

Central Composite Design Method Development, Optimization, and Validation of Metformin, Vildagliptin, and Remogliflozin by Rp-Hplc Using an Analytical Quality by Design Approach

Praveen Manapragada and Lakshmi Bavisetti*

*Department of Chemistry, GSS, GITAM (Deemed to be) University, Visakhapatnam, AP, India, -530045

pmanapra@gitam.in

Correspondence Author: baviset@gitam.edu

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ABSTRACT

Type 2 diabetes is treated with metformin, vildagliptin, and remogliflozin. The present study intended to develop, optimise, and verify an RP-HPLC technique for simultaneous quantification of metformin, vildagliptin, and remogliflozin utilising an analytical quality by design (AQbD) methodology. This approach develops and uses a cost-effective HPLC method for validation. In order to optimise the key method conditions based on chromatographic responses, such as retention time, resolution, and theoretical plate count, a central composite design was utilised to methodically examine the impact of specific chromatographic variables. Buffer and acetonitrile were employed in a 60:40 v/v ratio in an Inertsil C18 250 × 4.6 mm, 5 m column at a flow rate of 1 ml/min at 30 °C. The wavelength was detected at nm. Metformin, Vildagliptin, and Remogliflozin were shown to have retention times of 2.196 minutes, 2.621 minutes, and 3.060 minutes, respectively. With resolution values of 2.9 and 3.0 and hypothetical plate counts of 4667.1, 5847.4, and 4690.9 for metformin, vildagliptin, and remogliflozin, respectively, the optimised technique successfully separated the three analytes. The system precision %RSD was determined to be 0.8%, 0.7%, and 0.7%, respectively. The method demonstrated satisfactory linearity, precision, accuracy, and robustness over the investigated analytical ranges. The LOD and LOQ values were 0.07 and 0.21 µg/mL for vildagliptin, 0.30 and 0.92 µg/mL for metformin, and 0.14 and 0.41 µg/mL for remogliflozin, respectively. Forced degradation studies demonstrated limited degradation under the applied stress conditions, supporting the applicability of the method for stability assessment. The applicability of the optimised settings was further validated by the strong agreement between the expected and experimentally observed chromatographic results. All things considered, the AQbD-driven RP-HPLC approach offers a simple, affordable, accurate, sensitive, and reliable analytical process for the simultaneous measurement of metformin, vildagliptin, and remogliflozin. It may be used for regular quality control of their combination pharmaceutical formulations.

Keywords: Metformin, Remogliflozin, Vildagliptin, RP-HPLC, QbD

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INTRODUCTION

The decrease of patient mortality and morbidity is the aim of Type-II (DM2) management. Early, tailored blood glucose control without hypoglycemic episodes must be attained by pharmaceutical therapy, lifestyle changes, and relevant diabetes education [1]. Along with the right detection and therapy of the disease's consequences, which include the typical microvascular ones, like retinopathy and nephropathy, worldwide control of cardiovascular (CV) risk factors is also crucial [2]. Unlike prior recommendations, this article follows a different approach by offering a helpful text that highlights a number of distinct and often controversial aspects of Type II diabetes mellitus (DM2) [3]. PPAR γ agonists, which enhance the action of insulin; biguanides, which decrease the production of glucose in the liver; α -glucosidase

inhibitors, which prevent the stomach from absorbing glucose; and sulfonylureas, which enhance the release of insulin from pancreatic islets, were the main drug classes used to treat hyperglycemia in the past.2 For T2DM patients, the combined treatment of Vildagliptin, Remogliflozin, and Metformin is an efficient multi-mechanism method to enhance glycemic control [4].

The combination of the multifactorial character of the combination of the disease therapy, the multifactor pathophysiological therapy. The biguanide metformin mainly reduces hepatic glucose production and increases insulin sensitivity; the dipeptidyl peptidase-4 (DPP-4) inhibitor vildagliptin increases incretin-mediated insulin secretion and inhibits glucagon release in a glucose-dependent manner [5]; and the sodium-glucose cotransporter-2 (SGLT2) inhibitor remogliflozin etabonate

*Author for Correspondence: baviset@gitam.edu

increases urine glucose excretion by decreasing renal glucose reabsorption. Thus, the combination provides complementary mechanisms for controlling hyperglycaemia and can be incorporated into multidrug treatment strategies when additional glycaemic control is required [6].

Remogliflozin Etabonate lowers blood sugar levels and excretes excess sugar in the urine, which helps diabetics maintain glycaemic control [7]. Vildagliptin reduces the amount of glucose the liver makes by raising insulin levels and decreasing glucagon levels, a hormone that raises blood glucose [8]. Metformin limits the quantity of glucose absorbed from food and reduces the amount of glucose generated by the liver. Metformin also improves the body's response to insulin [9].

The simultaneous inclusion of several active pharmaceutical compounds into a pharmaceutical dosage form presents a significant analytical challenge, despite the fact that these chemicals have different pharmacological processes. Accurate measurement and simultaneous chromatographic separation may be challenging due to the significant differences in their physicochemical characteristics, polarity, chromatographic retention behavior, and dose levels. Consequently, an analytical procedure for such a multicomponent formulation should provide adequate selectivity and resolution while maintaining acceptable peak symmetry, efficiency, precision, accuracy, and robustness [10]. By successfully reducing HbA1c levels, postprandial plasma glucose (PPG), and fasting plasma glucose (FPG), this combo drug offers comprehensive glycaemic control. Experts have discovered that this medication combination helps the kidneys and heart while also improving glycaemic management. Additionally, the complementary mechanisms of action lower the risk of hypoglycemia and weight gain while improving glycaemic control.

The combination of these medications may be highly beneficial for patients with type 2 diabetes who are obese, have kidney disease, or are at high risk of cardiovascular events. The overall effectiveness of treatment is enhanced by the glycaemic benefits of vildagliptin and metformin as well as the ability of empagliflozin to reduce blood pressure and body weight.

[11].

The development of a reliable analytical procedure is therefore essential for quality control of such fixed-dose combination formulations, particularly for assay determination and routine batch-to-batch quality assessment. Conventional chromatographic method development frequently relies on experimentation one-factor-at-a-time, in which chromatographic variables are optimized independently [12]. Such an approach may require numerous experiments and, importantly, may fail to adequately identify interactions among chromatographic variables. In contrast, a design of experiments (DoE)-based approach enables simultaneous investigation of multiple analytical method variables and their interactions,

thereby providing a more systematic and statistically justified route to method optimization [13].

HPLC and HILIC-based methods for the simultaneous measurement of metformin, vildagliptin, and remogliflozin have been documented in earlier analytical studies. Response-surface methodology is applicable for simultaneous determination of these three analytes using an HILIC method that uses a face-centered central composite design. There are more recent studies that have been published. However, the continued development of robust RP-HPLC procedures using a clearly defined analytical quality by design (AQbD) framework remains relevant, particularly when the analytical procedure is developed by systematically defining the analytical target profile, identifying critical method variables and critical method attributes, modelling their relationships, and establishing an appropriate analytical design space [11-13].

Analytical Quality by Design provides a science- and risk-based framework for analytical procedure development in which method performance is considered during the entire development process rather than being evaluated only after optimization [14]. Prior knowledge, risk assessment, multivariate experiments, modelling, the identification of critical analytical methodology, and the formation of a method operable design area or analytical control strategy may all be integrated into an enhanced analytical procedure method of development in compliance with the principles outlined in ICH Q14. The Procedure is the final proof that it can be validated [15]. Because it can assess the linear, interaction, and quadratic impacts of certain analytical method variables within a predetermined experimental domain, central composite design (CCD), a response-surface design, is very helpful for chromatographic optimization [16]. Such data are useful for determining an optimum region rather than just a single experimental condition and for understanding how variations in chromatographic conditions affect simultaneous separation performance. Therefore, combining CCD with AQbD principles can offer a more empirically effective and scientifically sound approach for creating a reliable RP-HPLC procedure. A systematic RP-HPLC development strategy that selects critical analytical variables based on risk assessment, quantitatively optimizes them using CCD, and then confirms them through model-based prediction and experimental verification is still required, even though reported chromatographic procedures for this drug combination are available. During routine pharmaceutical quality control, this strategy can improve technique comprehension and boost confidence in method robustness.

Thus, utilizing an AQbD-based methodology, the current work was conducted to design, optimize, and verify a simultaneous RP-HPLC technique for the measurement of metformin, vildagliptin, and remogliflozin. The specific goals were as follows: (i) define the analytical target profile and identify potential critical method parameters

and critical method performance attributes; (ii) use a central composite design to methodically investigate the effects and interactions of specific chromatographic variables; (iii) create mathematical response-surface models and determine the optimal chromatographic conditions/analytical design space; (iv) verify the predicted optimum conditions experimentally; and (v) validate the optimized method for pertinent analytical performance characteristics in compliance with current ICH recommendations.

For the simultaneous measurement of the three antidiabetic drugs in pharmaceutical formulations, the established process is meant to offer a quick, accurate, precise, robust, and selective analytical method. It is anticipated that the systematic AQBd framework will improve technique comprehension, lessen reliance on empirical trial-and-error experimentation, and facilitate dependable routine quality control of multicomponent T2DM compositions.

MATERIALS AND METHODS

Chemicals and reagents

Vildagliptin, Metformin, and Remogliflozin. Methanol, Acetonitrile, ortho-phosphoric acid (OPA), HPLC water.

Instrumentation

Testing was carried out utilising a Waters Acquity HPLC 2695 SYSTEM with an auto-injector and Acquity TUV detector with Empower 3 software. The absorbance of solutions containing metformin, vildagliptin, and

remogliflozin was measured using a UV-VIS spectrophotometer (PG Instruments T60) equipped with a matched quartz cell and a specific bandwidth of 2mm and 10mm. a

Chromatographic Conditions

An Inertsil C18 column (4.6 mm × 250 mm, 5 µm particle size) kept at 30°C was used to perform chromatographic separation. Buffer and acetonitrile (ACN) in a 60:40 (v/v) ratio made up the mobile phase. The detection was done at 211 nm, and the flow rate was kept at 1 mL/min. The injection volume was 20 µL.

METHODS

Central Composite Design (CCD)

CCD was employed for the systematic optimization of the Metformin, Vildagliptin, and Remogliflozin Metformin simultaneous estimation using the RP-HPLC technique Organic phase, flow rate, and column temperature were used as independent variables (**Table 1**), which were adjusted across three levels: high, medium, and low. Various ranges of four parameters, specifically 51.6-68.4%, 0.1% OPA, column temperatures of 24.95 °C and 35.05 °C, and flow rates of 0.73-1.07ml/min, were utilised. Subsequently, contour and 3D surface plots illustrating the impact of each parameter on retention time, resolution, and theoretical plates were produced. A desirability function was utilised under optimised conditions to forecast retention time, resolution, and theoretical plates.

Table 1: Coded levels of independent variables

Factor	Level (-1)	Level (0)	Level (+1)
Flow rate (ml/min)	0.8	0.9	1.0
Organic Phase	55	60	65
Temperature	27	30	33

PREPARATION OF SOLUTIONS

Preparation of diluent

MeCN and HPLC water were mixed together at a ratio of 50:50 and used as a diluent.

Preparation of Buffer (0.01N KH₂PO₄)

Potassium dihydrogen phosphate 1.36 gm was collected in a 1000 mL volumetric flask, and 900 mL of Milli-Q water was incorporated and sonicated for 5- 10 min to degas; finally, a volume was made up to the mark with water after sonication, then a subsequent addition of 1 mL of trimethylamine, and the pH was changed to 3.5 with OPA.

Standard stock solutions

After weighing and transferring 125 mg of metformin, 12.5 mg of vildagliptin, and 25 mg of remogliflozin into 3 separate 50 mL volumetric flasks, 10 mL of diluent was then added to each, and the containers were kept under sonication before being filled with diluent (MeCN along with water in a 50:50 ratio). They were then designated as standard stock solutions 1, 2, and 3. They contained 250 ppm of vildagliptin, 500 ppm of remogliflozin, and 2500 ppm of metformin. In a 10 mL volumetric flask, one milliliter of each standard stock solution was pipetted out,

incorporated, and combined with diluent. Metformin, Vildagliptin, and Remogliflozin had final concentrations of 250 ppm, 25 ppm, and 50 ppm, respectively.

Sample stock solutions

The five tablets were ground into a powder after being weighed. A 100 mL volumetric flask was filled with the weight of one pill. After adding the diluent to 3/4 of the flask, the medications were stirred for 50 minutes until they were fully dissolved. The mixture was filled to the volume mark, purified, and transferred to a volumetric flask. The concentrations of metformin, vildagliptin, and remogliflozin were 5000, 500, and 1000 parts per million, respectively. Vildagliptin, Remogliflozin, and Metformin final concentrations of 25, 50, and 250 ppm were obtained by extracting a 0.5 mL solution from the filtered solution, transferring it to a 10 mL volumetric flask, and diluting it.

Method Validation

Method validation was undertaken using ICH Q2(R2) recommendations to test specificity and system appropriateness, linearity, precision, medium-to-high precision, robustness, and solution stability [17].

System Suitability

Standard solutions of metformin (5000 ppm), vildagliptin (500 ppm), and remogliflozin (1000 ppm) were prepared in order to evaluate the system suitability characteristics. Peak tailing, resolution, and USP plate count were measured after the solutions were injected six times. The percentage RSD should always be less than 2.0%. Each chromatogram contained the acceptance criteria for standards in system suitability.

Specificity

To assess interference at each drug's retention period, chromatograms of blank, standard, and sample were compared. Specificity was confirmed by the absence of baseline aberrations or co-eluting peaks. The absence of matrix interference is consistent with results from previous anti-diabetic stability-indicating studies [18].

Precision

The above-prepared sample stock solutions were injected six times for each sample, and the percentage RSD was examined; in order to satisfy the acceptance criteria, it should not exceed 2%.

Linearity

Linearity was evaluated by preparing six concentration levels in the range of (25, 50, 75, 100, 125, and 150%). Peak area versus concentration was plotted to create calibration curves after each concentration was injected three times. The least squares method was used to compute the regression equations, and correlation coefficients (R^2) were generated to evaluate linearity [19].

Accuracy

Sample stock solutions comprising Vildagliptin, Remogliflozin, and Metformin at levels of 25, 50, and 250 ppm, respectively, were employed, and the investigation was completed by recovery evaluation. The % recovery for each level should be 98.0 and 102.

Robustness

By carefully adjusting chromatographic parameters such as temperature, mobile phase ratio, and flow rate, robustness was evaluated. We assessed whether these modifications affected the tailing factor, peak area, and retention time. To ascertain technique dependability under various circumstances, the %RSD values were computed [20]. All metrics were within the acceptable range as defined by ICH recommendations, and there was no significant difference in drug elution.

Forced Degradation study

Studies on forced degradation were conducted in acidic, basic, neutral, reactive, oxidative, photolytic, and dry heat degradation environments. The percentage of degradation and peak purity were tested to verify the stability-indicating character of the devised RP-HPLC method [21]. Below is a summary of the sample preparation details.

Oxidation

One millilitre of 20% H₂O₂ and one millilitre of the metformin, vildagliptin, and remogliflozin stock solutions were added separately. For thirty minutes, the solutions were kept at 60°C. The final solution was diluted to yield (250 ppm, 25 ppm, and 50 ppm) solutions for the HPLC analysis. After injecting the 10 µg/mL sample, the chromatogram was produced.

Acid Degradation Studies

Separately, 1 mL of 2N HCl and 1 mL of the stock solutions of Metformin, Vildagliptin, and Remogliflozin were added. For thirty minutes, the solutions were refluxed at 60°C. The final solution was then diluted to yield (250 ppm, 25 ppm, and 50 ppm) solutions to conduct the HPLC analysis. After injecting the 10 µg/mL sample, the chromatogram was produced.

Alkaline degradation

1 mL of 2N NaOH and 1 mL of Metformin, Vildagliptin, and Remogliflozin stock solutions were each added individually. For thirty minutes, the solutions were kept at 60°C. The ultimate diluted (250, 25, 50 ppm respectively). The chromatogram was created by injecting a 10 µg/mL sample.

Dry heat deminutesgradation

The normal pharmaceutical solution was roasted at 105°C for six hours to investigate dry heat degradation. After diluting the sample to (250 ppm, 25 ppm, and 50 ppm) solutions, 10 µL was injected into the system to evaluate its stability for HPLC analysis. Following that, the chromatograms were recorded.

Photostability

By exposing the (5000 ppm, 500 ppm, and 1000 ppm) solutions to ultraviolet light and keeping the beaker in a UV chamber for seven days, or 200 watt-hours per square metre in a photostability lab, the drug's photochemical stability was further examined. An HPLC was used to dilute 10 µL of the resultant solution into solutions with concentrations of 250 ppm, 25 ppm, and 50 ppm in order to assess the stability of the sample. Following that, chromatograms were recorded.

Neutral degradation

Drug stress testing was conducted in neutral conditions by refluxing the drug in 60°C water for six hours. After diluting the solution to 250, 25, and 50 ppm concentrations, 10 µL was injected into the HPLC system to test the sample's stability. Following that, chromatograms were recorded.

RESULTS AND DISCUSSION

Optimized Chromatogram

The developed method for Remogliflozin, Metformin, and Vildagliptin showed well-resolved peaks, and they eluted at 2.196, 2.621, and 3.060 minutes, respectively (**Figure 1**). The USP plate count and TF were highly satisfactory, leading to the optimisation and validation.

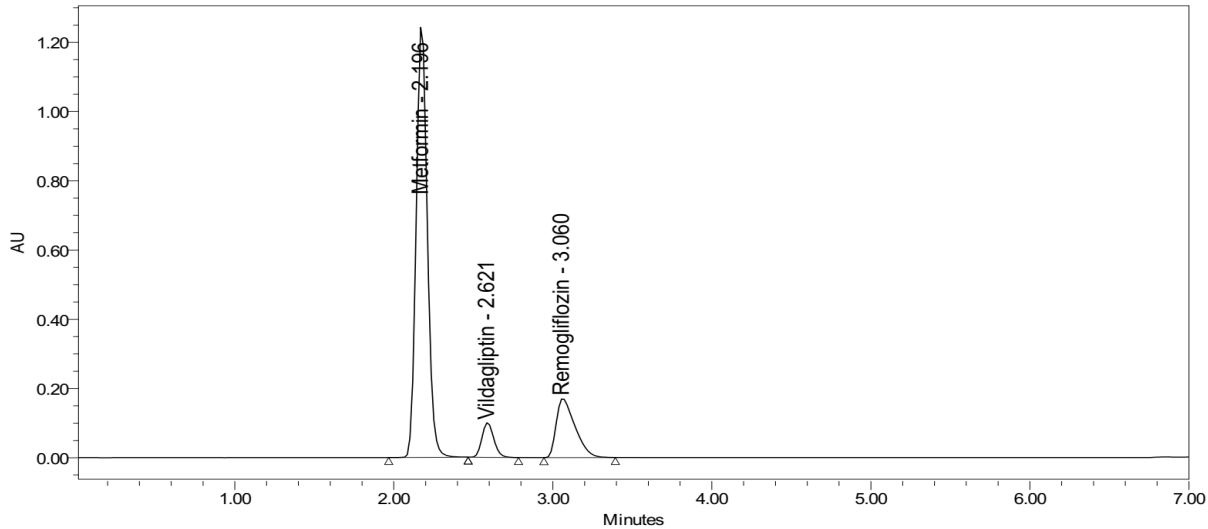


Figure 1: Optimized Chromatogram

Linearity

Linearity is one important factor for the validity of an analytical method. The detector response was shown to be linear in the concentration range of Metformin, Vildagliptin, and Remogliflozin; peak areas were measured. The calibration curves of Metformin, Vildagliptin, and Remogliflozin are shown in Figures 2-4, respectively, and calibration data are listed in Table 2. This shows that the analytical tool's response is exactly

proportionate to the analyte's concentration within a given range. The developed RP-HPLC technique for three drugs was tested for linearity in the concentration range as mentioned in Table 2. Over the entire range under investigation, there was good linearity between the concentration and the chromatographic result. The linear increase in the average peak area demonstrated that the analyte concentration was exactly proportionate to the detector response.

Table 2: Calibration data of Metformin, Vildagliptin and Remogliflozin

	Metformin		Vildagliptin		Remogliflozin	
s.no	Conc (µg/mL)	area	Conc (µg/mL)	area	Conc (µg/mL)	area
1.	62.5	397936	6.25	42619	12.5	117744
2.	125	814930	12.5	84347	25	233974
3.	187.5	1224814	18.75	125644	37.5	348228
4.	250	1606474	25	169615	50	464377
5.	312.5	2011935	31.25	211274	62.5	584284
6.	375	2427089	37.5	251207	75	692800
Conc (µg/mL)	62.5-375		6.25-37.5		12.5-75	
Regression Equation	$y = 6457.6x + 1081.9$		$y = 6721.1x + 363.57$		$y = 9262.5x + 1427.9$	
R ²	0.999		0.999		0.999	

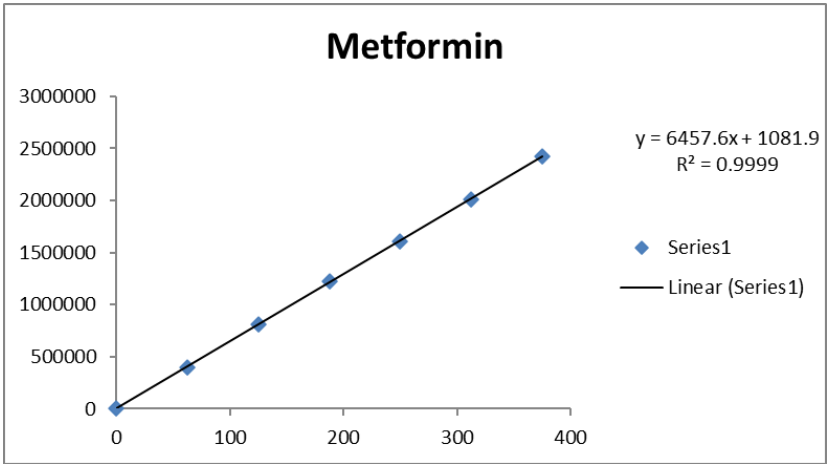


Figure 2: Calibration curve of Metformin

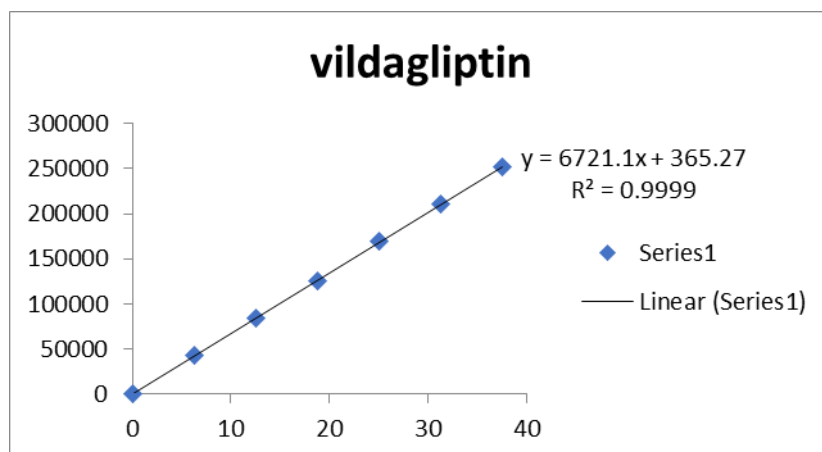


Figure 3: Calibration curve of Vildagliptin

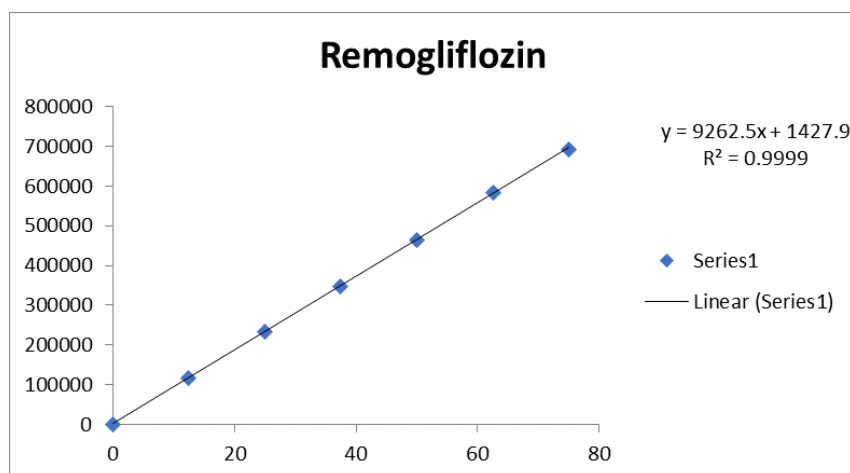


Figure 4: Calibration curve of Remogliflozin

Precision

Six replicate samples of metformin, vildagliptin, and remogliflozin under the same chromatographic circumstances were analysed to assess the system precision of the developed RP-HPLC method. The mean results collected are reported in Table 3.

The corresponding %RSD values were 0.8% for metformin, 0.7% for vildagliptin, and 0.7% for remogliflozin, demonstrating low variability among replicate measurements. Table 4 summarises the good results of the intra- and inter-day precision investigation.

For intra-day precision, the equivalent %RSD values were found to be 0.3% for metformin, 0.7% for vildagliptin, and 0.2% for remogliflozin, demonstrating low variability among replicate measurements. Similarly, for interday precision, % RSD of 0.2 % for Metformin, 0.4% for vildagliptin, and 0.4 % for Remogliflozin was observed. These findings validate the chromatographic system's consistency for the simultaneous measurement of all three analytes by showing that the devised RP-HPLC method has acceptable system precision and satisfactory repeatability.

Table 3: System Precision of Metformin, Vildagliptin and Remogliflozin

s.no	Metformin	Vildagliptin	Remogliflozin
1.	1648511	170888	465558
2.	1644355	168378	462758
3.	1660352	168407	467704
4.	1656944	167834	469335
5.	1622019	170487	461245
6.	1644403	169409	462535
Avg	1646097	169234	464856
Std dev	13508.1	1242.0	3209.2
%RSD	0.8	0.7	0.7

Table 4: Intraday precision and interday precision

s.no	Intra-day Precision			Inter-day Precision		
	Metformin	Vildagliptin	Remogliflozin	Metformin	Vildagliptin	Remogliflozin
1.	1639511	170843	462708	1617366	170166	464702
2.	1638476	168093	463207	1622219	169873	464798
3.	1644403	170599	463511	1619715	168214	460169
4.	1634612	168657	464901	1625258	169315	463262
5.	1638208	170130	465121	1625503	168630	461342
6.	1646100	170461	463077	1618649	169011	464470
Avg	1640218	169797	463754	1621452	169202	463124
Std dev	4268.8	1139.5	1009.5	3436.7	739.2	1950.9
%RSD	0.3	0.7	0.2	0.2	0.4	0.4

Accuracy:

The accuracy of the devised RP-HPLC method was verified using recovery experiments at three concentration levels: 50%, 100%, and 150% of the target concentration. At each level, three samples were examined, and the % recovery was determined. According to Table 5, excellent recoveries were obtained at each level. A good agreement between the amount of Metformin, Vildagliptin, and Remogliflozin added and recovered by the analytical method was demonstrated by the individual sample

recovery values, which ranged from 99.59% to 99.91%. The findings demonstrate that the method can accurately measure metformin, vildagliptin, and remogliflozin over a wide range of concentrations without interference from formulation excipients or analytical variations. The recoveries validated the accuracy, reliability, and suitability of the established RP-HPLC technique for routine quantitative measurement of Metformin, Vildagliptin, and Remogliflozin in standard pharmaceutical dosage forms.

Table 5: Accuracy of Metformin, Vildagliptin, and Remogliflozin

% Level	Metformin			Vildagliptin			Remogliflozin		
	Amount taken (µg/mL)	recovered (µg/mL)	% Recovery	Amount taken (µg/mL)	recovered (µg/mL)	% Recovery	Amount taken (µg/mL)	recovered (µg/mL)	% Recovery
50%	125	125.71	100.56	12.5	12.52	100.20	25	24.94	99.76
	125	125.43	100.34	12.5	12.48	99.80	25	24.81	99.23
	125	124.58	99.66	12.5	12.43	99.48	25	24.90	99.62
100%	250	249.29	99.72	25	24.86	99.46	50	49.61	99.22
	250	251.09	100.44	25	24.78	99.12	50	49.43	98.85
	250	249.85	99.94	25	24.83	99.31	50	49.79	99.58
150%	375	372.28	99.27	37.5	37.68	100.49	75	75.31	100.41
	375	373.44	99.58	37.5	37.25	99.35	75	74.89	99.85
	375	373.81	99.68	37.5	37.42	99.80	75	74.83	99.78
% recovery	99.91%			99.67%			99.59		

Limits of detection and quantification

The sensitivity of the new RP-HPLC method was determined by computing the LOD and LOQ using S/N ratio measurements. The LOD and LOQ results demonstrated the high sensitivity of the developed RP-HPLC method for the simultaneous determination of metformin, remogliflozin, and vildagliptin. The LOD values were 0.30, 0.14, and 0.07 µg/mL, while the corresponding LOQ values were 0.92, 0.41, and 0.21

µg/mL for metformin, remogliflozin, and vildagliptin, respectively (**Table 6**). The chromatographic response at these concentrations showed that the method could accurately identify low amounts of vildagliptin, remogliflozin, and metformin. The results show that the developed RP-HPLC method is sensitive enough to identify and quantify metformin, remogliflozin, and vildagliptin, and that it may be successfully applied for routine quality control and stability studies where low-level concentration analysis is required.

Table 6: LO-D and LOQ of Metformin, Vildagliptin and Remogliflozin

Molecule	LOD	LOQ
Metformin	0.30 µg/ml	0.92 µg/ml
Remogliflozin	0.14 µg/ml	0.41 µg/ml
Vildagliptin	0.07 µg/ml	0.21 µg/ml

Robustness

By purposefully varying the flow rate, column temperature, and mobile-phase composition around the optimised values, the robustness study parameters for this proposed RP-HPLC method were established. For metformin, vildagliptin, and remogliflozin, these intentional modifications very slightly altered peak area, RT, theoretical plate count, and tailing factor (Table 7). Variation in flow rate from 0.9 to 1.1 mL/min resulted in predictable changes in RT, although the tailing factors remained within an acceptable range (about 1.08–1.39). Similarly, chromatographic performance remained

unchanged when column temperature was changed from 27 to 33°C and mobile-phase composition was changed from 55:45 to 65:35. The peak responses exhibited comparatively little fluctuation across the conditions under investigation, and the theoretical plate counts remained sufficient. Overall, the small, intentional changes in the chosen method parameters do not significantly impact the developed method, indicating its robustness and suitability for routine simultaneous determination of metformin, vildagliptin, and remogliflozin. This is demonstrated by the limited changes found in the evaluated chromatographic parameters.

Table 7: Robustness study data

Conditions	Optimized method	Condition applied	Area	RT	Plate Count	Tailing factor
Flow rate (±0.1ml/min)	1	Metformin				
		0.9	1661024	2.501	5023	1.10
		1.0	1648511	2.183	4651	1.09
		1.1	1580264	2.085	4291	1.08
		Vildagliptin				
		0.9	169866	2.970	7864	1.15
		1.0	170888	2.587	5886	1.12
		1.1	163685	2.468	6190	1.13
		Remogliflozin				
		0.9	466476	3.573	6339	1.39
		1.0	465558	3.134	4691	1.34
		1.1	462049	3.031	5704	1.31
Column temp. (±30c)	30°C	Metformin				
		27°C	1669884	2.212	5025	1.10
		30°C	1644355	2.185	4633	1.10
		33°C	1535696	2.312	4998	1.07
		Vildagliptin				
		27°C	169174	2.784	6667	1.12
		30°C	168378	2.588	5854	1.13
		33°C	164880	2.681	6714	1.13
		Remogliflozin				
		27°C	468684	3.466	5903	1.36
		30°C	462758	3.136	4686	1.32
		33°C	460526	3.234	5948	1.32
Mobile phase Composition (5%v/v)	60:40	Metformin				
		55:45	1675905	2.121	5040	1.14
		60:40	1660352	2.185	4629	1.10
		65:35	1586782	2.300	5197	1.09
		Vildagliptin				
		55:45	170339	2.671	7132	1.14
		60:40	168407	2.588	5861	1.14
		65:35	168194	2.624	6804	1.15
		Remogliflozin				
		55:45	469941	3.402	5830	1.40
		60:40	467704	3.137	4660	1.33
		65:35	469679	3.132	6076	1.29

Forced Degradation Studies

The stability-indicating efficacy of the developed RP-HPLC method was assessed using forced degradation

studies under a variety of stress conditions, including acidic, alkaline, NaOH, thermal, photolytic, and humidity stress. Degliflozin is visible in the chromatogram in Table 8. Figure 5.

Table 8: Degradation data of Metformin, Vildagliptin and Remogliflozin

Degradation condition	Metformin		Vildagliptin		Remogliflozin	
	% drug degraded	% drug Undegraded	% drug degraded	% drug Undegraded	% drug degraded	% drug Undegraded
2N HCl, 30 min	3.80	96.20	5.08	94.92	6.34	93.66
2N NaOH, 30 min	5.96	94.04	7.16	92.84	7.26	92.74
Oxidative, 30 min	5.58	94.42	6.76	93.24	7.51	92.49
Thermal, 6 hrs	2.68	97.32	3.54	96.46	3.45	96.55
Photo, 7 days	1.95	98.05	2.08	97.92	1.63	98.37
Neutral, 6 hrs	0.65	99.35	0.22	99.78	0.58	99.42

The forced degradation study demonstrated that metformin, vildagliptin, and remogliflozin exhibited limited to moderate degradation under the investigated stress conditions, indicating adequate stability under most conditions. Alkaline conditions showed the greatest deterioration (5.96%, 7.16%, and 7.26%, respectively), followed by oxidative conditions (5.58%, 6.76%, and 7.51% for metformin, vildagliptin, and remogliflozin, respectively). Acidic treatment produced comparatively lower degradation of 3.80%, 5.08%, and 6.34%. Under thermal and photolytic conditions, degradation remained

relatively low, ranging from 1.63–3.54%, while the drugs showed minimal degradation under neutral conditions (0.22–0.65%). Overall, the percentage of undegraded drug remained above 92% under all stress conditions, demonstrating the relative stability of the three analytes. The observed degradation under different stress conditions also indicates that the developed RP-HPLC method can effectively assess the stability of metformin, vildagliptin, and remogliflozin and may therefore be considered suitable for stability-indicating analysis of the combined formulation.

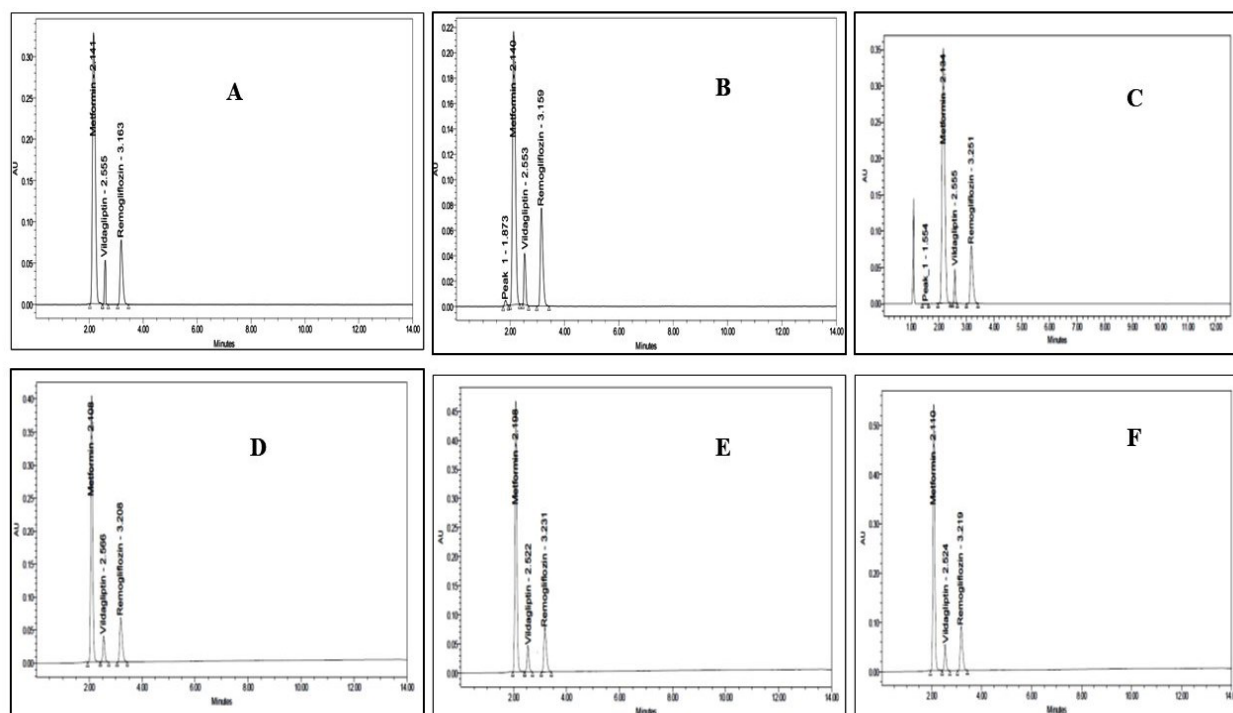


Figure 5: Forced degradation study chromatograms: A: Acid degradation; B: Base degradation; C: Peroxide degradation; D: Thermal degradation; E: UV degradation; F: Water degradation

QbD Overall desirability of final method

The optimized chromatographic responses for retention time (RT), resolution (RS), and theoretical plate number (NTP) are listed in **Table 9** and **Figure 5**. The observed retention times for the three analytes were 2.196 min (RT1), 2.621 min (RT2), and 3.060 min (RT3), which were close to the corresponding predicted values of 2.184, 2.580, and 3.045 min, respectively. The nearby analyte peaks were satisfactorily separated, as shown by the measured resolution values of 2.9 (RS1) and 3.0 (RS2). For each of the three analytes, the observed theoretical

plate counts were 4667.1, 5847.4, and 4690.9, indicating sufficient column efficiency. Additionally, all replicates' observed values were within the corresponding 95% prediction ranges, demonstrating the generated models' predictive power. In addition to demonstrating the efficacy of the CCD-based optimisation method in obtaining acceptable separation, resolution, and chromatography efficiency of metformin, vildagliptin, and remogliflozin, the strong correlation between predicted and experimentally observed results validates the suitability of the optimised chromatographic conditions.

Table 9: RT, NTP, RS values of Metformin, Vildagliptin and Remogliflozin

Response of 1 – 100 sol	Estimated Mean	Estimated Median	Observed	Std Dev	n	SE Pred	95% PI low	95% PI high
RT1	2.1839	2.1839	2.196	0.00499476	1	0.00549371	2.17166	2.19614
RT2	2.58034	2.58034	2.621	0.0208181	1	0.0228977	2.52932	2.63136
RT3	3.04511	3.04511	3.060	0.0115618	1	0.0127168	3.01678	3.07345
RS1	2.83092	2.83092	2.9	0.0581575	1	0.0639672	2.68839	2.97345
RS2	2.87117	2.87117	3.0	0.0762982	1	0.0839201	2.68419	3.05816
NTP1	4650.51	4650.51	4667.1	16.8945	1	18.5821	4609.11	4691.91
NTP2	5857.89	5857.89	5847.4	15.5121	1	17.0617	5819.87	5895.9
NTP3	4630.09	4630.09	4690.9	37.5622	1	40.5164	4542.56	4717.62

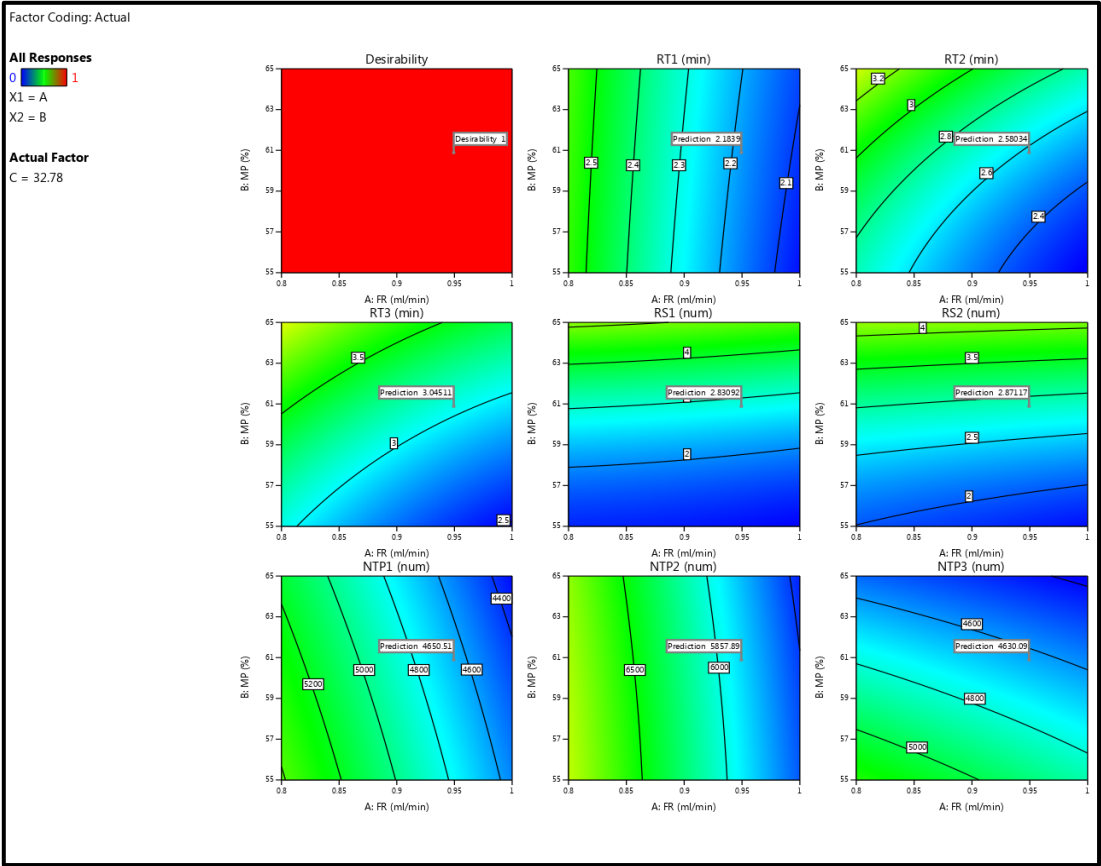


Figure 5: Desirability plot for RT 1,2,3 and NTP 1,2,3, and RS 1,2 graphs

CONCLUSION

The development of an HPLC method has made it possible to assess metformin, vildagliptin, and remogliflozin simultaneously in pharmaceutical formulations utilising a robust and reliable analytical approach. By effectively separating the components with high resolution, the validated method ensures accurate and dependable measurements across a range of concentrations. The optimised parameters—flow rate, mobile phase composition, and detection wavelength—allow for effective detection without interference from excipients or degradation products. This method can be used for routine quality control and stability studies, as well as to verify the efficacy and integrity of combination medications used to treat Type 2 Diabetes Mellitus (T2DM). The suggested HPLC technique satisfies the requirements for linearity,

precision, accuracy, and robustness since it follows ICH principles.

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