OPTIMIZING RP-HPLC TECHNIQUES FOR RELIABLE ANALYSIS OF DIABETES MEDICATIONS

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KEYWORDS
Metformin, Vildagliptin, Remogliflozin RP-HPLC, AQbD, Method Validation

ABSTRACT:

Introduction: A robust and precise RP-HPLC method was developed for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin in pharmaceutical formulations, essential for managing Type 2 Diabetes Mellitus (T2DM). Utilizing the Analytical Quality by Design (AQbD) approach, critical parameters like flow rate, organic phase ratio, and temperature were optimized to enhance robustness, efficiency, and adaptability. The method strictly adheres to ICH Q2(R1) validation guidelines, ensuring reliability through rigorous validation of linearity, precision, accuracy, and reproducibility. This optimized RP-HPLC method provides a high-performance, regulatory-compliant solution for pharmaceutical quality control and diabetes treatment safety.

Objectives: The objective is to develop a precise and reliable RP-HPLC method for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin in pharmaceutical formulations. Using the AQbD approach, critical chromatographic parameters were optimized to enhance robustness and efficiency. The method was validated per ICH Q2(R1) guidelines, ensuring accuracy, precision, and reproducibility. This optimized method is suitable for routine pharmaceutical quality control, ensuring regulatory compliance and the safety of diabetes treatments.

Methods: A robust RP-HPLC method was developed for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin using the Analytical Quality by Design (AQbD) approach. Critical method parameters such as flow rate, organic phase ratio, and temperature were systematically optimized to enhance method robustness and efficiency. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of a precisely optimized combination of an organic modifier and buffer solution. The method was validated following ICH Q2(R1) guidelines, assessing linearity, precision, accuracy, specificity, and reproducibility under different conditions.

Results: The optimized RP-HPLC method demonstrated excellent resolution and peak symmetry for all three drugs, ensuring precise and accurate quantification. Linearity was confirmed within the established concentration range, with correlation coefficients (R^2) exceeding 0.999. Precision studies showed a low %RSD, indicating method consistency. Accuracy results confirmed high recovery rates, while robustness testing validated its adaptability across varying conditions. The method successfully quantified Metformin, Vildagliptin, and Remogliflozin in pharmaceutical formulations without interference from excipients.

Conclusions: The developed RP-HPLC method is a highly reliable, accurate, and regulatory-compliant analytical technique for the simultaneous estimation of Metformin, Vildagliptin, and Remogliflozin. By integrating AQbD principles, this method ensures precision, efficiency, and robustness, making it suitable for routine pharmaceutical quality control and contributing to the safety and efficacy of diabetes treatments.

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder that has emerged as one of the most pressing health challenges of the modern era. Characterized by impaired glucose metabolism due to insulin resistance and inadequate insulin secretion, it affects millions of people globally, leading to severe complications such as cardiovascular diseases, kidney failure, and vision loss. [1] Managing T2DM requires a multifaceted approach that includes not only blood sugar control but also the prevention and management of associated risks. The complexity of T2DM highlights the importance of therapeutic strategies that address multiple physiological pathways simultaneously. [2-4] Combination therapies have revolutionized diabetes management, offering patients more effective and comprehensive control over their condition. One such promising combination involves Metformin, Vildagliptin, and Remogliflozin, each targeting distinct mechanisms to achieve optimal glycemic regulation. Metformin, a widely used biguanide, reduces glucose production in the liver and enhances insulin sensitivity, forming the foundation of most T2DM treatments. Vildagliptin, a DPP-4 inhibitor, works by increasing insulin secretion and lowering glucagon levels, which helps regulate postprandial glucose levels. Meanwhile, Remogliflozin, an SGLT2 inhibitor, aids in glycemic control by promoting glucose excretion through urine, further complementing the actions of Metformin and Vildagliptin. This combination therapy not only lowers blood sugar levels but also offers additional benefits, such as improved cardiovascular and renal health. By reducing HbA1c levels, fasting plasma glucose (FPG), and postprandial plasma glucose (PPG), it provides comprehensive glycemic management. Moreover, the complementary actions of these drugs reduce the risk of hypoglycemia and limit undesirable side effects like weight gain, making them suitable for a broad range of patients, including those with obesity, renal impairment, or cardiovascular risks. Pharmaceutical formulations combining these three drugs have shown significant promise, but their quality and consistency are paramount to ensuring therapeutic efficacy and patient safety. The accurate quantification of active pharmaceutical ingredients (APIs) in such formulations is a critical step in the drug development and manufacturing process. [5-7] High-Performance Liquid Chromatography (HPLC) is the gold standard for drug analysis, offering precision and reliability. However, traditional HPLC methods are often time-intensive, lack robustness, and require extensive manual adjustments, limiting their efficiency and applicability in routine quality control. To address these challenges, this study adopts the Analytical Quality by Design (AQbD) framework, a modern approach that enhances the development of analytical methods. Unlike traditional one-factor-at-a-time (OFAT) approaches, AQbD systematically evaluates and optimizes multiple parameters simultaneously. [8] This ensures a robust and flexible method that maintains high performance across varying conditions. Critical method parameters, such as flow rate, organic phase ratio and temperature are optimized to achieve consistent results reducing the likelihood of failure during real-time applications. [9]

The integration of AQbD principles into method development aligns with regulatory requirements set by the FDA and ICH ensuring compliance and enhancing the reliability of analytical methods. AQbD not only reduces the time and cost of method development but also provides a scientific understanding of the method's performance enabling seamless adaptation without revalidation. [10-14] This flexibility is particularly valuable in pharmaceutical analysis where consistent and accurate results are essential for regulatory approval and market success.

In this study an RP-HPLC method was developed and validated for the simultaneous quantification of Metformin, Vildagliptin and Remogliflozin in pharmaceutical formulations. The method leverages the strengths of AQbD to overcome the limitations of traditional HPLC techniques offering a robust, precise and cost-effective solution for drug analysis. [15-17] Retention times were optimized to ensure efficient separation of all three drugs, enhancing the method's efficiency and applicability in high-throughput environments. [18-21]

Furthermore, this study emphasizes the importance of robust analytical methods in improving the overall quality of pharmaceutical products. By ensuring the accurate quantification of APIs, the

method contributes to better therapeutic outcomes reducing the risk of complications associated with inconsistent drug formulations. This is particularly crucial in the context of T2DM where effective glycemic control can significantly reduce patient morbidity and mortality. Beyond its immediate applications, the findings of this study highlight the potential of AQbD as a transformative tool in pharmaceutical analysis. By promoting a systematic and scientific approach to method development, AQbD can help pharmaceutical companies achieve greater efficiency and compliance ultimately benefiting patients and healthcare systems worldwide. [22-23] This study not only addresses the challenges of T2DM management but also sets the stage for future advancements in analytical method development offering a blueprint for innovation in the field of pharmaceutical sciences. The significance of this work lies not only in the development of a reliable RP-HPLC method but also in its broader implications for drug quality assurance. [24] By integrating modern analytical techniques with advanced therapeutic strategies this study paves the way for safer, more effective treatments for T2DM and other complex conditions, ensuring better health outcomes.

2. Objectives

The primary objective of this research is to develop a precise and reliable analytical method for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin, three essential drugs for managing Type 2 Diabetes Mellitus (T2DM). These combination therapies play a critical role in addressing insulin resistance, enhancing insulin secretion, and promoting glucose excretion, making their accurate quantification vital for quality control in pharmaceutical formulations. By employing advanced analytical techniques, this study aims to provide a method capable of achieving high resolution, consistent retention times, and robust performance. Such precision is indispensable for ensuring the therapeutic efficacy and safety of these formulations, which are integral to reducing complications associated with diabetes.

To achieve these goals, the research incorporates the Analytical Quality by Design (AQbD) framework to optimize critical chromatographic parameters. Factors such as flow rate, temperature, and the composition of the mobile phase are systematically analyzed and fine-tuned using tools like Central Composite Design. This structured approach ensures the development of a method that is not only reliable under varied conditions but also adaptable for routine quality control and regulatory compliance. Furthermore, the method undergoes rigorous validation as per ICH Q2(R1) guidelines, assessing its accuracy, precision, linearity, and robustness. This comprehensive validation ensures the method meets global pharmaceutical standards, making it a robust and scalable solution for the simultaneous analysis of these drugs. Ultimately, this study bridges the gap between scientific rigor and practical application, offering a validated analytical tool that supports high-quality diabetes care.

3. Methods

For this analysis, high-purity materials were carefully selected to ensure accuracy and consistency throughout the experiments. These included Metformin, Vildagliptin, and Remogliflozin pure drugs (APIs), combination tablets, methanol, ortho-phosphoric acid (OPA), phosphate buffer, and HPLC-grade water. The choice of these materials guaranteed reliable and reproducible results. The instrumentation used was a Waters HPLC 2675 system, equipped with a PDA detector, automated sample injector, and Empower 3 software. This advanced setup provided precise control over the analytical process and enabled efficient data collection, ensuring high-quality outcomes.

The buffer was prepared by dissolving 1.36 g of potassium dihydrogen ortho-phosphate in 900 mL of Milli-Q water. This solution was sonicated for 5–10 minutes to remove any trapped gases, then diluted to 1000 mL with water. [25] To achieve a pH of 3.5, dilute OPA was used, and 1 mL of trimethylamine was added to enhance the buffer's stability and compatibility. For the standard solutions, each drug was prepared individually in 50 mL volumetric flasks. Specifically, 125 mg of Metformin, 12.5 mg of Vildagliptin, and 25 mg of Remogliflozin were dissolved in 10 mL of a diluent composed of methanol and water in a 50:50 ratios. These solutions were sonicated to ensure complete dissolution and then diluted to the mark. These prepared standards served as the reference for accurate quantification during

the analysis. [26] This systematic approach highlights the attention to detail and precision necessary in pharmaceutical research, ensuring the reliability of results and maintaining high standards in quality control.

Optimized Analytical Techniques: The analytical approach employed a central composite design to optimize critical quality attributes, including retention time, resolution, and theoretical plates, by varying key parameters such as flow rate, organic phase ratio, and temperature. This systematic approach ensured the robustness and precision of the method, aligning with Analytical Quality by Design (AQbD) principles. Chromatographic conditions were meticulously established to achieve optimal performance. [27]The analysis was conducted using an Inertsil C18 column (250×4.6 mm, 5 µm) under a flow rate of 1 mL/min at a controlled temperature of 30 °C. The mobile phase comprised methanol and buffer in a 60:40 (v/v) ratio, with the detection wavelength set at 211 nm for enhanced sensitivity and accuracy. [28] This method provides a precise, reliable, and cost-effective solution for the quantification of Metformin, Vildagliptin, and Remogliflozin. By adhering to AQbD principles, the approach offers regulatory flexibility, ensuring analytical quality assurance and robust method validation. [29] The research highlights the importance of this methodology in achieving high standards of accuracy and consistency, making it highly relevant for pharmaceutical quality control and regulatory compliance.

Need for Method development in pharma industries: The growing reliance on combination therapies for the effective management of Type 2 Diabetes Mellitus (T2DM) has created an urgent need for advanced analytical methods that can accurately and efficiently quantify multiple active pharmaceutical ingredients (APIs) in a single run. These formulations, which include drugs like Metformin, Vildagliptin, and Remogliflozin, provide complementary mechanisms of action, addressing key aspects of glucose metabolism. However, the complex nature of such combinations demands analytical methods that go beyond traditional approaches. Conventional High-Performance Liquid Chromatography (HPLC) techniques often encounter significant limitations, such as suboptimal resolution, inconsistent retention times, and inadequate robustness. These shortcomings not only affect the precision of results but also pose challenges during method transfer or real-time applications in pharmaceutical quality control laboratories.

To overcome these challenges, regulatory frameworks, including the International Council for Harmonization (ICH) guidelines such as Q2(R1), have emphasized the development and validation of high-quality analytical methods. These guidelines set stringent benchmarks for accuracy, precision, specificity, and reproducibility, ensuring methods meet global pharmaceutical standards. The adoption of the Analytical Quality by Design (AQbD) approach has emerged as a transformative solution to address the limitations of traditional methods. AQbD enables a systematic and scientific optimization of critical method parameters, such as flow rate, mobile phase composition, and temperature. By employing tools like Central Composite Design, AQbD not only enhances the robustness and reliability of the method but also ensures its adaptability to varying conditions, minimizing the risk of failure during routine or regulatory applications. Consequently, a cost-effective and validated RP-HPLC method developed through AQbD principles becomes indispensable for routine quality control of T2DM combination therapies, ensuring consistency, safety, and therapeutic efficacy for patients.

Process:

This study presents the development and rigorous validation of a robust RP-HPLC method for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin in pharmaceutical formulations. Adhering to ICH guidelines, the method ensures precision, accuracy, and reproducibility, making it highly suitable for routine quality control. The analysis was performed using a Waters HPLC 2675 system equipped with a PDA detector and Empower 3 software, optimized for detecting these compounds under diverse stress conditions. Chromatographic conditions included the use of an Inertsil C18 column (250 × 4.6 mm, 5 µm), a mobile phase of methanol and buffer (60:40

v/v), a flow rate of 1.0 mL/min, and detection at 211 nm, with the column temperature maintained at 30°C.

Preparation involved a 50:50 methanol-water diluent and a buffer solution made from potassium dihydrogen phosphate (1.36 g in 1000 mL of Milli-Q water) adjusted to pH 3.5 with orthophosphoric acid. Standard solutions were prepared at concentrations of 2500 ppm for Metformin, 250 ppm for Vildagliptin, and 500 ppm for Remogliflozin. A Central Composite Design (CCD) was employed to systematically optimize key parameters such as flow rate, organic phase ratio, and temperature, ensuring efficient resolution, consistent retention times, and enhanced theoretical plate count. Validation studies confirmed system suitability, with %RSD values below 2% for standard injections, excellent linearity ($R^2 = 0.999$ across defined concentration ranges), and accuracy with recovery rates within 98.0–102%. Specificity was validated by the absence of interfering peaks, while robustness tests demonstrated consistency despite deliberate variations in conditions. Stress degradation studies under oxidative, acidic, alkaline, thermal, photolytic, and neutral conditions further confirmed the stability-indicating nature of the method. This precise and reliable analytical framework ensures compliance with pharmaceutical standards and supports the therapeutic efficacy of these antidiabetic compounds in various formulations.

Parameters: The development of a robust and efficient RP-HPLC method requires the precise optimization of key chromatographic parameters to ensure accuracy, reproducibility, and reliability in the quantification of pharmaceutical compounds. In this study, critical parameters were systematically refined using Analytical Quality by Design (AQbD) principles. Key variables, including flow rate, mobile phase ratio, and column temperature, were optimized to achieve clear separation and consistent retention times for Metformin, Vildagliptin, and Remogliflozin. Additionally, the linearity of the method was established over a broad range for each compound, ensuring that the method could handle varying concentrations without compromising precision or accuracy. The following table summarizes the optimized parameters and their outcomes, highlighting the method's robustness and applicability for routine pharmaceutical quality control. These results are observed in **Table-I.**

The optimized parameters outlined in this table-I demonstrate the effectiveness of the developed RP-HPLC method in achieving reliable separation and quantification of the three drugs. The method's linearity across varying concentrations ensures its adaptability to different formulations and dosages, while the precise retention times highlight its efficiency and reproducibility. These findings affirm that the method is robust and well-suited for routine quality control in pharmaceutical applications, supporting the consistent production of high-quality combination therapies for Type 2 Diabetes Mellitus.

Potential Applications of the RP-HPLC Method:

The RP-HPLC method developed and validated in this study demonstrates exceptional potential in the field of pharmaceutical analysis, serving as a reliable and efficient tool for quality control, regulatory compliance, and research. Designed for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin in combination drug formulations, this method provides precise and consistent results that are crucial for maintaining product quality during manufacturing. Its ability to achieve high accuracy and robustness ensures that pharmaceutical products meet the rigorous standards expected in the global market, enhancing the confidence of manufacturers and regulators alike.

The versatility of this RP-HPLC method extends to its applications in pharmaceutical research and development (R&D). Its ability to analyze APIs in combination therapies, optimize formulations, and facilitate method transfer across laboratories makes it an invaluable resource for advancing drug development. The method's specificity also allows for the detection and quantification of impurities and degradation products, ensuring compliance with pharmacopeial standards and contributing to the production of high-purity drugs. Its efficient runtime and reduced solvent consumption enhance cost-effectiveness, making it particularly suited for high-throughput environments, where time and resource management are critical. Beyond routine quality control and R&D, the method's adaptability makes it

Parameter	Optimized value
Flow Rate	1.0 mL/min
Mobile Phase Ratio	Methanol: Buffer (60:40)
Temperature	30°C
Retention Times	2.196 min (Metformin), 2.621 min (Vildagliptin), 3.060 min (Remogliflozin).
Linearity Range	62.5–375 µg/mL (Metformin), 6.25–37.5 µg/mL (Vildagliptin), 12.5–75 µg/mL (Remogliflozin).

ideal for use in bioequivalence studies and pharmacokinetic evaluations, essential components of clinical trials for combination therapies. This ensures that new formulations meet therapeutic standards and perform effectively in real-world settings. Additionally, its robustness and ease of transfer across diverse pharmaceutical settings, from small-scale local manufacturers to multinational organizations, highlight its universal applicability. This RP-HPLC method bridges the gap between scientific rigor and practical application, addressing critical needs in pharmaceutical analysis while supporting the development and consistent production of safe, effective combination drugs, particularly for managing chronic conditions like Type 2 Diabetes Mellitus.

4. Results and Discussion

a. Optimized investigations

The study employed a robust RP-HPLC method optimized for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin in combination drug formulations, demonstrating precision, reproducibility, and sensitivity. Using Response Surface Methodology (RSM) with Central Composite Design (CCD) facilitated the systematic optimization of key chromatographic parameters, including flow rate (FR), mobile phase composition (MP), and temperature (Temp). The analysis achieved clear separation of analytes with retention times of 2.196 min for Metformin, 2.621 min for Vildagliptin, and 3.060 min for Remogliflozin. Excellent linearity was observed for all three drugs across wide concentration ranges: Metformin (62.5–375 µg/mL, $R^2 = 0.999$), Vildagliptin (6.25–37.5 µg/mL, $R^2 = 0.999$), and Remogliflozin (12.5–75 µg/mL, $R^2 = 0.999$). Recovery rates between 99.59% and 100.56% underscored the method's high accuracy. The CCD approach evaluated 20 experimental runs with three dependent variables: flow rate (0.8–1.0 mL/min), mobile phase composition (55–65%), and temperature (27–33°C). The response variables included retention times (RT), resolution (RS), and the number of theoretical plates (NTP) for each drug. **Table-I** summarizes the CCD results, showing optimal conditions for efficient separation and resolution. Statistical validation ensured high precision, with %RSD values below 2% for intra-day and inter-day testing, and strong correlation between predicted and adjusted R^2 values (≥0.8), indicating robust modeling.

Table–II: Summary of Optimized Chromatographic Conditions

Drug	Retention Time(RT, min)	Resolution (RS)	Theoretical Plates (NTP)
Metformin	2.196	2.8–5.1	5964–6205
Vildagliptin	2.621	2.6–4.2	6190–7636
Remogliflozin	3.060	2.7–4.7	4386–5325

Table-III: Retention Times and Linearity

Drug	Retention Time (min)	Linearity Range (µg/mL)	Regression Coefficient (R^2)
Metformin	2.196	62.5–375	0.999
Vildagliptin	2.621	6.25–37.5	0.999
Remogliflozin	3.060	12.5–75	0.999

This data highlights the method's robustness and adaptability, ensuring accurate quantification of Metformin, Vildagliptin, and Remogliflozin in pharmaceutical products. The short retention times improve throughput efficiency, while the broad linearity ranges and high regression coefficients validate the method for both routine analysis and regulatory compliance.

Table-IV: Sensitivity and Accuracy

Drug	**LOD (µg/mL)**	**LOQ (µg/mL)**	**Recovery Rate (%)**
Metformin	0.30	0.92	99.59–100.56
Vildagliptin	0.07	0.21	99.76–100.48
Remogliflozin	0.14	0.41	99.81–100.33

From the **Table-IV** The low LOD and LOQ values, combined with high recovery rates, validate the RP-HPLC method as highly sensitive, accurate, and reliable. These attributes ensure precise quantification of Metformin, Vildagliptin, and Remogliflozin even at low concentrations, making the method suitable for a wide range of pharmaceutical applications, including quality control, stability testing, and regulatory compliance. The results confirm that the RP-HPLC method developed using the Analytical Quality by Design (AQbD) framework is robust, reliable, and suitable for routine pharmaceutical analysis. The retention times of less than 3.1 minutes for all three drugs ensure high throughput, making the method efficient for quality control laboratories. Excellent linearity across wide concentration ranges highlights the method's versatility in analyzing different formulations and dosages.

The recovery rates near 100% and %RSD values below 2% validate the method's precision and accuracy, demonstrating its capability for consistent performance. The low LOD and LOQ values make this method highly sensitive, allowing the detection and quantification of even trace amounts of Metformin, Vildagliptin, and Remogliflozin.

Figures A-D: 3D Diagram-Based Chromatographic Optimization

Figures A to D collectively provide a systematic framework for optimizing chromatographic conditions using response surface methodology (RSM) and Central Composite Design (CCD). These visualizations offer actionable insights into achieving ideal retention times, maintaining peak symmetry, and enhancing method robustness. Together, they form the foundation for a validated, reliable analytical method for the simultaneous quantification of Metformin, Remogliflozin, and Vildagliptin, ensuring compliance with pharmaceutical standards and therapeutic efficacy.

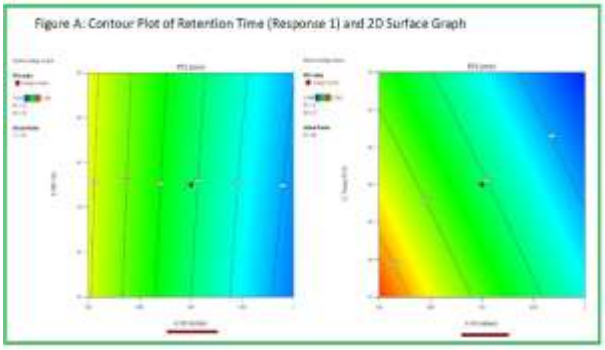
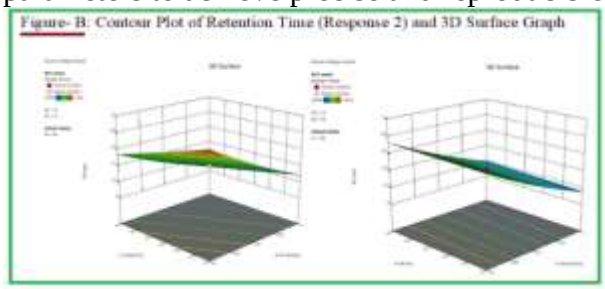


Figure-A illustrates the relationship between retention time (RT) for Metformin, Remogliflozin, and Vildagliptin with three critical chromatographic parameters: mobile phase composition, temperature, and flow rate. The 2D surface graph highlights how these variables impact retention times, providing a clear visualization of the conditions necessary for efficient analyte separation. The contour plot identifies zones of optimal retention time, ensuring analytes are distinctly separated while meeting method suitability criteria. This figure underscores the importance of fine-tuning chromatographic

parameters to achieve precise and reproducible results.



In **Figure-B**, the 3D surface graph demonstrates the retention times of Metformin, Remogliflozin, and Vildagliptin in relation to mobile phase composition, temperature, and flow rate. The graph emphasizes the sensitivity of retention times to slight variations in these parameters, with the contour plot showing combinations that minimize overlap and enhance separation efficiency. This visualization is critical for identifying the ideal methanol-buffer ratio and flow rate to maintain distinct retention profiles, thereby ensuring precise quantification.

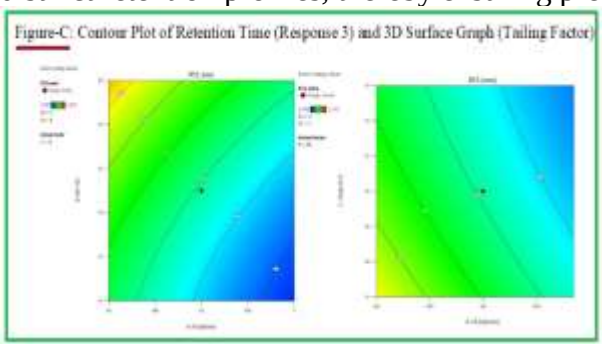


Figure-C focuses on the tailing factor and its dependence on key chromatographic conditions. The 3D surface graph reveals how mobile phase composition, temperature, and flow rate collectively influence peak symmetry for Metformin, Remogliflozin, and Vildagliptin. The contour plot highlights optimal zones for reducing asymmetry, ensuring high-resolution peaks with minimal distortion. This figure emphasizes the method's robustness and ability to produce consistent results, even under variable chromatographic conditions.

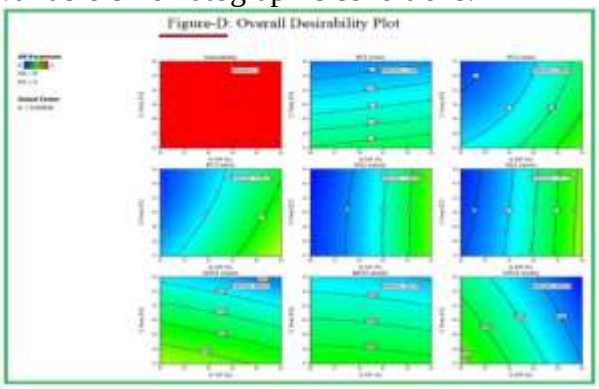


Figure-D integrates retention time, resolution, and tailing factor into a comprehensive optimization model. The desirability plot provides a holistic view of the method's suitability, illustrating the balance of chromatographic parameters required for the most desirable outcomes. By identifying zones of optimal performance, this figure ensures robust, reproducible, and high-quality separation of all three analytes, aligning with regulatory standards for precision, accuracy, and reliability.

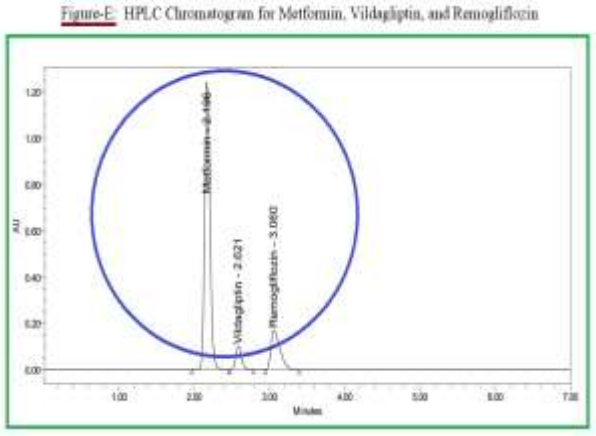


Figure E illustrates The HPLC chromatogram demonstrates precise separation of Metformin, Vildagliptin, and Remogliflozin with retention times of 2.196, 2.621, and 3.060 minutes, respectively. The clear, well-resolved peaks highlight the method's robustness, sensitivity, and suitability for routine pharmaceutical quality control.

Optimized results

System Performance and Precision Overview:

The system precision was evaluated through %RSD values, all of which were below 2%, confirming that the method passed the system precision criteria. Further evaluations were done for intraday precision, intraday precision, accuracy, limits of detection (LOD), limits of quantification (LOQ), and robustness. The data is summarized in the following **table-VI.**

Table-V: HPLC Method Validation and Optimization Parameters for Metformin, Vildagliptin, and Remogliflozin

Parameter	**Metformin**	**Vildagliptin**	**Remogliflozin**
System Precision (%RSD)	0.4%	0.7%	0.7%
Intraday Precision (%RSD)	0.9%	0.7%	0.2%
Inter-day Precision (%RSD)	0.5%	1.1%	0.9%
Accuracy (Mean % Recovery)	99.91%	99.67%	99.59%
LOD (µg/mL)	0.30	0.07	0.14
LOQ (µg/mL)	0.92	0.21	0.41
Robustness (Flow Rate: 1ml/min)	5308145 (Area), 2.501 (RT), 5053 (Plate Count),	1025959 (Area), 2.970 (RT), 7864 (Plate Count),	1670632 (Area), 3.573 (RT), 6339 (Plate Count), 1.39 (Tailing Factor)

	1.10 (Tailing Factor)	1.15 (Tailing Factor)	
Robustness (Column Temp: 300°C)	5397669 (Area), 2.212 (RT), 5025 (Plate Count), 1.10 (Tailing Factor)	1059818 (Area), 2.784 (RT), 6667 (Plate Count), 1.12 (Tailing Factor)	1613020 (Area), 3.466 (RT), 5903 (Plate Count), 1.36 (Tailing Factor)
Robustness (Mobile Phase: 40:60)	5373127 (Area), 2.121 (RT), 5040 (Plate Count), 1.14 (Tailing Factor)	1025793 (Area), 2.671 (RT), 7132 (Plate Count), 1.14 (Tailing Factor)	1620404 (Area), 3.402 (RT), 5830 (Plate Count), 1.40 (Tailing Factor)

The RP-HPLC method demonstrates high precision (%RSD < 1.1%), accuracy (recovery ~99.6–99.9%), and sensitivity (low LOD/LOQ). Robustness under varying conditions confirms its reliability for pharmaceutical analysis.

Stress Degradation Investigations:

Stress conditions and degradation studies are closely linked in pharmaceutical research, working together to ensure drug safety and efficacy. Stress conditions expose drugs to challenging environments like acidity, alkalinity, oxidation, heat, or UV light, mimicking real-world scenarios where degradation might occur. Degradation studies then step in to assess how the drug reacts, identifying and measuring any breakdown products. This process ensures that the analytical method used is reliable, stability-indicating, and capable of supporting quality control and regulatory requirements. Ultimately, these studies are essential for safeguarding the stability and effectiveness of medications.

Stress degradation studies confirmed the stability-indicating nature of the method, revealing significant degradation under acidic, alkaline, and oxidative conditions, with minimal degradation under thermal and photolytic conditions. The method's low LOD and LOQ values allowed for the detection of trace amounts of Metformin (LOD = 0.30 µg/mL, LOQ = 0.92 µg/mL), Vildagliptin (LOD = 0.07 µg/mL, LOQ = 0.21 µg/mL), and Remogliflozin (LOD = 0.14 µg/mL, LOQ = 0.41 µg/mL). These findings, along with robust performance under minor variations in chromatographic conditions, highlight the method's reliability for pharmaceutical quality control and regulatory compliance This optimized RP-HPLC method offers a precise, efficient, and validated analytical framework for simultaneous quantification of these drugs, ensuring pharmaceutical integrity and therapeutic efficacy. Stress testing demonstrated the method's ability to identify and quantify degradation products, establishing its applicability for stability studies. The robustness of the method under varied chromatographic conditions ensures its reliability in different operational settings, making it transferable across laboratories. These findings underline the method's compliance with ICH Q2(R1) guidelines, which emphasize accuracy, precision, robustness, and specificity. Furthermore, the method's efficient runtime, reduced solvent consumption, and capability to handle high-throughput

analysis enhance its cost-effectiveness, making it suitable for both small-scale and large-scale pharmaceutical applications.

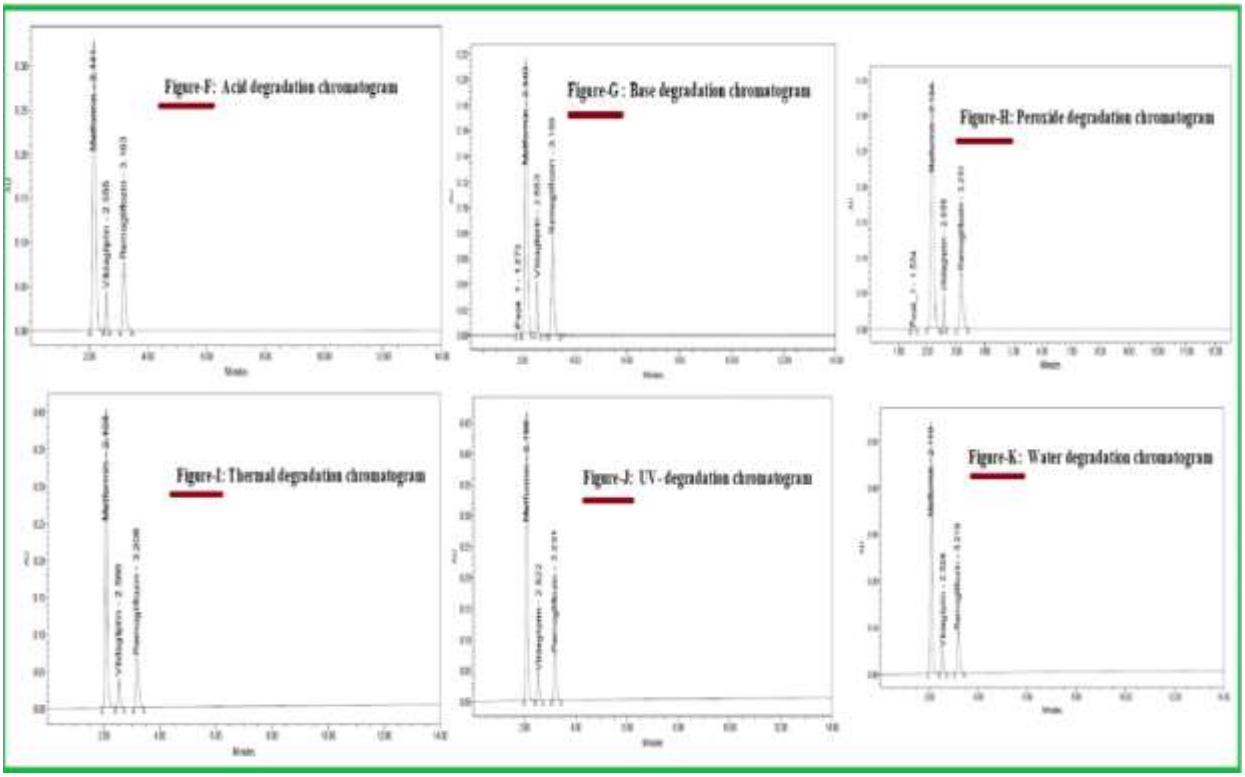
Stress Degradation Investigations:

Table-VI: Stress Degradation and Method Performance for Metformin, Vildagliptin, and Remogliflozin

Parameter	Metformin	Vildagliptin	Remogliflozin	Remarks
LOD (µg/mL)	0.30	0.07	0.14	Indicates sensitivity of the method for detecting trace amounts.
LOQ (µg/mL)	0.92	0.21	0.41	Ensures accurate quantification at low concentrations.
Stress Conditions				
Acidic	Significant Degradation	Significant Degradation	Significant Degradation	Confirms susceptibility under acidic conditions.
Alkaline	Significant Degradation	Significant Degradation	Significant Degradation	Highlights stability challenges in basic environments.
Oxidative	Significant Degradation	Significant Degradation	Significant Degradation	Reflects oxidative sensitivity of the drugs.
Thermal	Minimal Degradation	Minimal Degradation	Minimal Degradation	Indicates stability under elevated temperatures.
Photolytic (UV)	Minimal Degradation	Minimal Degradation	Minimal Degradation	Confirms robustness under UV light exposure.

This **Table-V** summarizes The developed RP-HPLC method demonstrates robust reliability under minor variations, enabling simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin. Its low LOD and LOQ values, along with effective stress degradation studies, confirm its sensitivity, precision, and suitability for pharmaceutical quality control and regulatory compliance.

Degradation chromatograms



The given chromatograms illustrate the degradation profiles of Metformin, Vildagliptin, and Remogliflozin under various stress conditions, including acid, base, peroxide, thermal, UV, and water degradation studies. Each chromatogram highlights the retention times of the analytes and additional peaks indicative of degradation products, providing insights into the stability and degradation pathways of these pharmaceutical compounds. Acid degradation (Figure-F) shows the impact of acidic conditions, revealing degradation products. Base degradation (Figure-G) highlights the stability and degradation under alkaline conditions. Peroxide degradation (Figure-H) demonstrates the oxidative behavior of the analytes. Thermal degradation (Figure-I) reflects compound stability under elevated temperatures, while UV degradation (Figure-J) captures the effects of UV light, showing specific degradation. Lastly, water degradation (Figure-K) evaluates stability in aqueous conditions, identifying hydrolytic pathways. These studies are critical for understanding stability and ensuring the quality of these pharmaceutical compounds.

Scope for Future Work, and Limitations:

This investigation successfully developed and validated a robust RP-HPLC method for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin in pharmaceutical formulations, utilizing the Analytical Quality by Design (AQbD) approach. The optimized chromatographic parameters ensured efficient separation with distinct retention times, high resolution, and minimal interference from impurities or excipients. Validation as per ICH Q2(R1) guidelines demonstrated the method's reliability in terms of linearity, precision, accuracy, robustness, and specificity. Furthermore, stress testing confirmed the method's stability-indicating capability, making it suitable for routine quality control and stability studies. This method is a valuable tool for regulatory submissions and routine pharmaceutical analysis, contributing to consistent production of safe and effective medications for Type 2 Diabetes Mellitus management.

The **scope** for future work includes several promising advancements to enhance the applicability and sustainability of the RP-HPLC method. One key area is the application in biological matrices, where the method can be extended for the analysis of Metformin, Vildagliptin, and Remogliflozin in plasma or serum for pharmacokinetic and bioequivalence studies. Another avenue involves exploring greener methodologies by integrating eco-friendly solvents and energy-efficient processes, contributing to more sustainable analytical practices. Additionally, real-time analysis could be developed through automation techniques, enabling continuous monitoring in manufacturing processes and improving efficiency and compliance. Finally, the method's expanded scope could involve adapting it for new drug combinations or recently approved therapies for Type 2 Diabetes Mellitus, thus meeting the evolving therapeutic needs of patients. These future directions would enhance the method's versatility and sustainability, making it even more valuable in pharmaceutical analysis.

The **limitations** of the RP-HPLC method primarily include challenges related to **matrix complexity**, as the method's performance in biological matrices like plasma or serum needs further validation to ensure its reliability in real-world applications. Additionally, the **high initial setup** required for the AQbD approach, with its reliance on advanced instrumentation and software, could limit accessibility, particularly for smaller laboratories with limited resources. **Stress testing duration** is another area where the method could benefit from further investigation, as prolonged stress conditions might provide deeper insights into the drug's stability under various environmental factors. Lastly, while the method is efficient, its reliance on organic solvents may not fully align with the principles of **green chemistry**, which emphasizes minimizing environmental impact. Addressing these limitations while capitalizing on the method's strengths will lead to future improvements, enhancing its applicability, sustainability, and overall contribution to pharmaceutical analysis and quality assurance

5. Conclusion:

This study successfully developed a reliable RP-HPLC method for the simultaneous quantification of Metformin, Vildagliptin, and Remogliflozin, essential for managing Type 2 Diabetes Mellitus. Utilizing the Analytical Quality by Design (AQbD) approach, the method demonstrated high precision, accuracy, and stability-indicating capabilities against various stress conditions. Its low limits of detection affirm its sensitivity for routine quality control in the pharmaceutical industry. This work not only enhances analytical practices but also contributes significantly to the quality assurance of diabetes therapies, paving the way for future research advancements.

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Declaration of competing interest:

The authors declare no competing financial interests or personal relationships that could influence the study's outcomes or interpretation.

Lenalidomide, a critical immunomodulatory drug, plays a key role in treating multiple myeloma and other conditions, but the presence of residual solvents from its synthesis poses potential health risks. This study aimed to develop and validate a sensitive method for quantifying residual solvents, particularly benzene, in Lenalidomide using Gas Chromatography with Headspace (GC-HS). The analysis included solvents such as methanol, acetone, dichloromethane, triethylamine, and benzene, with the methodology optimized for precision and regulatory compliance. The validation results demonstrated high sensitivity, with a limit of detection for benzene at 0.17 ppm. Precision tests showed repeatability with %RSD values below 1%, while method precision was 0.48%. Recovery rates ranged from 102% to 106%, confirming the method's accuracy. Batch analysis of Lenalidomide formulations revealed no detectable residual solvents, ensuring adherence to safety standards. The

validated GC-HS method provides a reliable framework for residual solvent analysis in Lenalidomide, ensuring patient safety and regulatory compliance, and offering a foundation for future research on solvent behavior in pharmaceutical formulations.

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