycemic agents with unique mechanisms of action (Ahsan *et al*., 2021).

A combination of teneligliptin and Remogliflozin etabonate is often prescribed when other antidiabetic drugs fail to achieve adequate glycaemic control. Remogliflozin ($C_{26}H_{38}N_2O_9$) acts as an SGLT2 inhibitor, lowering blood glucose by promoting urinary glucose excretion. Teneligliptin ($C_{22}H_{30}N_6OS$), a DPP-4 inhibitor, increases active GLP-1 and GIP levels, enhancing insulin secretion and reducing glucagon in a glucose-dependent manner. Together, these agents offer an effective dual mechanism for improving glycaemic control in type 2 diabetes patients (Li *et al*., 2021).

Effective separation analytical methods such as High-Performance Liquid Chromatography can be utilized to quantify drug concentration. Numerous RP-H-P-L-C techniques have been documented in the literature18-35 for assessing the dosage forms of Remogliflozin, Teneligliptin, and several other antiretroviral medications, either individually or in combination. In the pursuit of more economical methods through the literature review, there is a necessity to develop and validate a straightforward, cost-effective stability-indicating simultaneous estimation

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of Remogliflozin and Teneligliptin using RP-H-P-L-C in pharmaceutical dosage forms, adhering to ICH guidelines (Q2 specification) (Kasturi J *et al*., 2021).

MATERIALS AND METHODS

Spectrum Pharma Research Aq. sols provided the pure forms of remogliflozin and teneligliptin. Acetonitrile and methanol of HPLC quality were purchased from Rankem Chemical Division in India. Pure milli-Q H_2O was used with the use of 0.45 µ Millipore filters (Rankem, India), and sodium hydrogen phosphate was procured from Rankem, India.

Ethical Statement

All analytical procedures were conducted with integrity and transparency. Certified pure standards of remogliflozin and teneligliptin were obtained from Spectrum Pharma Research Aq. sols, and only high-quality HPLC-grade solvents (acetonitrile and methanol) from Rankem Chemical Division, India, were utilized. Milli-Q water and 0.45 µm Millipore filters were employed to ensure accuracy and reliability, and sodium hydrogen phosphate was sourced responsibly from Rankem, India. All materials were used strictly for research purposes, adhering to standard laboratory safety and ethical guidelines.

Instrumentation and Chromatographic Conditions

For method development and validation, the Waters H-P-L-C system, model 2695, with an automated specimen injector and a photodiode array detector, was employed. A 150 x 4.6 x 5 µm Discovery C18 column was used for the separation procedure. M.P. A was a mobile phase made up of 0.01N Na_2HPO_4, and M.P. B was acetonitrile in a 65:35 ratio. With an injection vol. of 10 µL and a rate of flow 1.0 mL/min, the analysis was carried out in isocratic mode. The entire run period was six min, and the column temp was kept at 30°C. Empower 2 software was used to acquire data at an uncovering wavelength of 210 nm.

Prep of Aq.sols

Diluent: Ratio of 50:50v/v Mixed H_2O and Acetonitrile.

Prep of buffer

Buffer (0.01N Na_2HPO_4 Buffer): Precisely measure 1.41 g of Disodium hydrogen phosphate. In a 1000 mL volumetric flask, add approximately 900 mL of milli-Q H_2O, then degas by sonication. Finally, adjust the vol. with H_2O to achieve a 0.01N Disodium hydrogen phosphate buffer.

Prep of Std. aq. sol: Accurately Weighed and transferred 5 mg of Teneligliptin and 25 mg of Remogliflozin working Std. into 50 mL clean dry volumetric flasks, add 10 mL of diluent, sonicated for 10 min and make up to the final vol. with diluents. (100 µg/mL of Teneligliptin and 500 µg/mL of Remogliflozin).

Prep of Std. Effective aq. sol: A 10 mL volumetric flask was filled with 1 mL of the filtered specimen stock aq.sol, which was then diluted. 50 µg/mL of Remogliflozin and 5 µg/mL of Teneligliptin.

Prep of Specimen stock aq. sol: A 100 mL volumetric flask was filled with a precisely measured equivalent weight of the combination powder sample. 50 mL of diluent were introduced, and the mixture was subjected to sonication for 25 min. The volume was subsequently adjusted with diluent and filtered using HPLC filters (100 µg/mL Teneligliptin and 1000 µg/mL Remogliflozin).

Prep of Std. effective aq. sol: 0.5 mL of stock aqueous filtered specimen Sol. was moved to a 10-mL volumetric flask and diluted. Teneligliptin (5 µg/mL) and Remogliflozin (50 µg/mL).

To show that the HPLC method is suggested for routine analysis, it was validated for the simultaneous measurement of the drug substances Remogliflozin (Figure 1) and Teneligliptin (Figure 2) in accordance with ICH criteria.

System Suitability

Remogliflozin 50 µg/mL and Teneligliptin 5 µg/mL were injected into a system suitability aq. sol to test for system compatibility for each validation parameter.

Specificity

Verifying that the optimised approach is free of interference. When these medications are retained using this approach, we shouldn't see interfering peaks in the blank and placebo. Thus, it was claimed that this approach was specific.

To assess the stability-indicating properties of the H-P-L-C method, Remogliflozin, and Teneligliptin, specimens were exposed to base, acid, oxidation, heat, light and H_2O stress. Deteriorating specimens were analysed using a photodiode-array detector.

The data showed that despite the specimens being subjected to acid, base, hydrolysis, heat, light, and H_2O, no degradation was seen. No degradants co-eluted with the dynamic medication peaks, according to the stress investigation (Panigrahy *et al*., 2015).

Limit of Quantification and Limit of Detection

The detection limit is the extremely low quantity of an analyte in a specimen that is detectable but not always quantifiable. The L-O-Q has the lowest conc. of an analyte in a specimen that can be detected with a method's acceptable accuracy and precision (Prashanthi *et al*., 2023).

Linearity

Linearity for Remogliflozin and Teneligliptin was evaluated by analyzing solutions at concentrations ranging from 25% to 150% of the target level (Table 1). The calibration curves showed good

linearity with correlation coefficients (R2) of 0.999 for both Remogliflozin and Teneligliptin, indicating excellent agreement between concentration and peak area response.

Recovery

Aq. Sol. containing spiked Remogliflozin and Teneligliptin specimens at 50%, 100%, and 150% of the working strength were used to assess the method's accuracy. Every aq. Sol. was made three times and examined.

System Precision

To ascertain the system precision, six injections which were replicated of standard aq. sol at 100% of the limit which is specified with respect to the working strength of the Remogliflozin and teneligliptin were examined.

Method Precision

The std. deviation of an observational population is one of the most often used statistical terms. When the number of outcomes in the set is less than one, the std. deviation is calculated by taking the square root of the sum of squares of the deviations of each individual result for the mean. The std. deviation S can be found using:

$$S = \sqrt{\frac{\sum_{i=1}^{n}(x - x')^2}{(n-1)}}$$

The units of the std. deviation match those of the property being measured.

Variance (S2) is the square of the std. deviation. The std. deviation stated as a fraction of the mean, or S/x, is known as the relative std. deviation. It is sometimes stated as a percentage of the relative std. deviation and multiplied by 100. It becomes a more trustworthy way to represent the accuracy. % Std. Deviation Relative equals to examining a Remogliflozin and Teneligliptin specimen, the method's accuracy was established. (Six separate specimen preparations).

Intermediate Precision

Intermediate precision (Table 2) differs from repeatability, as it refers to the precision achieved within a single laboratory over an extended duration (typically several months or more) and considers a greater number of variables than repeatability. Specifically, this includes different analysts, calibration standards, reagent batches, columns, spray needles, and so forth. These elements remain constant throughout a single day (i.e., they exhibit systematic behavior on a daily timescale) but are not stable over a longer timeframe, thus acting as random factors in the context of intermediate precision.

Robustness

The chromatographic settings intentionally changed in order to estimate the resilience of the existing process. To confirm the technique's capability, system suitability aq. sol is arranged in accordance with the methodology and introduced into HPLC under several modified conditions, such as R.O.F. (10%), column oven temp (5°C), and M.P. (10%) from actual method settings.

RESULTS

System Suitability

Figure 3 displayed the system suitability chromatogram, and Table 3 lists the values.

Specificity

Table 4 presents experimental data. According to the chromatogram above, no interference was seen in the blank or placebo aq. Sol. During the retention periods of Remogliflozin and teneligliptin.

The peak purity test was conducted and passed by remogliflozin and teneligliptin. The conditions for forced degradation are listed

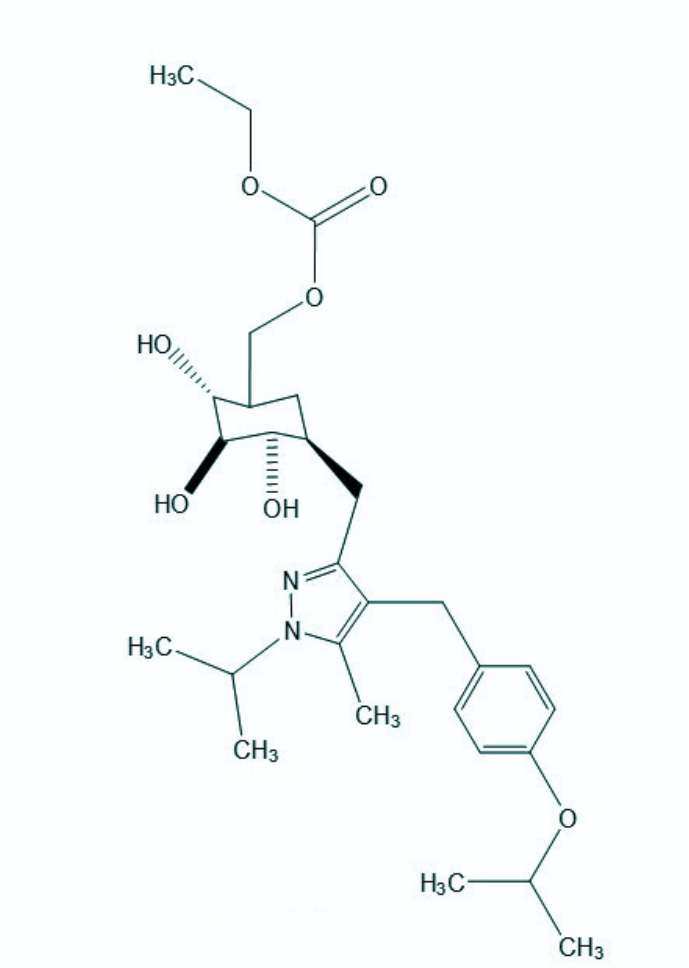

Figure 1: Remogliflozin.

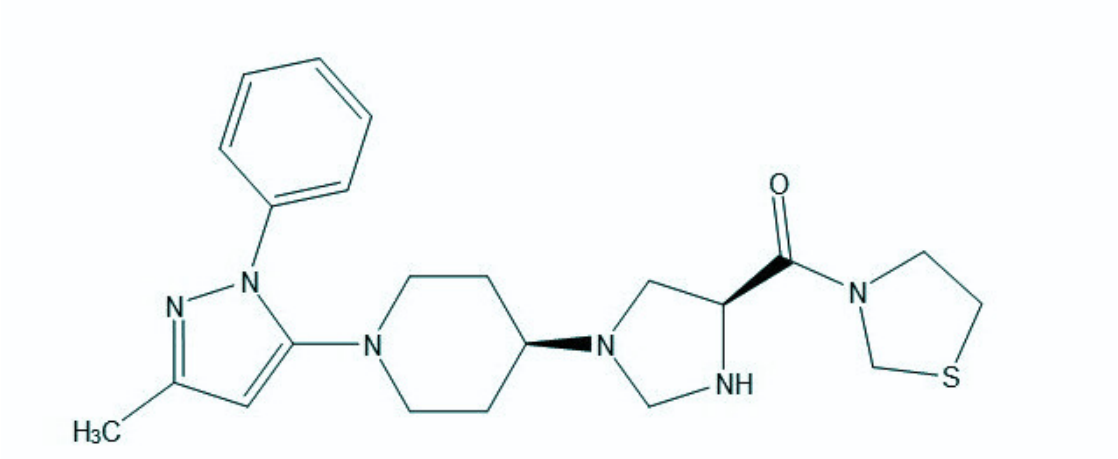

Figure 2: Teneligliptin.

Table 1: Linearity data.

% Level	Remogliflozin		Teneligliptin	
	Conc (µg/mL)	Area	Conc (µg/mL)	Area
25%	12.5	418605	1.25	51826
50%	25	823205	2.5	105308
75%	37.5	1254969	3.75	152041
100%	50	1623740	5	202500
125%	62.5	2021886	6.25	252937
150%	75	2381855	7.5	300593

Table 2: Intermediate precision data.

Injection	Remogliflozin	Teneligliptin
1	1661743	206606
2	1649679	202157
3	1678328	201211
4	1698803	203085
5	1692192	205832
6	1694647	203962
Avg	1679232	203809
Std. dev	19842.7	2094.9
% RSD	1.2	1.0

in Table 5 and the Degradation profile results are listed in Table 6. The Peak purity plots were plotted in the Figures 4-6.

Limit of Quantification and Limit of Detection

The L-O-D readings for Remogliflozin and Teneligliptin are listed in Table 7, and the relevant example chromatogram is shown in Figures 7 and 8.

Linearity

The linearity plots are presented in Figures 9-10.

Recovery

Table 8 showed the % recovery outcomes for each contaminant.

System Precision

The peak area results are summarized in Table 9. After six replicate injections of std. aq. sol, the percentage R-S-D for the peak areas of remogliflozin and teneligliptin was within the acceptable range.

Method Precision

Table 10: A summary of the data provides and collected.

Based on aforementioned findings, method precision study's percentage R-S-D for remogliflozin and teneligliptin was within the acceptable range.

Intermediate Precision

Due to the inclusion of more influencing factors, the value of intermediate precision, represented as standard deviation (as discussed in the following section), is greater than that of the repeatability standard deviation.

Robustness

This is done in order to assess the method's robustness. The protocol is followed and no noticeable changes are observed when flow, temp, M.P., and system suitability are altered. The robustness

Table 3: System suitability table.

Remogliflozin			Teneligliptin			
RT (min)	TP	Tailing	RT (min)	TP	Tailing	RS
2.274	5827	1.49	2.673	7100	1.37	3.2
2.275	5745	1.48	2.675	7080	1.38	3.2
2.281	5689	1.41	2.689	7059	1.37	3.2
2.282	5858	1.42	2.706	7065	1.36	3.3
2.285	6287	1.44	2.709	7029	1.39	3.3
2.287	6151	1.49	2.715	7033	1.36	3.3

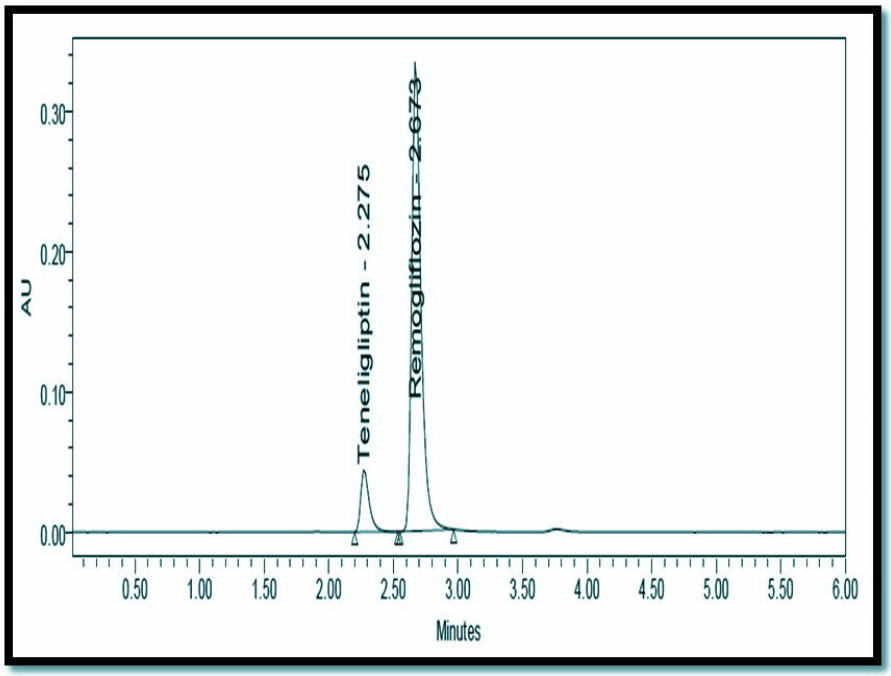

Figure 3: System Suitability Chromatogram of Remogliflozin and Teneligliptin.

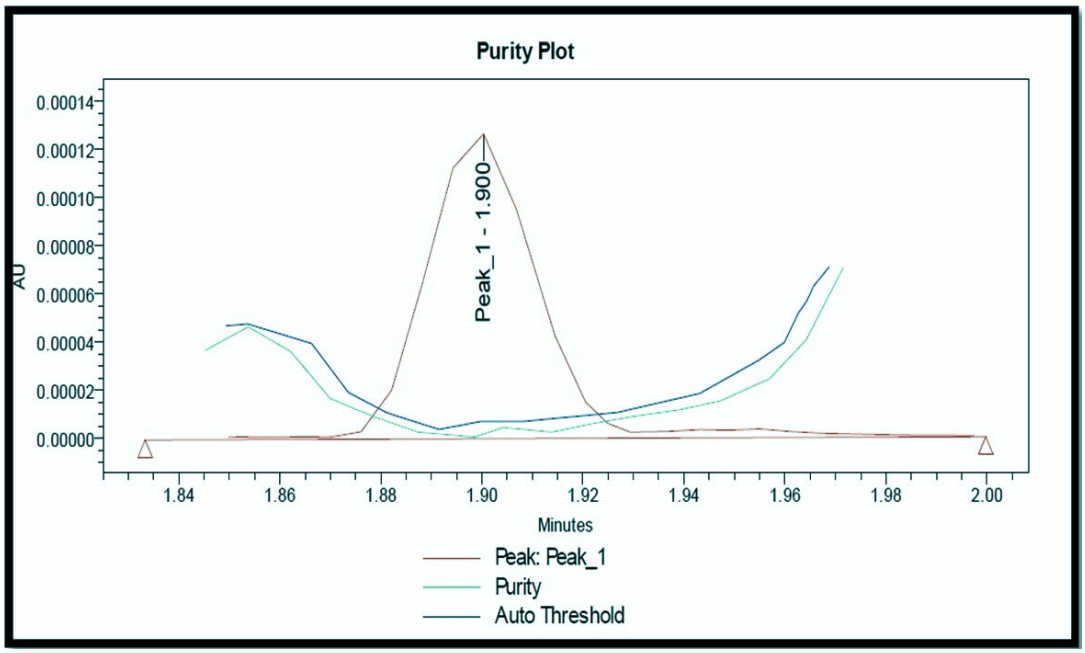

Figure 4: Purity Plots of forced Degradation studies (Acid).

results are summarized in Table 11. The robustness analysis's conclusions align with the standards for system suitability.

DISCUSSION

The validated RP-HPLC method demonstrated strong analytical performance, and the results clearly support its suitability for simultaneous estimation of Remogliflozin and Teneligliptin. The low % RSD values (0.7% for Remogliflozin and 0.8% for Teneligliptin) indicate excellent repeatability and fall well within the ICH acceptance limit of <2%, confirming the method's high precision. Recovery values close to 100% further affirm its accuracy and lack of interference. The sensitivity achieved-reflected by the low LOD and LOQ values-is comparable to or better than several methods previously reported in the literature (References 18-35), demonstrating the method's capability to detect both analytes at very low concentrations. Additionally, the short retention times (2.28 min and 2.68 min) significantly improve run-time efficiency compared to earlier published procedures, many of which report longer chromatographic windows. This reduction in analysis time enhances throughput and minimizes solvent consumption, aligning with the goal of developing an economical, routine QC-friendly method. Overall, the results collectively show that the proposed method not only meets ICH validation criteria but also offers improved simplicity, rapidity, and efficiency over existing reported methods.

Table 4: **Specificity data.**

Name	RT (min)
Remogliflozin	2.200
Teneligliptin	2.700

Statistical Analysis

Precision (Repeatability)

The % RSD values for both drugs indicates excellent precision. Since the acceptance criterion for method precision is typically % RSD <2%, the results confirm that the method is highly precise.

Teneligliptin: %RSD=0.8%.

Remogliflozin: %RSD=0.7%.

Accuracy or Recovery Studies

Recovery values close to 100% show good accuracy. These values fall within the acceptable ICH range of 98-102%, indicating that the method is accurate and free from interference.

Table 5: **Forced degradation conditions for Remogliflozin and Teneligliptin.**

Stress condition	Solvent	Temp (°C)	Exposed time
Acid	2N HCL	60°C	30 min
Base	2N NAOH	60°C	30 min
Oxidation	20% H_2O_2	60°C	30 min
Thermal	Diluent	105°C	6 hr
Photolytic	Diluent	-	-
Hydrolytic	Water	60°C	

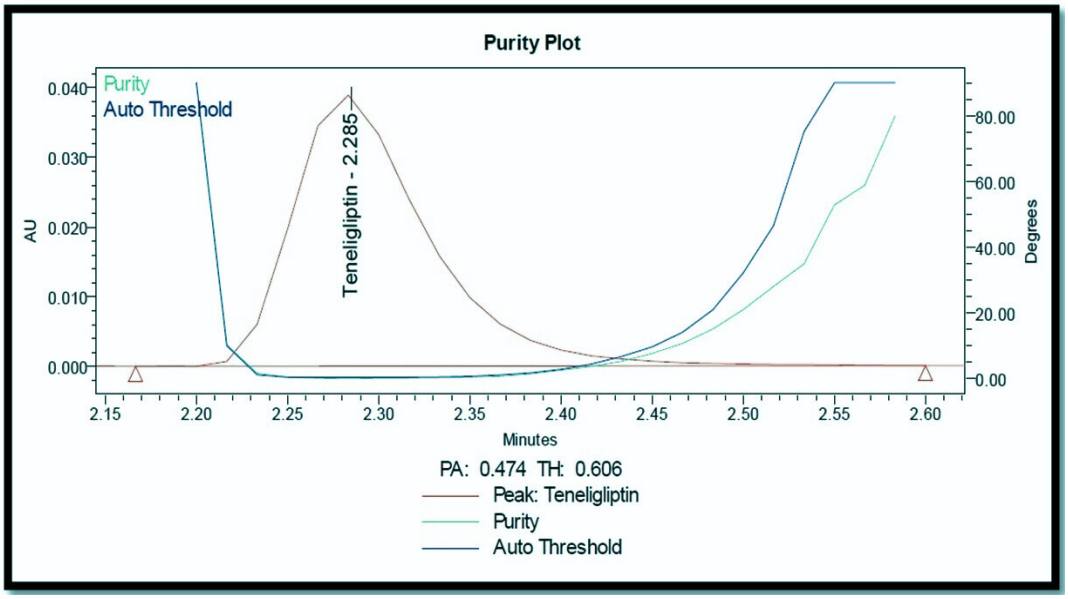

Figure 5: Purity Plots of forced Degradation studies (Acid).

Table 6: Degradation profile results.

Degradation condition	Remogliflozin % v Undegraded	Teneligliptin % Undegraded
Acid	95.68	94.59
Base	93.50	93.95
Oxidation	98.55	97.71
Thermal	97.93	97.91
Photolytic	98.80	98.96
Hydrolytic	99.35	99.88

Table 7: Summary of limit of Quantification andlimit of Detection.

Specimen	LOQ		LOD	
	Peak area	S/N Ratio	Peak area	S/N Ratio
Remogliflozin	88071	21.6	57863	7.0
Teneligliptin	34388	36.5	37293	9.4

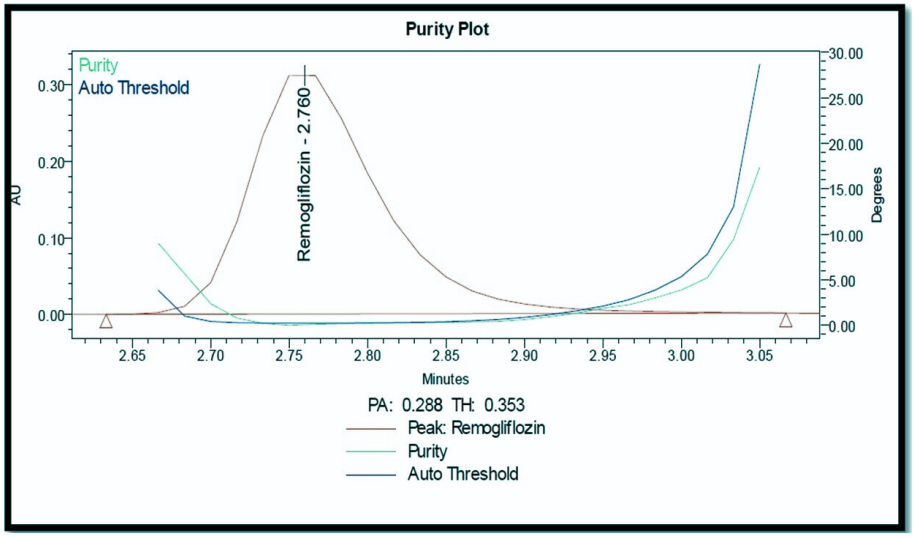

Figure 6: Purity Plots of forced Degradation studies (Acid).

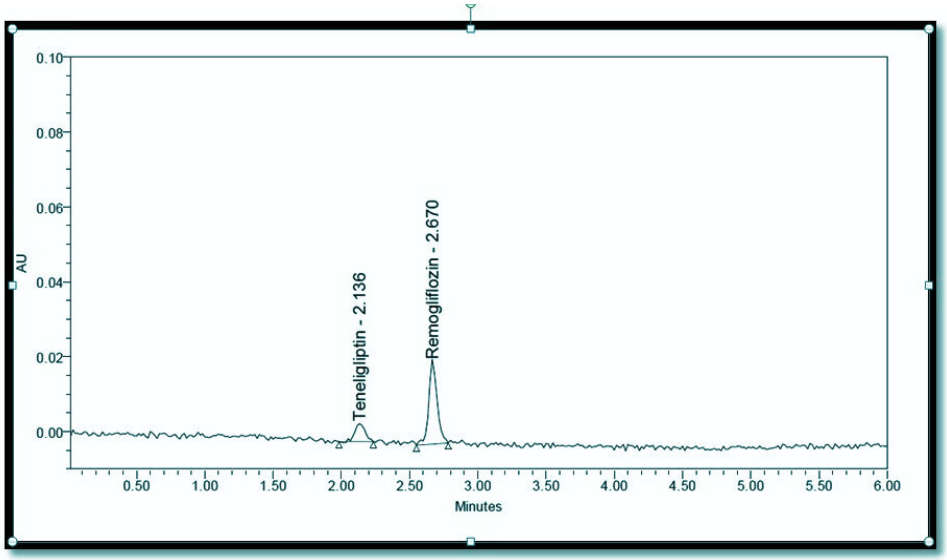

Figure 7: A standard depiction of the HPLC chromatogram for the LOQ solution. According to the results mentioned above, the signal-to-noise ratio for each component fell within the acceptable limits.

Table 8: % Recovery data.

% Level	% Recovery	
50% Level	Remogliflozin	Teneligliptin
	100.36	99.65
	99.05	100.09
	99.22	100.73
100% Level	99.09	99.12
	99.66	99.05
	100.07	99.67
150% Level	100.76	100.68
	100.46	99.62
	100.53	99.33
Mean %	99.91	99.77

Table 9: System precision data.

Injection	Remogliflozin	Teneligliptin
1	1652641	204085
2	1649535	203538
3	1648980	205156
4	1640960	203833
5	1622602	205450
6	1653186	200837
Avg	1644651	203817
Std. dev	11653.9	1642.6
% RSD	0.7	0.8

Table 10: Method precision data.

Injection	Remogliflozin	Teneligliptin
1	1631527	203084
2	1651800	204718
3	1642528	205060
4	1635365	204109
5	1633111	202375
6	1640378	204653
Avg	1639118	204000
Std. dev	7507.9	1054.2
%RSD	0.5	0.5

Table 11: Robustness results.

Chromatographic condition	Remogliflozin (RSD)	Teneligliptin (RSD)
Flow (-)	0.5	0.9
Flow (+)	1.0	1.1
Temp (-)	1.2	1
Temp (+)	0.5	0.5
Mobile phase (-)	0.6	0.6
Mobile phase (+)	0.8	1.2

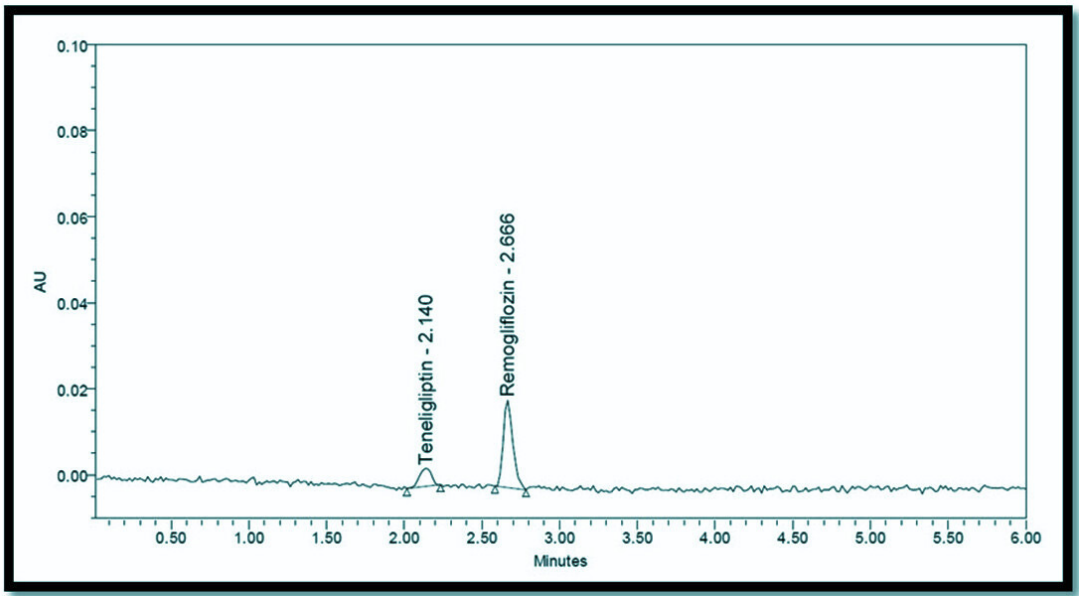

Figure 8: A standard depiction of the HPLC chromatogram for the LOD solution. According to the results presented above for LOD, the signal-to-noise ratio for each component remained within the acceptable limits.

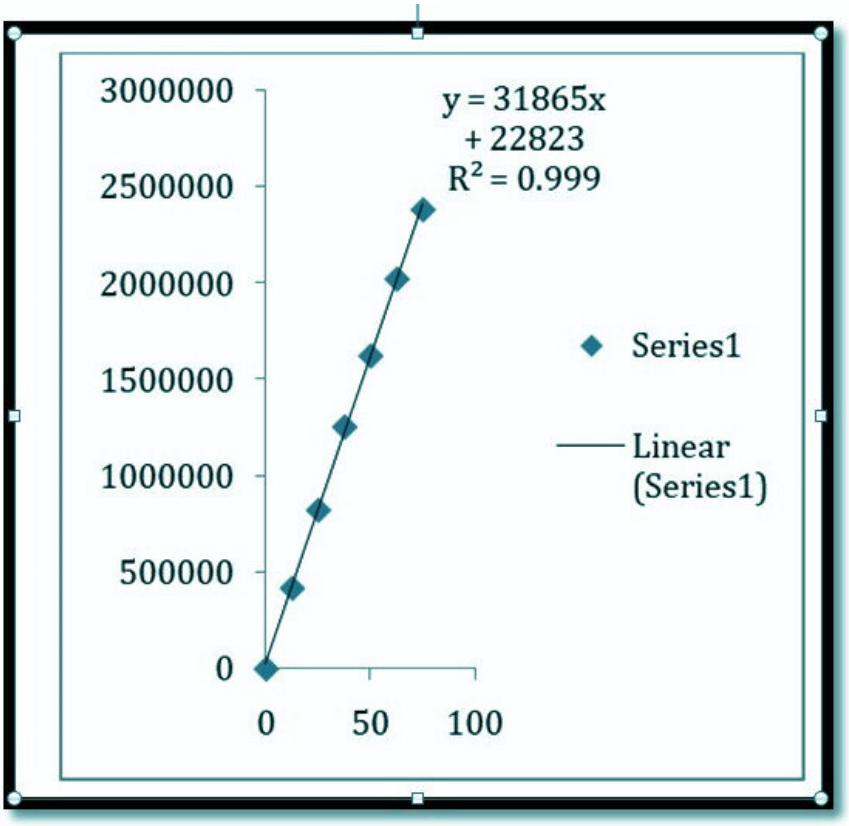

Figure 9: Linearity plot of Remogliflozin.

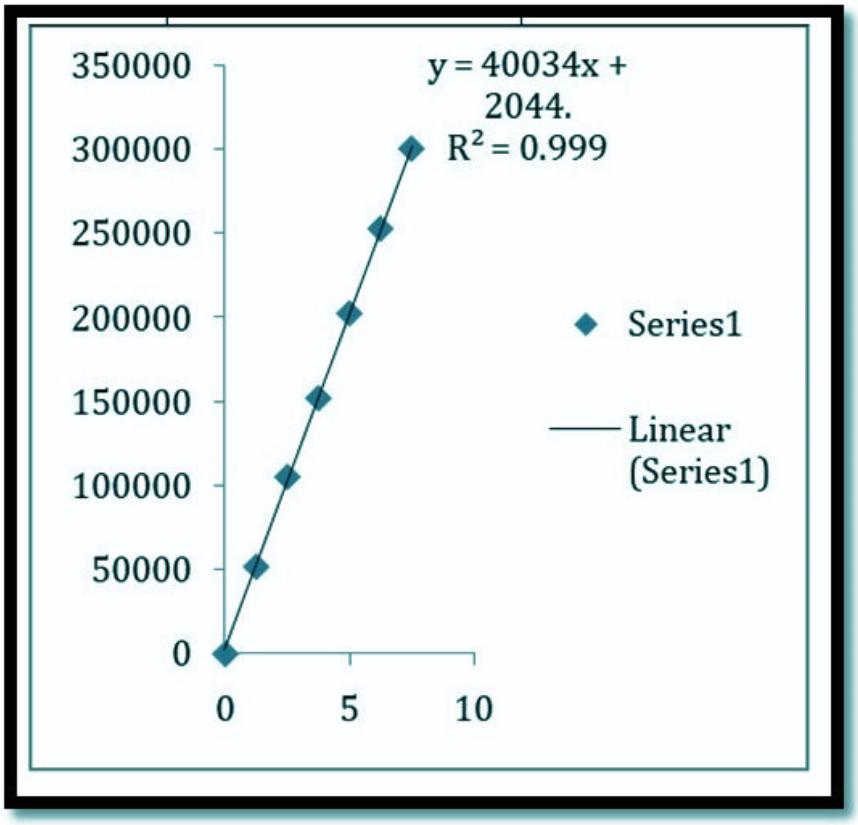

Figure 10: Linearity plot of Teneligliptin.

Teneligliptin: 99.77%.

Remogliflozin: 99.91%.

Sensitivity (LOD and LOQ)

The method demonstrates good sensitivity, evidenced by low LOD and LOQ values. These low values indicate the method can detect and quantify even small amounts of each drug.

LOD

Teneligliptin: 0.04 µg/mL.

Remogliflozin: 0.30 µg/mL.

LOQ

Teneligliptin: 0.13 µg/mL.

Remogliflozin: 0.89 µg/mL.

Linearity

The regression equations demonstrate strong linear relationships between concentration and peak area. The high slope values and consistent intercepts suggest excellent linearity, suitable for quantification over the tested range.

- **Teneligliptin**

$$y=40034x + 2044.4$$

- **Remogliflozin**

$$y=31865x + 22823$$

Retention Time and Method Efficiency

The short retention times contribute to a reduced run time, increasing throughput and making the method cost-effective and suitable for routine QC analysis.

Teneligliptin RT: 2.282 min.

Remogliflozin RT: 2.684 min.

CONCLUSION

The present study successfully developed and validated a robust, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of Remogliflozin and Teneligliptin in bulk and pharmaceutical dosage forms. The chromatographic separation was efficiently achieved using a Discovery C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase comprising 0.01N sodium phosphate dibasic buffer (pH 4.6) and acetonitrile in the ratio of 65:35 v/v. Optimal detection at 210 nm, along with a flow rate of 1.0 mL/min and column temperature of 30°C, provided sharp, symmetrical peaks with average retention times of 2.282 min for Teneligliptin and 2.684 min for Remogliflozin.

The method demonstrated excellent system suitability, with RSD values of 0.8% for Teneligliptin and 0.7% for Remogliflozin, indicating high precision. Accuracy was confirmed through recovery studies, yielding values of 99.77% and 99.91% for Teneligliptin and Remogliflozin, respectively. Sensitivity of the method was established through LOD and LOQ values of 0.04 µg/mL and 0.13 µg/mL for Teneligliptin, and 0.30 µg/mL and 0.89 µg/mL for Remogliflozin. The regression equations y=40034x + 2044.4 for Teneligliptin and y=31865x + 22823 for Remogliflozin-confirmed excellent linearity across the tested concentration ranges.

Forced degradation studies demonstrated that the method effectively separated the parent drugs from their degradants, confirming its stability-indicating capability. Additionally, the reduced retention times significantly shortened the overall run time, enhancing method efficiency and reducing analytical costs.

Overall, the proposed RP-HPLC method is simple, rapid, cost-effective, and highly reliable for routine quality control analysis of Remogliflozin and Teneligliptin in pharmaceutical formulations. Its precision, accuracy, sensitivity, and degradation-separation efficiency make it a valuable analytical tool for industrial and regulatory applications.

ACKNOWLEDGEMENT

Appreciation to Spectrum Pharma Research Solutions for generously supplying the pure samples of Remogliflozin and Teneligliptin utilized in this study. Their assistance was crucial for the successful execution of this research.

ABBREVIATIONS

M.P: Mobile Phase; **R-O-F:** Rate of flow; **Prep:** Preparation; **Max:** Maximum; **and:** And; **L-O-D:** Limit of Detection; **L-O-Q:** Limit of Quantification; **Fig.:** Figure; **H_2O:** Water; **Aq. Sol:** Solution; **RT:** Retention Time; **RP-H-P-L-C:** Reverse Phase High-Performance Liquid Chromatography; **H-P-L-C:** High-Performance Liquid Chromatography; **R-S-D:** Relative Standard Deviation; **N:** Normality (as in 0.01 N); **ICH:** International Council for Harmonisation; **PDA:** Photodiode Array; **S/N Ratio:** Signal-to-Noise Ratio; **C18:** Octadecyl carbon chain; **TP:** Theoretical Plates; **RS:** Resolution; **Std dev:** Standard Deviation; **HCl:** Hydrochloric Acid; **NaOH:** Sodium Hydroxide; **H_2O_2:** Hydrogen Peroxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Badupula Sravan Kumar: Conceptualization of the study, method development, experimental work, data analysis, and drafting of the manuscript.

Roopa Redamala: Supervision, validation of analytical methodology, critical review of results, and revision of the manuscript for intellectual content.

Both authors read and approved the final version of the manuscript.

SUMMARY

This investigation aimed to validate RP-HPLC method has been developed and validated for the simultaneous estimation of Remogliflozin and Teneligliptin in bulk and pharmaceutical dosage forms, including forced degradation studies. The stationary phase utilized for separation is Discovery (150 mm × 4.6 mm 5 µm) C18, while the mobile phase consists of 0.01N Dibasic Sodium Phosphate Buffer (Na_2HPO_4) at pH 4.6 and acetonitrile in a 65:35 ratio. The method was conducted at a maximum wavelength of 210 nm, with a flow rate of 1.0 mL/min and a temperature of 30°C. The average retention times for Teneligliptin and Remogliflozin were determined to be 2.282 and 2.684 min, respectively. The Relative Standard Deviations (RSD) for Teneligliptin and Remogliflozin were found to be 0.8 and 0.7, respectively. The recovery rates for Teneligliptin and Remogliflozin were 99.77% and 99.91%, respectively. The regression equations for Teneligliptin and Remogliflozin provided LOD and LOQ values of 0.04, 0.13, and 0.30, 0.89, respectively. The regression equation for Remogliflozin is y=31865x + 22823, while for Teneligliptin it is y=40034x + 2044.4. The reduction in retention times also led to a decrease in run time, indicating that the developed method is both simple and cost-effective, making it suitable for routine quality control testing in the industry.

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