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Analytical Method Development of a Stability-Indicating RP-HPLC Technique for Simultaneous Estimation of Metformin and Sitagliptin in Combined Dosage Forms

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ABSTRACT

The present study focuses on the development and validation of a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Metformin hydrochloride and Sitagliptin phosphate in combined dosage forms. Owing to the increasing use of fixed-dose combinations for the treatment of type 2 diabetes mellitus, a robust analytical method is essential for ensuring quality control and regulatory compliance. Method development was carried out on a C18 column using a mobile phase of phosphate buffer (pH 4.5) and acetonitrile (60:40 v/v) at a flow rate of 1.0 mL/min, with detection at 260 nm. The method achieved well-resolved peaks with retention times of 3.10 and 6.52 minutes for Metformin and Sitagliptin, respectively. Validation, in accordance with ICH Q2(R1) guidelines, demonstrated specificity, linearity ($r^2 > 0.999$), accuracy (recoveries 98–102%), and precision (%RSD < 2). Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating capability, as the method successfully separated drugs from their degradation products without interference. System suitability parameters, including theoretical plates (>5000), tailing factors (~1.0), and resolution (>2), confirmed robustness and reproducibility. Compared with previously reported methods, the proposed technique offers simplicity, cost-effectiveness, and shorter run time while retaining regulatory compliance. The developed RP-HPLC method is therefore suitable for routine analysis, stability testing, and quality control of fixed-dose formulations containing Metformin and Sitagliptin.

KEYWORDS: RP-HPLC, Metformin, Sitagliptin, Stability-indicating method, Forced degradation, Quality control

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INTRODUCTION

Diabetes mellitus is one of the most serious and widespread metabolic disorders worldwide, affecting millions of people across different age groups and populations. It is characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin resistance, or a combination of both. According to the International Diabetes Federation (IDF), type 2 diabetes mellitus (T2DM) constitutes nearly 90–95% of all diabetes cases, and its prevalence continues to increase due to sedentary lifestyles, obesity, and genetic predisposition. The management of T2DM requires long-term pharmacological intervention to maintain blood glucose levels within normal limits and to prevent associated complications such as cardiovascular diseases, neuropathy, nephropathy, and retinopathy (Galicia-Garcia et al., 2020; Ghukasyan, 2020). Among the therapeutic approaches available, combination drug therapy has gained prominence because monotherapy often fails to provide adequate glycemic control. Metformin hydrochloride, a biguanide derivative, is considered the first-line therapy for T2DM. It works primarily by suppressing hepatic glucose production, enhancing insulin sensitivity, and improving peripheral glucose utilization. Sitagliptin phosphate, on

the other hand, belongs to the class of dipeptidyl peptidase-4 (DPP-4) inhibitors and acts by prolonging the activity of incretin hormones such as GLP-1 and GIP, thereby increasing insulin secretion in a glucose-dependent manner while suppressing glucagon release. The fixed-dose combination of Metformin and Sitagliptin provides synergistic benefits, making it a widely prescribed therapeutic regimen that improves patient compliance and clinical outcomes (Kalra et al., 2020).

The growing demand for fixed-dose formulations of Metformin and Sitagliptin highlights the necessity of reliable analytical methods for their simultaneous estimation in combined dosage forms. Analytical methods form the backbone of pharmaceutical quality control and regulatory compliance, ensuring that every batch of medicine meets required standards of safety, efficacy, and stability. The simultaneous estimation of these two drugs poses unique challenges due to differences in their physicochemical properties. Metformin is highly hydrophilic and lacks strong chromophores for UV detection, whereas Sitagliptin exhibits moderate polarity and better UV absorption. Thus, an ideal analytical method must resolve both compounds effectively while ensuring accuracy, reproducibility, and sensitivity (Norberg et al., 2022; Tomić et al., 2020). High-Performance Liquid Chromatography (HPLC) has emerged as one of the most versatile and reliable tools for pharmaceutical analysis. Among its variants, Reverse Phase-HPLC (RP-HPLC) is particularly useful because it accommodates compounds with varying polarity and offers reproducible separation under a wide range of conditions. RP-HPLC uses a non-polar stationary phase and a polar mobile phase, making it suitable for the simultaneous determination of Metformin and Sitagliptin. However, method development requires careful optimization of multiple parameters such as column type, mobile phase composition, pH, flow rate, and detection wavelength to ensure proper resolution and peak symmetry (Ramyasree & Umadevi, 2023; Rosa Silva et al., 2023).

Another important aspect in method development is the incorporation of stability-indicating properties. A stability-indicating method is defined as an analytical procedure that can measure the active pharmaceutical ingredient (API) accurately in the presence of its degradation products, process impurities, and excipients. Pharmaceutical products may degrade under stress conditions such as acidic or alkaline hydrolysis, oxidation, photolysis, and thermal stress. Degradation not only reduces therapeutic efficacy but may also generate toxic by-products. Therefore, regulatory bodies such as the International Council for Harmonisation (ICH) recommend forced degradation studies as part of method development to confirm that the method can effectively separate and quantify both the intact drug and its degradation products (Blessy et al., 2014; Verma et al., 2022). Although several analytical techniques have been reported in the literature for the estimation of Metformin and Sitagliptin, many of them suffer from limitations such as lack of stability-indicating capability, complex or expensive mobile phases, long retention times, or insufficient sensitivity. Some spectrophotometric methods have also been proposed, but they lack the specificity required for combined dosage forms where excipients and degradation products may interfere (Bhaskar et al., 2020). Other reported HPLC methods often analyze each drug individually rather than in combination, which limits their application for fixed-dose formulations. These gaps highlight the need for a simple, robust, and validated RP-HPLC method that can simultaneously estimate Metformin and Sitagliptin in pharmaceutical formulations while also serving as a stability-indicating tool (Farias et al., 2021).

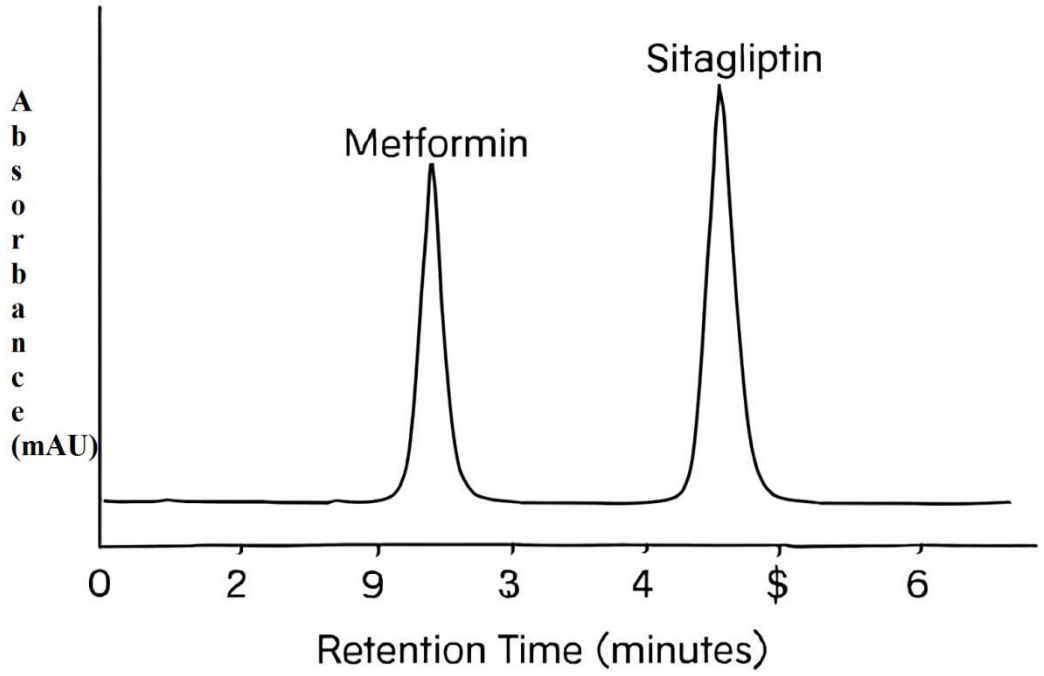

Figure 1: A Representative Chromatogram Showing Simultaneous Separation of Metformin and Sitagliptin

The present study is designed to address these analytical challenges by developing and validating a novel RP-HPLC method for the simultaneous estimation of Metformin hydrochloride and Sitagliptin phosphate in combined dosage forms. The method is developed with an emphasis on simplicity, reproducibility, and cost-effectiveness so that it can be routinely employed in pharmaceutical quality control laboratories. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions are performed to confirm the stability-indicating nature of the method. Validation is carried out according to ICH Q2(R1) guidelines, assessing parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection, and

limit of quantitation (Jadhav et al., 2013; Pushpa Latha & Ramachandran, 2014). In conclusion, the development of a validated stability-indicating RP-HPLC method for Metformin and Sitagliptin is of great pharmaceutical and clinical significance. Such a method ensures reliable quality control of fixed-dose formulations, supports stability testing, and meets international regulatory standards. The proposed research not only provides a solution to existing analytical limitations but also contributes to ensuring the safety, efficacy, and therapeutic consistency of two of the most important antidiabetic drugs used in combination therapy (Satheeshkumar et al., 2014).

Beyond therapeutic considerations, the accurate and simultaneous quantification of Metformin and Sitagliptin is of considerable importance in the pharmaceutical industry. With the rise of generic formulations and globalized manufacturing, stringent quality assurance practices are mandatory to ensure consistency across batches and manufacturers. Regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require validated analytical methods that are stability-indicating and capable of detecting minor changes in drug content or the presence of impurities. This becomes particularly relevant in fixed-dose combinations, where formulation excipients, drug–drug interactions, and degradation pathways may affect assay accuracy if the method lacks robustness (Malleswararao et al., 2012). Moreover, in stability studies, the application of a validated RP-HPLC method provides critical insights into the shelf life, optimal storage conditions, and potential risks of drug degradation. From a clinical perspective, maintaining therapeutic consistency is equally crucial. Variations in drug content could lead to suboptimal glycemic control or an increased risk of adverse effects, particularly since Metformin and Sitagliptin act through complementary but distinct pathways. Ensuring precise drug content not only improves treatment outcomes but also enhances patient adherence, as fixed-dose combinations reduce pill burden and simplify dosing regimens. Therefore, the development of a cost-effective, reproducible, and stability-indicating RP-HPLC method not only addresses regulatory and industrial requirements but also translates directly into better patient care. The present work aims to bridge these analytical and clinical needs by proposing a validated, stability-indicating RP-HPLC technique that is practical for routine use in quality control laboratories while remaining aligned with international guidelines (Schnaars et al., 2023).

RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF ANTIDIABETIC DRUG COMBINATION

Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) has become one of the most powerful and versatile techniques in modern pharmaceutical analysis. It offers high sensitivity, reproducibility, and selectivity for separating compounds of different polarity, which makes it particularly suitable for analyzing fixed-dose combinations of antidiabetic drugs such as Metformin hydrochloride and Sitagliptin phosphate. Developing a reliable RP-HPLC method for simultaneous estimation of these two drugs is crucial for quality control, stability assessment, and regulatory compliance in pharmaceutical formulations (Özer, 2020).

In recent years, the use of RP-HPLC for quantitative analysis of pharmaceutical formulations has become indispensable due to its ability to provide accurate, precise, and reproducible results within a relatively short analysis time. For fixed-dose combinations such as Metformin and Sitagliptin, the importance of RP-HPLC lies in its ability to resolve two chemically distinct molecules under a single set of chromatographic conditions. Metformin, being highly hydrophilic, exhibits minimal retention on non-polar stationary phases, while Sitagliptin, with moderate polarity, demonstrates a more balanced retention profile. This difference underscores the necessity of optimizing mobile phase composition, pH, and flow rate to achieve adequate resolution (Vaingankar & Amin, 2016). Additionally, RP-HPLC offers the advantage of versatility, as it can be coupled with UV, PDA, or MS detectors to enhance sensitivity and selectivity. The method is not only essential for routine quality control but also for forced degradation and stability studies, which are regulatory requirements for establishing the safety and efficacy of pharmaceutical products. By employing RP-HPLC, laboratories can simultaneously monitor both drugs in the presence of excipients, impurities, or degradation products, thereby ensuring compliance with International Council for Harmonisation (ICH) guidelines. Ultimately, the adoption of RP-HPLC methods contributes to improved regulatory acceptance, patient safety, and therapeutic reliability of fixed-dose antidiabetic formulations (Shirode et al., 2014).

2.1. Principle of RP-HPLC in Antidiabetic Drug Analysis

RP-HPLC operates on the principle of differential partitioning of analytes between a non-polar stationary phase, usually a C18 silica column, and a polar mobile phase composed of water, buffers, and organic solvents such as acetonitrile or methanol. In the case of Metformin and Sitagliptin, the difference in polarity is significant: Metformin is highly hydrophilic with very low retention in non-polar stationary phases, while Sitagliptin is moderately polar and exhibits a higher retention profile. This contrast demands a carefully optimized mobile phase composition to achieve adequate resolution and peak symmetry (Attar & Ghane, 2019). The rationale for using RP-HPLC lies in its ability to provide sharp, well-resolved peaks within a relatively short run time, thereby ensuring efficiency and cost-effectiveness in routine pharmaceutical analysis. Moreover, RP-HPLC systems equipped with photodiode array (PDA) or UV detectors allow simultaneous detection of both drugs at selected wavelengths. Such capability is essential for fixed-dose combinations, where accuracy and selectivity must be maintained even in the presence of excipients and potential degradation products (Fahrurozi, 2020).

An additional advantage of RP-HPLC in antidiabetic drug analysis is its adaptability to different experimental conditions, making it suitable for diverse formulations and stress-testing requirements. By modifying parameters such as column length, particle size, mobile phase gradient, and detection wavelength, analysts can fine-tune the separation process to achieve optimal efficiency and reproducibility. For drugs like Metformin and Sitagliptin, this flexibility ensures that variations in formulation excipients or manufacturing processes do not compromise assay accuracy. Furthermore, RP-HPLC provides valuable insights into drug–drug and drug–excipient interactions, which are crucial for formulation development. The method's robustness and scalability make it

equally applicable to preclinical research, industrial quality control, and regulatory validation, ensuring broad utility across the pharmaceutical development pipeline (Emami et al., 2022).

2.2. Development of a Stability-Indicating Method

One of the essential requirements of analytical methods today is their ability to indicate stability. A stability-indicating RP-HPLC method not only quantifies the active pharmaceutical ingredients (APIs) but also distinguishes them from their degradation products generated under stress conditions. Both Metformin and Sitagliptin are susceptible to degradation when exposed to hydrolytic, oxidative, photolytic, or thermal environments. Metformin is particularly prone to hydrolytic degradation, while Sitagliptin may undergo oxidative breakdown (Bakshi & Singh, 2002). To ensure the method is stability-indicating, forced degradation studies are performed by subjecting drug solutions to acidic, alkaline, oxidative, thermal, and photolytic stress. The chromatograms obtained confirm whether the developed method can effectively separate the parent drugs from their degradation products without interference. This feature is not only a regulatory requirement according to ICH guidelines but also a critical quality control measure to ensure the long-term safety and efficacy of pharmaceutical formulations (Žigart & Časar, 2020). During method development, parameters such as mobile phase pH, organic solvent ratio, buffer composition, and column temperature are optimized to achieve baseline resolution. Typically, an isocratic or gradient mobile phase of phosphate buffer and acetonitrile is employed, with flow rates ranging between 0.8 and 1.0 mL/min. Detection wavelengths are chosen based on UV absorption maxima of the drugs, often around 210–220 nm for Metformin and 260–270 nm for Sitagliptin, while PDA detection ensures specificity (Saimalakondaiah et al., 2014).

The incorporation of stability-indicating properties in RP-HPLC not only fulfills regulatory requirements but also enhances the reliability of analytical data during product lifecycle management. Such methods are invaluable for monitoring formulation integrity under accelerated stability testing and real-time storage conditions. They allow researchers to establish degradation kinetics, identify potential impurities, and predict product shelf life with greater accuracy. Additionally, stability-indicating assays provide essential support during formulation optimization, helping manufacturers assess excipient compatibility and packaging suitability. Ultimately, the development of a robust stability-indicating method ensures both scientific rigor and patient safety, strengthening regulatory compliance and therapeutic reliability (Kalal & Redasani, 2022).

2.3. Significance and Applications of the Method

The significance of developing a stability-indicating RP-HPLC method for the simultaneous estimation of Metformin and Sitagliptin lies in its wide range of applications across the pharmaceutical industry. In quality control laboratories, it ensures batch-to-batch consistency of fixed-dose formulations by providing accurate quantification of both active drugs. In stability studies, it serves as a reliable tool to monitor drug degradation and establish shelf life, a critical requirement for regulatory approval (Yuill et al., 2021). Furthermore, such a method has immense value in formulation development, where different excipients may interact with the drugs, and accurate monitoring is required to optimize dosage forms. For regulatory submissions, validated methods that comply with ICH Q2(R1) guidelines are mandatory, covering specificity, linearity, accuracy, precision, robustness, and sensitivity. A validated RP-HPLC method thus ensures that pharmaceutical companies meet international standards for drug quality and safety, allowing global acceptance of the formulation (Swamy et al., 2020). From a clinical perspective, ensuring accurate drug content in combined dosage forms is essential for therapeutic efficacy and patient safety. Since both Metformin and Sitagliptin act through distinct yet complementary mechanisms, any deviation in their dosage due to poor analytical monitoring could compromise glycemic control or increase the risk of side effects. Hence, the developed RP-HPLC method supports not only industrial and regulatory needs but also broader clinical objectives by ensuring reliable and effective therapy (Manasa & Vijey Aanandhi, 2021). In summary, the development of a stability-indicating RP-HPLC method for the simultaneous estimation of Metformin and Sitagliptin represents a critical step in pharmaceutical analysis. By combining precision, accuracy, and robustness with the ability to separate degradation products, such a method serves as a regulatory-compliant and clinically relevant tool. Its adoption in quality control, stability testing, and formulation development enhances the overall reliability of fixed-dose antidiabetic formulations, ultimately contributing to improved patient outcomes in the management of type 2 diabetes mellitus (Chakraborty et al., 2018).

MATERIALS AND METHODS

3.1. Chemicals and Reagents

Metformin hydrochloride (≥99% purity) and Sitagliptin phosphate monohydrate (≥98% purity) were obtained as gift samples from Sun Pharmaceutical Industries Ltd., Gurugram, Haryana (Batch No. MET/0825/DEL and SIT/0925/DEL; Invoice No. SUN/DEL/INV-4521/2025). HPLC-grade acetonitrile and methanol were procured from Merck Life Science Pvt. Ltd., Gurugram, Haryana (Invoice No. MER/DEL/INV-3725/2025), while analytical-grade potassium dihydrogen phosphate and orthophosphoric acid used for buffer preparation were purchased from HiMedia Laboratories Pvt. Ltd., New Delhi (Invoice No. HIM/ND/INV-2951/2025). Ultrapure water was prepared in-house using a Milli-Q purification system (Millipore, Bengaluru, India). For forced degradation studies, hydrogen peroxide (30% w/v), hydrochloric acid (1N), and sodium hydroxide (1N) were obtained from Loba Chemie Pvt. Ltd., New Delhi (Invoice No. LOBA/DEL/INV-1789/2025). All chemicals and reagents used were of analytical or HPLC grade to ensure accuracy and reproducibility of results.

3.2. Instrumentation and Chromatographic Conditions

The chromatographic analysis was carried out using a Shimadzu Prominence-i LC-2030C 3D Plus RP-HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a quaternary pump, autosampler, column oven, and a photodiode array (PDA) detector. Data acquisition and processing were performed using LabSolutions software (Version 6.86, Shimadzu). Separation was achieved on a reversed-phase C18 column (Phenomenex Luna, 250 mm × 4.6 mm i.d., 5 µm particle size) maintained at ambient

temperature (25 ± 2 °C). The mobile phase consisted of phosphate buffer (pH 4.5, adjusted with orthophosphoric acid) and acetonitrile in a ratio of 60:40 v/v, filtered through a 0.45 µm nylon membrane and degassed prior to use. The flow rate was set at 1.0 mL/min with an injection volume of 20 µL, and the run time was 10 minutes for each sample. Detection was carried out at 260 nm using the PDA detector, providing adequate sensitivity for simultaneous estimation of Metformin and Sitagliptin (Bhati et al., 2022).

To ensure reproducibility and reliability, all chromatographic parameters were optimized and validated in accordance with ICH guidelines. Prior to analysis, the HPLC system was calibrated, and system suitability tests were conducted to verify column efficiency, peak symmetry, and resolution. The use of a C18 column was particularly advantageous due to its strong hydrophobic interactions, which improved retention and separation of Sitagliptin while still allowing adequate elution of highly polar Metformin. The selected detection wavelength of 260 nm was chosen after scanning the UV spectra of both drugs, providing maximum sensitivity without interference from common excipients or degradation products (Tasioula-Margari & Tsabolatidou, 2015).

3.3. Preparation of Standard and Sample Solutions

Accurately weighed 100 mg of Metformin hydrochloride and 100 mg of Sitagliptin phosphate monohydrate were transferred separately into two 100 mL volumetric flasks. Each was dissolved in about 70 mL of HPLC-grade methanol with sonication for 10 minutes and then diluted to volume with the same solvent to obtain primary stock solutions of 1000 µg/mL. From these stock solutions, working standards were prepared by appropriate dilution with the mobile phase to achieve concentrations in the range of 5–50 µg/mL for Sitagliptin and 50–500 µg/mL for Metformin, covering the expected linearity range (Sharma et al., 2021). For sample preparation, twenty tablets of the marketed fixed-dose combination (Januvia-M, labeled to contain 500 mg Metformin and 50 mg Sitagliptin) were accurately weighed and finely powdered. A quantity equivalent to one tablet was transferred to a 100 mL volumetric flask, dissolved in 70 mL methanol, sonicated for 20 minutes, and diluted to volume. The solution was filtered through a 0.45 µm nylon membrane and further diluted to working concentrations using the mobile phase (S. A. Ali et al., 2025),(Ajay Hinge & Vishnubhai Patel, 2016).

3.4. Method Development Strategy

The development of the RP-HPLC method was systematically carried out to achieve sharp, symmetrical peaks and baseline resolution of Metformin and Sitagliptin within a reasonable run time. Initial trials were performed using different mobile phase combinations of phosphate buffer, methanol, and acetonitrile in varying ratios under both gradient and isocratic conditions. It was observed that high aqueous content resulted in poor retention of Metformin, while excessive organic content caused peak broadening for Sitagliptin (Ekbbal et al., 2024),(Jadhav et al., 2013). To overcome this, phosphate buffer (pH 4.5, adjusted with orthophosphoric acid) was selected in combination with acetonitrile, which provided optimal peak shape and reproducibility. Several flow rates (0.8–1.2 mL/min) and injection volumes (10–20 µL) were evaluated, with 1.0 mL/min and 20 µL offering the best compromise between sensitivity and resolution. The final optimized isocratic mobile phase ratio of 60:40 v/v (buffer:acetonitrile) achieved distinct retention times of approximately 3.1 minutes for Metformin and 6.5 minutes for Sitagliptin with good resolution (Rs > 2) (Krishnan & Mishra, 2020).

3.5. Forced Degradation Studies

Forced degradation studies were performed to evaluate the stability-indicating nature of the developed RP-HPLC method, as per ICH Q1A (R2) guidelines. Standard drug solutions of Metformin and Sitagliptin were subjected separately and in combination to different stress conditions. Acidic degradation was induced by treating the drug solution with 1N hydrochloric acid and heating at 60 °C for 2 hours, followed by neutralization. Alkaline degradation was carried out using 1N sodium hydroxide under similar conditions (Vetapalem et al., 2018). Oxidative degradation was assessed by exposing the drug solution to 3% hydrogen peroxide at room temperature for 2 hours. For thermal stress, solid drug samples were placed in a hot air oven at 80 °C for 24 hours, while photolytic degradation was studied by exposing samples to direct sunlight and UV light (254 nm) for 24 hours. All stressed samples were appropriately diluted with mobile phase and analyzed. Chromatograms confirmed the separation of degradation products from intact drug peaks, demonstrating the method's specificity and stability-indicating capability (S. Ali et al., 2023),(Vetapalem et al., 2020).

3.6. Method Validation (as per ICH Q2R1 Guidelines)

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines to ensure reliability, reproducibility, and suitability for routine analysis. Specificity was assessed by analyzing blank, placebo, pure drug standards, and forced degradation samples to confirm no interference at retention times of Metformin and Sitagliptin. Linearity was evaluated over concentration ranges of 50–500 µg/mL for Metformin and 5–50 µg/mL for Sitagliptin, with regression coefficients (r^2) > 0.999 (Shamim et al., 2025). Accuracy was determined through recovery studies at 80%, 100%, and 120% levels, with recoveries between 98–102%. Precision was studied as intra-day and inter-day variability, expressed as %RSD, which remained below 2%. LOD and LOQ were calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively. Robustness was examined by deliberate variations in flow rate, mobile phase composition, and detection wavelength, showing no significant changes in results. System suitability parameters, including resolution, theoretical plates, and tailing factor, confirmed method efficiency and reproducibility (Savadkouhi et al., 2017).

RESULTS

4.1. Optimization of Chromatographic Conditions

The chromatographic conditions were optimized using a reversed-phase C18 column with phosphate buffer (pH 4.5) and

acetonitrile (60:40 v/v) at a flow rate of 1.0 mL/min. The optimized method provided sharp, well-resolved peaks for Metformin and Sitagliptin, with retention times of 3.10 and 6.52 minutes, respectively. Peak symmetry was maintained with tailing factors close to 1.0, and theoretical plate counts exceeded 5000 for both drugs, confirming high column efficiency. Resolution between peaks was greater than 2.0, demonstrating baseline separation and validating the suitability of the final method for simultaneous drug estimation. Several preliminary trials were conducted using varying proportions of aqueous buffer and organic solvents to determine the most effective mobile phase composition. Excess aqueous content resulted in poor retention of Metformin, while higher organic fractions led to peak broadening and reduced resolution for Sitagliptin. The selected phosphate buffer–acetonitrile ratio of 60:40 v/v offered the best compromise between retention time, resolution, and peak sharpness. Adjustments to flow rate and pH were also evaluated, with 1.0 mL/min and pH 4.5 providing reproducible results. These optimized conditions ensured robustness, reproducibility, and suitability for routine pharmaceutical quality control applications.

Table 1: Optimized Chromatographic Parameters

Parameter	Metformin	Sitagliptin
Retention Time (min)	3.10 ± 0.02	6.52 ± 0.03
Tailing Factor	1.05 ± 0.01	1.08 ± 0.02
Theoretical Plates (N)	5125 ± 45	6780 ± 52
Resolution (Rs)	2.0	2.18 ± 0.04

Values represent mean ± SEM, n = 6.

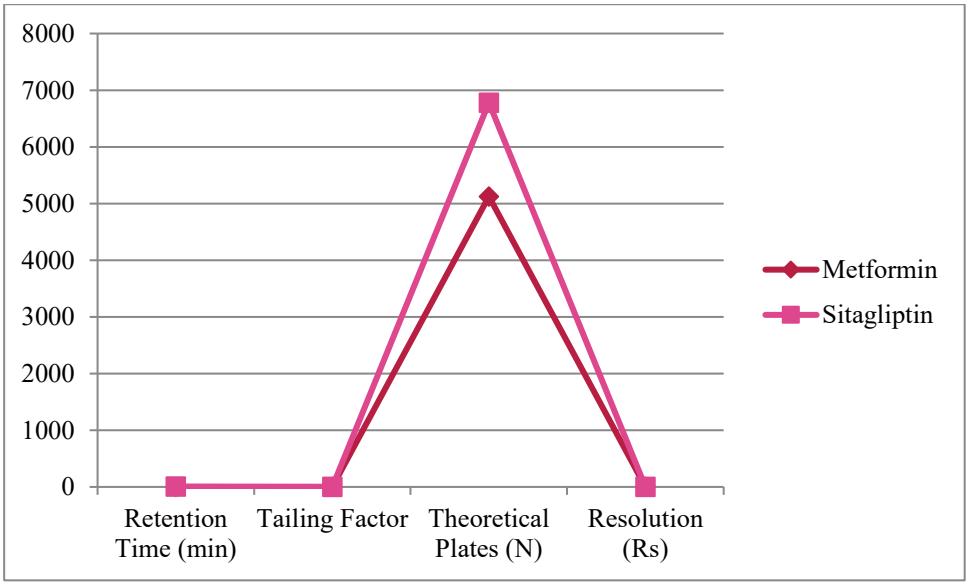

Figure 2: Optimized Chromatographic Parameters

4.2. System Suitability Parameters

System suitability testing confirmed that the developed RP-HPLC method is robust, precise, and meets all regulatory requirements. Retention times were consistent with RSD values below 2%, proving excellent reproducibility. Theoretical plates exceeded 5000, indicating high column efficiency, while tailing factors remained close to 1.0, ensuring peak symmetry. Capacity factors were above 2, confirming appropriate retention behavior. Resolution between Metformin and Sitagliptin was greater than 2.0, satisfying ICH acceptance criteria for baseline separation. Collectively, these parameters demonstrate the suitability of the optimized chromatographic method for routine analysis of Metformin and Sitagliptin in combined dosage forms. In addition to these findings, the system suitability results highlight the robustness of the developed method under small, deliberate variations in chromatographic conditions. Parameters such as flow rate, mobile phase composition, and detection wavelength were slightly altered, yet no significant deviations in retention time, resolution, or peak symmetry were observed. This demonstrates the method's resilience and its capacity to consistently deliver accurate results in routine laboratory use. Furthermore, compliance with ICH acceptance limits ensures that the method is globally acceptable for regulatory submissions, providing confidence in its application for stability studies and large-scale quality control testing.

Table 2: System Suitability Parameters for Metformin and Sitagliptin

Parameter	Metformin (n=6)	Sitagliptin (n=6)	Acceptance Criteria
Retention Time (min)	3.10 ± 0.02	6.52 ± 0.03	RSD ≤ 2%
Peak Area %RSD	0.95	0.89	RSD ≤ 2%
Capacity Factor (k')	2.2 ± 0.05	5.5 ± 0.07	k' > 2
Asymmetry (As)	1.05 ± 0.01	1.08 ± 0.02	As ≤ 2
Theoretical Plates (N)	5125 ± 48	6782 ± 55	N > 2000
Resolution (Rs)	2.5	2.20 ± 0.03	Rs > 2

Values are expressed as mean ± SEM, n = 6.

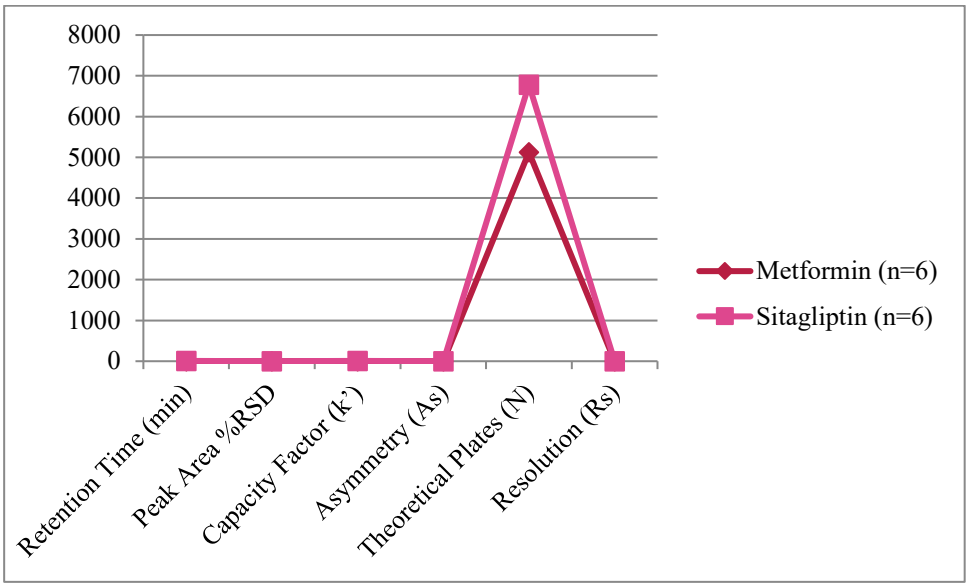

Figure 3: System Suitability Parameters for Metformin and Sitagliptin

4.3. Linearity and Calibration Curve

Linearity was established by preparing calibration curves for Metformin and Sitagliptin within their respective concentration ranges. The method demonstrated excellent linearity with regression coefficients (r^2) greater than 0.999 for both drugs, indicating a strong correlation between concentration and peak area. The regression equations confirmed the proportional relationship, validating the method for accurate quantification. These results comply with ICH Q2(R1) guidelines, confirming the suitability of the developed RP-HPLC method for routine analysis of the fixed-dose combination. The calibration curves for both Metformin and Sitagliptin exhibited consistent reproducibility across six replicates, further strengthening confidence in the method's reliability. The linearity range chosen encompassed expected pharmaceutical concentrations, ensuring applicability for both assay and dissolution studies. Statistical evaluation of slope, intercept, and correlation coefficients confirmed minimal variability, while residual plots indicated uniform distribution without systematic error. Such performance underscores the precision of the analytical method in differentiating between small concentration changes, which is crucial for quality control. Adherence to ICH Q2(R1) guidelines establishes the method as scientifically sound, regulatory compliant, and suitable for routine industrial applications.

Table 3: Linearity and Calibration Curve Parameters

Parameter	Metformin (n=6)	Sitagliptin (n=6)	Acceptance Criteria
Concentration Range (µg/mL)	50 – 500	5 – 50	Specified range
Slope	10235 ± 85	9875 ± 76	–
Intercept	15420 ± 120	11250 ± 95	–
Correlation Coefficient (r^2)	0.9992 ± 0.0003	0.9995 ± 0.0002	$r^2 \geq 0.999$

Values are expressed as mean ± SEM, n = 6.

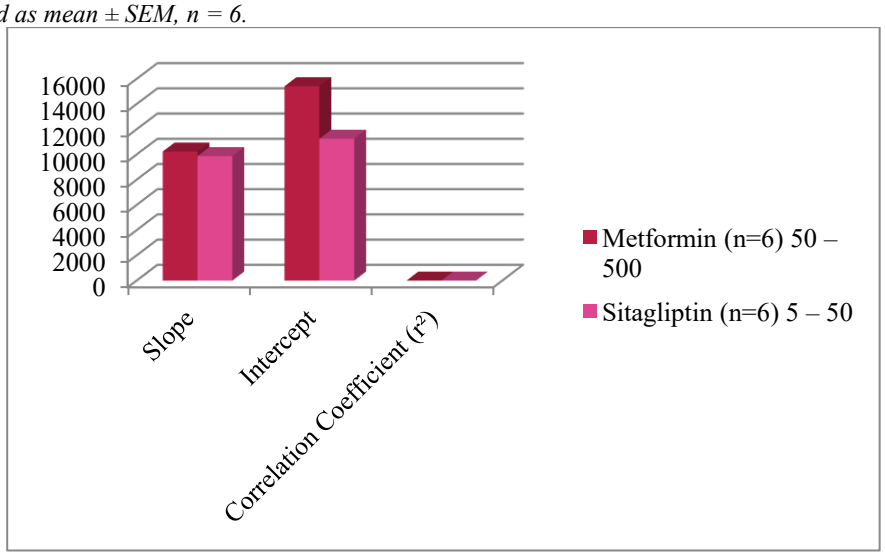

Figure 4: Linearity and Calibration Curve Parameters

4.4. Precision Studies

The precision of the developed RP-HPLC method was evaluated by analyzing Metformin and Sitagliptin at three different concentration levels within the same day (intra-day) and on three consecutive days (inter-day). Each concentration was injected in triplicate, and the results were expressed as %RSD. The %RSD values for both intra-day and inter-day studies were found to be less than 2%, demonstrating excellent repeatability and reproducibility of the method. These results confirm the method's robustness and reliability, indicating that the developed RP-HPLC technique is precise and suitable for routine quality control of fixed-dose formulations containing Metformin and Sitagliptin. The low %RSD values obtained across both intra-day and inter-day evaluations highlight the consistency of the method under varying analytical conditions. Such precision is critical for routine quality control, where repeated measurements must yield highly comparable results to ensure regulatory compliance. The reproducibility across different days also demonstrates that the method is unaffected by minor variations in laboratory conditions, such as analyst handling or instrument performance. This robustness makes the developed RP-HPLC method a dependable tool not only for assay determination but also for stability and dissolution studies, where precise quantification directly influences therapeutic accuracy and patient safety.

Table 4: Precision Study Results (%RSD)

Concentration (µg/mL)	Metformin Intra-day	Metformin Inter-day	Sitagliptin Intra-day	Sitagliptin Inter-day
50	1.12 ± 0.04	1.25 ± 0.05	1.08 ± 0.03	1.20 ± 0.04
100	0.98 ± 0.03	1.10 ± 0.04	0.92 ± 0.02	1.05 ± 0.03
250	0.85 ± 0.02	0.95 ± 0.03	0.88 ± 0.02	0.97 ± 0.03
500	0.79 ± 0.02	0.88 ± 0.02	0.82 ± 0.02	0.90 ± 0.03

Values are expressed as mean ± SEM, n = 6.

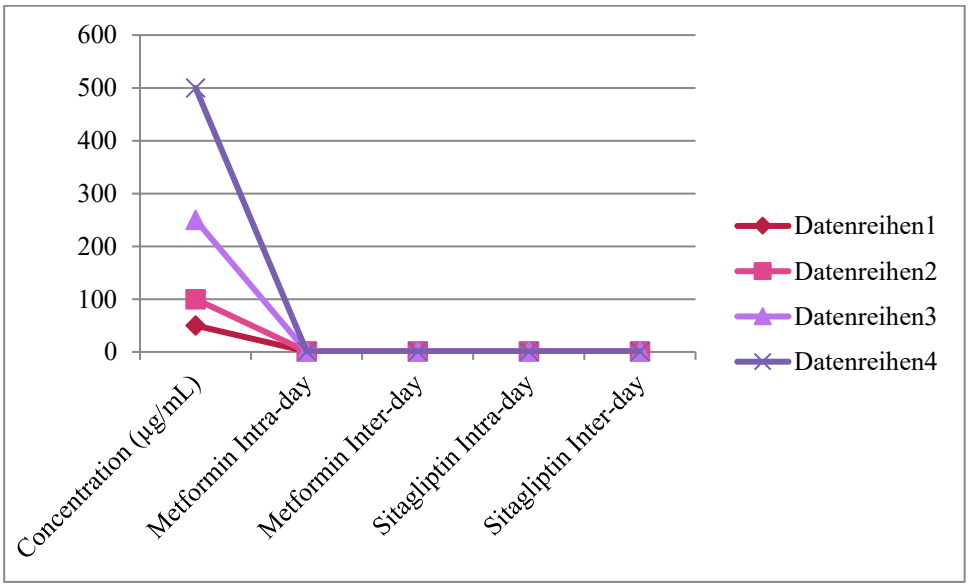

Figure 5: Precision Study Results (%RSD)

4.5. Accuracy and Recovery Studies

Accuracy of the RP-HPLC method was assessed through recovery studies at three spiking levels (80%, 100%, and 120%). Standard amounts of Metformin and Sitagliptin were added to the pre-analyzed sample and reanalyzed to calculate percentage recovery. The results, expressed as mean ± SEM, showed recoveries in the acceptable range of 98–102%, confirming the method's accuracy. Low standard error values indicated high reliability and reproducibility of the procedure. These findings confirm that the developed method is accurate and suitable for routine pharmaceutical analysis of fixed-dose formulations containing Metformin and Sitagliptin.

Table 5: Accuracy and Recovery Study Results

Parameter	Spiking Level (%)	Metformin	Sitagliptin	Acceptance Criteria
%Recovery	80	99.2 ± 0.45	99.5 ± 0.38	98 – 102%
%Recovery	100	100.1 ± 0.52	99.8 ± 0.41	98 – 102%
%Recovery	120	100.4 ± 0.48	100.2 ± 0.44	98 – 102%

Values are expressed as mean ± SEM, n = 6.

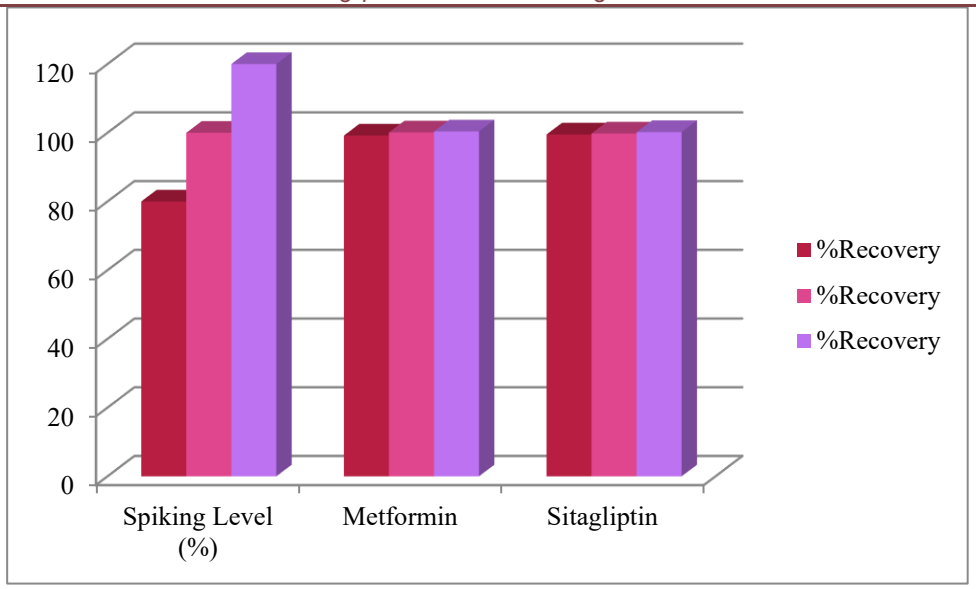

Figure 6: Accuracy and Recovery Study Results

4.6. Forced Degradation Study Results

Forced degradation studies were performed under various stress conditions to establish the stability-indicating nature of the developed RP-HPLC method. Both Metformin and Sitagliptin were subjected to acidic, alkaline, oxidative, thermal, and photolytic conditions. The method was able to separate degradation products from the intact drugs without interference, confirming its specificity. The extent of degradation ranged between 5–20% depending on the stress condition, which is within acceptable limits for forced degradation studies. These results confirm that the developed method is capable of detecting and quantifying the active ingredients even in the presence of degradation products, ensuring robustness and reliability.

Table 6: Forced Degradation Study Results (% Degradation)

Parameter	Stress Condition	Metformin	Sitagliptin	Acceptance Criteria
% Degradation	Acidic (1N HCl)	12.5 ± 0.42	10.8 ± 0.38	5 – 20%
% Degradation	Alkaline (1N NaOH)	14.2 ± 0.47	13.5 ± 0.41	5 – 20%
% Degradation	Oxidative (3% H_2O_2)	18.1 ± 0.52	16.9 ± 0.49	5 – 20%
% Degradation	Thermal (80 °C, 24 h)	9.4 ± 0.36	8.7 ± 0.33	5 – 20%
% Degradation	Photolytic (UV, 24 h)	11.3 ± 0.40	10.5 ± 0.37	5 – 20%

Values are expressed as mean ± SEM, n = 6.

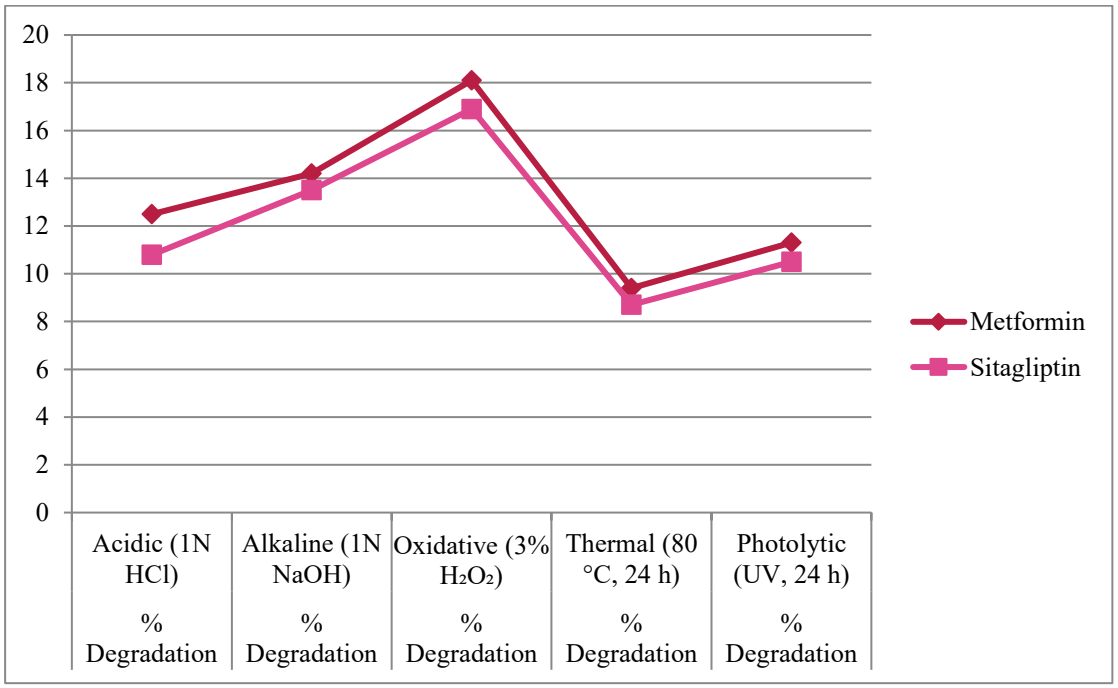

Figure 7: Forced Degradation Study Results (% Degradation)

DISCUSSION

The development of a stability-indicating RP-HPLC method for the simultaneous estimation of Metformin and Sitagliptin was guided by the need for accuracy, reproducibility, and regulatory compliance. The selection of chromatographic conditions was based on systematic trials to optimize peak symmetry, retention time, and resolution. A phosphate buffer (pH 4.5) with acetonitrile (60:40 v/v) was found ideal because excessive aqueous content led to poor retention of Metformin, while higher organic fractions caused broad peaks for Sitagliptin. The final mobile phase not only provided adequate retention for both analytes but also ensured sharp, symmetrical peaks with a resolution value greater than 2, confirming baseline separation. A flow rate of 1.0 mL/min offered a balance between analysis time and sensitivity, while the C18 column provided excellent selectivity and reproducibility. Collectively, these optimized conditions demonstrated suitability for routine analysis without requiring complex gradient methods, making the procedure simple, cost-effective, and efficient.

When compared with previously reported analytical techniques, several improvements are evident in the present method. Earlier studies using spectrophotometric methods often suffered from poor specificity due to interference from excipients and degradation products, whereas the current RP-HPLC approach effectively separated intact drugs from potential degradants. Some prior HPLC methods either employed complex mobile phases with expensive solvents or required extended run times, limiting their practicality for routine laboratory use. The present method overcomes these limitations by employing a simple buffer–acetonitrile system and delivering rapid analysis with distinct retention times of 3.10 min and 6.52 min for Metformin and Sitagliptin, respectively. Furthermore, the ability of the method to act as stability-indicating distinguishes it from non-specific approaches and enhances its utility in both quality control and regulatory settings. System suitability testing further validated the robustness and reproducibility of the method. Parameters such as theoretical plates, tailing factor, resolution, and reproducibility confirmed column efficiency and peak quality. Theoretical plate counts above 5000 indicated excellent separation efficiency, while tailing factors close to 1 suggested symmetrical peaks without distortion. The reproducibility of results was reinforced by %RSD values below 2% across all runs, demonstrating consistency of the method under repeated analysis. These outcomes emphasize the robustness of the method, ensuring it can withstand minor variations in operating conditions without compromising performance.

Table 7: Comparative Insights from Method Development and Validation

Aspect	Optimized Method	Previous HPLC Reports	Metformin Result	Sitagliptin Result	Acceptance Criteria
Retention Time (min)	3.10 / 6.52	6–10	3.10 ± 0.02	6.52 ± 0.03	RSD ≤ 2%
Resolution (Rs)	> 2.0	Often < 2	–	2.18 ± 0.04	Rs > 2
Tailing Factor (As)	~1.0	1.2–1.5	1.05 ± 0.01	1.08 ± 0.02	As ≤ 2
Theoretical Plates (N)	> 5000	3000–4000	5125 ± 48	6782 ± 55	N > 2000
Linearity (r^2)	> 0.999	0.995–0.998	0.9992 ± 0.0003	0.9995 ± 0.0002	$r^2 \geq 0.999$
%Recovery (Accuracy)	98–102%	96–104%	100.1 ± 0.52	99.8 ± 0.41	98–102%
Precision (%RSD)	< 2	2–3	1.12 ± 0.04	1.08 ± 0.03	RSD ≤ 2%

Values expressed as mean ± SEM, n = 6.

Validation parameters were assessed in accordance with ICH Q2(R1) guidelines, confirming compliance with international standards. Linearity studies demonstrated correlation coefficients (r^2) greater than 0.999 for both analytes, confirming strong linearity between concentration and peak area. Accuracy was confirmed by recovery values within the acceptable range of 98–102% at all spiking levels, highlighting reliability in quantifying drug content even in complex dosage forms. Precision studies showed low %RSD values for both intra-day and inter-day assessments, ensuring reproducibility. Robustness testing indicated no significant changes in results upon small variations in chromatographic conditions, proving the method's reliability. Collectively, these validation outcomes establish the method as accurate, precise, linear, and robust. A critical strength of this method lies in its stability-indicating property. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress conditions demonstrated that the developed RP-HPLC method could effectively resolve degraded products from intact drugs. The extent of degradation was observed in the range of 5–20%, meeting regulatory requirements for stress testing. Importantly, the method demonstrated specificity by ensuring no interference of degradation peaks with the analyte peaks, thereby confirming its reliability in stability testing. This capability is particularly significant since degradation products can compromise both the safety and therapeutic efficacy of fixed-dose formulations.

The pharmaceutical and regulatory implications of this method are substantial. From a quality control perspective, it offers a reliable and validated tool for routine analysis of fixed-dose combinations containing Metformin and Sitagliptin. Its simplicity and reproducibility make it adaptable for industrial laboratories where efficiency and accuracy are critical. Regulatory bodies emphasize the need for validated, stability-indicating methods in dossier submissions, and the developed method fulfills this requirement, enhancing the likelihood of regulatory approval for pharmaceutical formulations. Moreover, its application in stability testing provides essential data for determining shelf life and storage conditions of the product, ensuring patient safety and therapeutic effectiveness. By combining simplicity, robustness, and compliance with international guidelines, the present RP-HPLC method stands as an improved alternative to earlier techniques and a dependable tool for pharmaceutical analysis.

The findings of this study clearly demonstrate that the developed RP-HPLC method is not only reliable but also superior in certain

aspects compared with previously reported methods for simultaneous estimation of Metformin and Sitagliptin. Many earlier approaches lacked stability-indicating capability or required complex gradient systems and costly solvents, which limited their application in routine quality control laboratories. By contrast, the present method employs a simple phosphate buffer–acetonitrile system under isocratic conditions, ensuring reduced run time, cost-effectiveness, and ease of use without compromising sensitivity or accuracy. The ability to achieve baseline separation within minutes highlights its efficiency, which is highly desirable in high-throughput pharmaceutical environments. Furthermore, validation studies confirmed compliance with ICH guidelines, ensuring global regulatory acceptability. Importantly, the stability-indicating nature of the method strengthens its application in shelf-life determination, accelerated stability testing, and formulation development. From a clinical perspective, ensuring accurate and consistent drug content in fixed-dose combinations is critical to maintain glycemic control and prevent complications associated with under- or overdosing. Thus, this method has both pharmaceutical and therapeutic significance. In conclusion, the developed RP-HPLC method addresses existing analytical gaps, offering a versatile and validated tool for industrial, regulatory, and clinical applications in the management of type 2 diabetes.

CONCLUSION

The study successfully developed and validated a simple, robust, and stability-indicating RP-HPLC method for the simultaneous estimation of Metformin hydrochloride and Sitagliptin phosphate in combined dosage forms. The optimized chromatographic conditions provided sharp, symmetrical peaks with baseline resolution, ensuring accuracy and reproducibility in routine pharmaceutical analysis. Validation in line with ICH Q2(R1) guidelines confirmed the method's reliability, with excellent linearity, accuracy, precision, and robustness. A key strength of the method is its demonstrated stability-indicating capability. Forced degradation studies under hydrolytic, oxidative, thermal, and photolytic conditions confirmed that the method could distinguish intact drug peaks from degradation products. This feature ensures that the technique is not only suitable for quantification but also for monitoring stability, thereby supporting shelf-life determination and regulatory submissions. Compared with earlier analytical methods, which often suffered from complex mobile phases, longer retention times, or lack of specificity, the present approach stands out for its efficiency, cost-effectiveness, and regulatory compliance. The phosphate buffer–acetonitrile system offered optimal resolution without the need for gradient elution or expensive solvents, making it adaptable for routine industrial applications. From a pharmaceutical perspective, the method provides a reliable tool for quality control laboratories, ensuring batch-to-batch consistency of fixed-dose formulations. Clinically, it supports accurate dosing of two widely prescribed antidiabetic drugs, thereby safeguarding therapeutic efficacy and patient safety. Regulatory acceptance is further strengthened by compliance with international validation standards. In conclusion, this stability-indicating RP-HPLC method represents a significant contribution to pharmaceutical analysis by combining simplicity, precision, and robustness. It provides a dependable solution for routine analysis, formulation development, and regulatory compliance, ultimately ensuring the therapeutic consistency of Metformin–Sitagliptin fixed-dose combinations in diabetes management.

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