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SIMPLE COST-EFFECTIVE METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF REMOGLIFLOZIN ETABONATE AND VILDAGLIPTIN IN COMBINED DOSAGE FORM

Manish Kumar Kesharwani[1], B.K Dubey[1], Vivek Singh Thakur[1], Deepak Kumar Basedia[1], Prabhat Kumar Jain[2], Sunil Shah[2]

[1]Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)
[2]TIT- College of Pharmacy, Bhopal (M.P.)

Abstract:
A simple, rapid, and cost-effective high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous estimation of Remogliflozin Etabonate (RGE) and Vildagliptin (VGT) in combined tablet dosage form. Chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of 20 mM KH_2PO_4:acetonitrile (20:80, v/v) at a flow rate of 1.0 mL/min and detection at 254 nm. The method demonstrated good linearity over the concentration ranges of 2–10 µg/mL for RGE and 1–5 µg/mL for VGT, with correlation coefficients (r^2) of 0.999. Accuracy was confirmed through recovery studies (96.92–99.26% for RGE and 98.48–99.03% for VGT), while precision was verified by intra-day, inter-day, and analyst-to-analyst studies (%RSD <1.2%). The method was robust, sensitive (LOD 0.10 µg/mL for RGE and 0.15 µg/mL for VGT), and suitable for routine quality control analysis. The validated method was successfully applied to marketed tablet formulations, providing reliable quantification of both drugs.

Keywords: *Remogliflozin Etabonate, Vildagliptin, HPLC, Simultaneous Estimation, Method Validation, Linearity, Accuracy, Precision, Robustness, Tablet Formulation*

Corresponding author:
Manish Kumar Kesharwani,
Technocrats Institute of Technology-Pharmacy,
Bhopal (M.P.)
Mail id: pradeepkumarv376@gmail.com

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INTRODUCTION:

Diabetes mellitus, especially type 2 diabetes mellitus (T2DM), is a chronic metabolic disorder with a rapidly increasing global prevalence that leads to long-term complications including cardiovascular diseases, nephropathy, and neuropathy [1]. Combination therapy has become a cornerstone in diabetes management to achieve optimal glycemic control due to the complementary mechanisms of action offered by drug classes targeting different physiological pathways [2].

Remogliflozin etabonate is a selective inhibitor of the sodium-glucose co-transporter-2 (SGLT2) that reduces glucose reabsorption in the kidney, resulting in increased urinary glucose excretion and reduced blood glucose levels in diabetic patients [3]. This prodrug and its active form have shown efficacy and safety in clinical evaluations of diabetes management, with beneficial effects on plasma glucose and minimal hypoglycemic risk [4]. Vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, improves glycemic control by enhancing incretin hormone activity, increasing insulin secretion and suppressing glucagon release in a glucose-dependent manner, thereby complementing insulin action [5]. The fixed-dose combination of remogliflozin etabonate and vildagliptin provides a synergistic approach for improved glycemic regulation and patient compliance through dual mechanisms targeting renal glucose handling and incretin enhancement [6].

Analytical methods for the estimation of remogliflozin etabonate and vildagliptin have been reported using a variety of techniques, including stability-indicating high-performance thin-layer chromatography (HPTLC) methods for simultaneous estimation of both drugs with validation according to ICH guidelines [7] Reverse phase high-performance liquid chromatography (RP-HPLC) and ultra-performance liquid chromatography (UPLC) methods have also been developed for simultaneous quantification in bulk drug and formulations, demonstrating good resolution, precision, and robustness of analysis [8]. Moreover, spectrophotometric and HPLC-PDA techniques have been successfully applied for simultaneous estimation in fixed-dose tablet formulations, providing reliable methods for routine analysis [9].

Despite these significant advancements, many existing methods involve complex mobile phases, high costs, or sophisticated instrumentation that may not be practical for routine quality control settings. Therefore, there remains a need for a simple, cost-effective, precise, and stability-indicating RP-HPLC method for simultaneous estimation of remogliflozin etabonate and vildagliptin in combined dosage forms that adheres to the International Council for Harmonisation (ICH) guidelines for method validation. The present study aims to fulfill this need by developing and validating an economical and efficient RP-HPLC method suitable for routine quality assurance and stability evaluation of this combination therapy.

MATERIAL AND METHODS:

Material

The study utilized pure drug samples of Remogliflozin Etabonate (RGE) and Vildagliptin (VGT), along with their marketed tablet formulation. HPLC-grade solvents including acetonitrile, methanol, and 20 mM potassium dihydrogen phosphate were used as mobile phase and diluent. Analytical grade water was used throughout the study. Standard laboratory glassware, 0.45 µm membrane filters, and a C18 HPLC column (250 mm × 4.6 mm, 5 µm particle size) were employed for method development and validation. All chemicals and solvents were of analytical or HPLC grade to ensure accurate and reliable results.

Methods:

Selection of Mobile Phase

Several mobile phase compositions in different ratios were initially tested to optimize the estimation of Remogliflozin Etabonate (RGE) and Vildagliptin (VGT) in a fixed-dose combination. System suitability parameters such as retention time, tailing factor, number of theoretical plates, and HETP were considered for optimization. The most suitable mobile phase was found to be Acetonitrile: Methanol in a 50:50 v/v ratio. The mobile phase was filtered through a 0.45 µm membrane to remove particulate matter and degassed by sonication before use. A flow rate of 1.0 mL/min was employed throughout the analysis.

Selection of Diluent

The diluent used for sample preparation was compatible with the mobile phase and did not significantly affect the retention or resolution of the analytes. After various trials, the mobile phase itself was selected as the diluent for all sample preparations.

Preparation of Stock and Sub-Stock Solutions

Accurately weighed 10 mg of RGE and VGT were transferred into separate 10 mL volumetric flasks. Approximately 5 mL of mobile phase was added, and the solutions were sonicated for 20 minutes to ensure complete dissolution. The volumes were then made up to 10 mL with mobile phase to obtain stock solutions of 1000 µg/mL (Stock-A).

From Stock-A, 5 mL of each drug solution was pipetted into separate 50 mL volumetric flasks and

diluted to volume with mobile phase to prepare sub-stock solutions of 100 µg/mL (Stock-B).

Preparation of Working Solutions

From Stock-B, aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 mL were transferred to separate 10 mL volumetric flasks and diluted with mobile phase to prepare RGE solutions of 2, 4, 6, 8, and 10 µg/mL, respectively. Similarly, VGT solutions of 1, 2, 3, 4, and 5 µg/mL were prepared using the same procedure.

Linearity and Calibration

Linearity of the analytical method was established by preparing solutions in the concentration ranges of 2–10 µg/mL for RGE and 1–5 µg/mL for VGT. All solutions were filtered through a 0.45 µm membrane and injected into the HPLC system. Chromatograms were recorded at 254 nm, and each concentration was analyzed in quintuplicate. Calibration curves were plotted between mean peak area and respective concentration, and regression equations were derived to assess linearity.

System Suitability

System suitability parameters were evaluated by allowing the mobile phase to saturate the column at a flow rate of 1 mL/min. Six replicates of the working standard solutions (10 mg/mL for RGE and 5 mg/mL for VGT) were injected, and peak reports along with column performance parameters were recorded to ensure consistent resolution, retention time, and peak symmetry.

Validation of Developed Method [10]

Linearity: Linearity was assessed by analyzing five different concentrations of each drug within their respective ranges. Peak areas were recorded in triplicate, and mean values were used to calculate regression equations and correlation coefficients. The response ratio (response factor) was determined by dividing the area under the curve by the corresponding concentration.

Specificity: The method was evaluated for specificity to ensure that the analytes were accurately quantified in the presence of potential impurities, degradation products, and matrix components.

Accuracy: Accuracy was determined using recovery studies at 80%, 100%, and 120% levels by spiking known concentrations of standard drugs into pre-analyzed samples. The percentage recovery was calculated to confirm the reliability of the method.

Precision: Precision was evaluated at three levels:

Repeatability (Intra-day precision): Five replicates at five concentrations (5–25 µg/mL for RGE and 1–5 µg/mL for VGT) were analyzed under the same operating conditions over a short interval.

Intermediate Precision (Inter-day): Variability within the laboratory was assessed by performing the analysis on different days and by different analysts.

Analyst-to-Analyst Precision: The method was further validated by analyzing samples by two analysts and comparing the results.

Robustness: Deliberate small variations in mobile phase composition were applied to assess the method's ability to remain unaffected. The ratio of 20 mM KH_2PO_4: Acetonitrile was varied from 80:20 to 85:15 v/v.

Sensitivity: The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve.

Analysis of Tablet Formulation

The content of RGE and VGT in tablets was determined by weighing an amount equivalent to 10 mg of VGT, dissolving it in 10 mL of mobile phase, sonicating for 15 minutes, and centrifuging at 300 rpm. One milliliter of this solution was further diluted to 10 mL with mobile phase. The resulting solutions were injected into the HPLC system, and the peak areas were used to calculate the drug content using the regression equations. The procedure was repeated six times to ensure accuracy and reproducibility.

RESULTS AND DISCUSSION:

A simple, precise, and cost-effective HPLC method was developed for the simultaneous estimation of Remogliflozin Etabonate (RGE) and Vildagliptin (VGT) in combined dosage forms. The chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase composed of 20 mM KH_2PO_4 and acetonitrile (20:80, v/v), at a flow rate of 1.0 mL/min. The method provided sharp, well-resolved peaks with retention times of 3.454 ± 0.002 min for RGE and 5.617 ± 0.001 min for VGT, indicating efficient separation and minimal interference from excipients.

The method showed excellent linearity over the concentration ranges of 2–10 µg/mL for RGE and 1–5 µg/mL for VGT, with correlation coefficients (r^2) of 0.999 for both drugs. The response ratios exhibited low %RSD values (1.059 for RGE and 2.451 for VGT), confirming reproducibility of the detector response. System suitability parameters, including theoretical plates, tailing factor, and retention time, were within acceptable limits, indicating the robustness and reliability of the method.

Recovery studies demonstrated the method's accuracy, with mean recovery values ranging from 96.92% to 99.26% for RGE and 98.48% to 99.03% for VGT. The %RSD of recovery was below 1.2% for both drugs, confirming the precision of the assay. Intra-day (repeatability), inter-day (intermediate precision), and analyst-to-analyst variations showed %RSD values below 1.2%,

highlighting the method's excellent precision and reproducibility. Deliberate variations in the mobile phase composition during robustness studies resulted in minimal changes in retention times and peak areas, confirming the method's robustness.

The sensitivity of the method was demonstrated by low LOD and LOQ values: 0.10 µg/mL and 0.35 µg/mL for RGE, and 0.15 µg/mL and 0.40 µg/mL for VGT, respectively, which allows accurate detection and quantification even at trace levels. The assay of the marketed tablet formulation showed that the method could reliably quantify both drugs in combined dosage forms, with %assay values of 99.85% for RGE and 99.5% for VGT and %RSD < 0.23%, confirming its applicability for routine quality control.

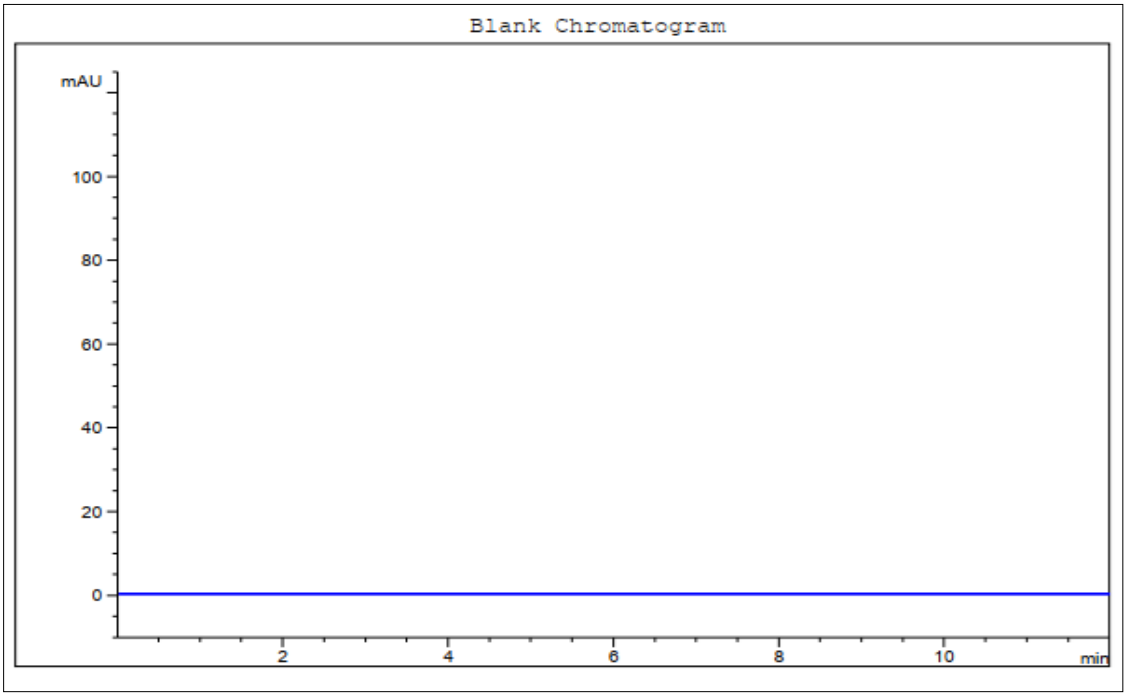

Figure 1: Chromatogram of Blank

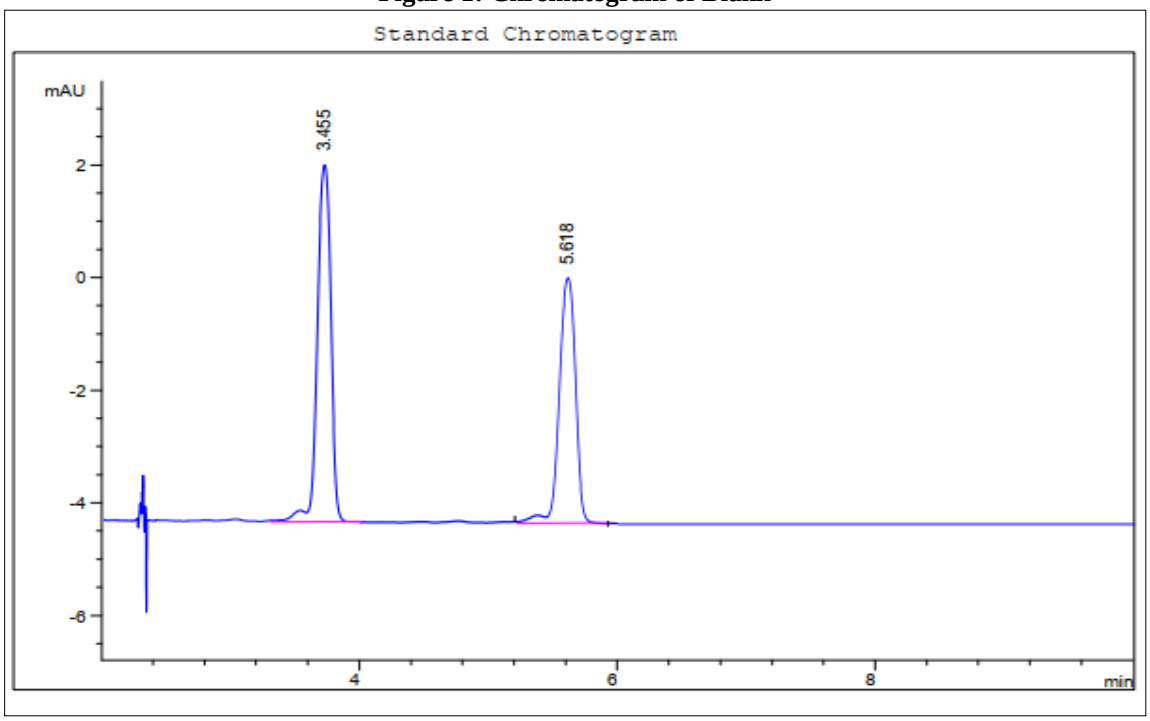

Figure 2: Chromatogram of Both the drug

Table 1: Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
20mM KH_2PO_4: Acetonitrile	20
Acetonitrile	80
Diluent	Mobile Phase
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	254nm
Retention time	
Remogliflozin etabonate	3.454± 0.002min.
Vildagliptin	5.617± 0.001min.

Table 2: Results of Method Validation Parameters for RGE and VGT

Parameter	RGE	VGT
Linearity Range (µg/mL)	2–10	1–5
Regression Equation	AUC = 1113 × C + 12.15	AUC = 1422 × C + 8.348
Correlation Coefficient (r^2)	0.999	0.999
Mean Response Ratio	1116.96	1420.065
%RSD of Response Ratio	1.059	2.451
System Suitability	RT: 3.4545 min AUC: 11151.178 Theoretical plates: 2704.83 Tailing factor: 1.05	RT: 5.617 min AUC: 7058.002 Theoretical plates: 2584 Tailing factor: 1.155
Recovery (%)	80%: 99.26 100%: 96.92 120%: 99.07	80%: 99.03 100%: 98.48 120%: 98.89
%RSD of Recovery	0.265–1.182	0.243–0.973
Repeatability (Intra-day)	1.97–9.88 µg/mL %RSD 0.078–0.152	0.97–4.88 µg/mL %RSD 0.085–0.143
Intermediate Precision (Day-to-Day)	1.96–9.78 µg/mL %RSD 0.131	0.98–4.79 µg/mL %RSD 0.094
Analyst-to-Analyst Precision	1.92–9.885 µg/mL %RSD 0.101	0.97–4.715 µg/mL %RSD 0.080
Robustness	1.914–9.97 µg/mL %RSD 0.112–1.034	0.97–4.918 µg/mL %RSD 0.055–0.150

Table 3: LOD and LOQ of RGE and VGT

Name	LOD (µg/ml)	LOQ (µg/ml)
RGE	0.10	0.35
VGT	0.15	0.40

Table 4: Result of assay of tablet formulation

	RGE*	VGT*
Label Claim (mg)	**100mg**	**50mg**
% Found (mg)	99.85	49.75
% Assay	99.85	99.5
% RSD	0.225	0.115

*Average of three determination

CONCLUSION:

A simple, rapid, and cost-effective HPLC method was successfully developed and validated for the simultaneous estimation of Remogliflozin Etabonate and Vildagliptin in combined tablet dosage form. The method demonstrated good linearity, accuracy, precision, robustness, and sensitivity**,** making it suitable for routine quality control analysis of marketed formulations.

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