

RESEARCH ARTICLE

Development and Validation of Stability-Indicating Methods for the Estimation of Antidiabetic Drugs (Repaglinide and Sitagliptin)Yashlok Sharma¹, Archana Tiwari², Ravinder Kaur², Dishant Gupta², P. K. Dubey²¹Department of Pharmaceutical Chemistry, Swami Vivekanand College of Pharmacy, Indore, Madhya Pradesh, India, ²Department of Pharmacy, Faculty of Pharmacy, Swami Vivekanand College of Pharmacy, Indore, Madhya Pradesh, India**Received: 17-07-2026; Revised: 14-08-2026; Accepted: 10-09-2026****ABSTRACT**

The present study aimed to develop and validate a stability-indicating reverse-phase high-performance liquid chromatographic method for simultaneous estimation of Repaglinide and Sitagliptin in pharmaceutical formulations. Chromatographic separation was achieved using a C18 column with an optimized phosphate-buffer/acetonitrile mobile phase under isocratic conditions and ultraviolet/photodiode array detection. The analytical procedure was evaluated for system suitability, specificity, linearity, accuracy, precision, sensitivity, and robustness in accordance with ICH validation principles. Forced degradation studies were performed under acidic, alkaline, oxidative, thermal, and photolytic conditions to establish the stability-indicating capability of the method. The developed procedure showed satisfactory system suitability, with theoretical plate counts above 2000, acceptable tailing factors, and resolution >2.0. Good linearity was obtained for Repaglinide (1–10 µg/mL; $r^2 = 0.9997$) and Sitagliptin (25–150 µg/mL; $r^2 = 0.9999$). Recovery studies demonstrated mean recoveries of approximately 99–101% with low percent relative standard deviation values. Forced degradation resulted in measurable degradation while maintaining separation of degradation products from the analyte peaks, with peak purity passing for both drugs. The validated method was therefore found to be suitable for routine quantitative analysis, quality control, and stability testing of Repaglinide and Sitagliptin pharmaceutical formulations.

Keywords: Method validation, repaglinide, reversed-phase high-performance liquid chromatography, sitagliptin, stability-indicating method

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and is associated with progressive complications involving the cardiovascular system, kidneys, eyes and nervous system. Type 2 diabetes accounts for the great majority of diabetes cases and continues to represent a major public-health challenge.^[1] Pharmacological management commonly requires agents with complementary mechanisms to achieve

adequate glycemic control. Repaglinide is a short-acting insulin secretagogue that lowers blood glucose by stimulating glucose-dependent insulin release through closure of ATP-sensitive potassium channels in pancreatic β -cells.^[2] Sitagliptin is a selective dipeptidyl peptidase-4 inhibitor that prolongs the activity of incretin hormones, thereby enhancing glucose-dependent insulin secretion and reducing glucagon release.^[3] The complementary actions of these drugs provide a rationale for their combined use in the management of type 2 diabetes.

Reliable analytical procedures are essential for identification, assay, quality control and stability assessment of pharmaceutical products. Reversed-

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phase high-performance liquid chromatography (RP-HPLC) is widely applied because it provides efficient separation, quantitative accuracy, and the ability to resolve active pharmaceutical ingredients from related substances and degradation products.^[3] Stability-indicating procedures are particularly important because they should distinguish the intact drug from products formed during storage or under stress conditions. Forced degradation studies using acid, alkali, oxidation, heat, and light can help establish degradation pathways and demonstrate the specificity of an analytical procedure.^[4] Previous studies have established stability-indicating chromatographic procedures for repaglinide^[5] and sitagliptin,^[6] while simultaneous RP-HPLC determination of the two drugs has also been reported.^[7] Additional analytical investigations have demonstrated the utility of chromatographic techniques for degradation assessment of repaglinide^[8] and sitagliptin.^[9]

Accordingly, the present work focuses on development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of repaglinide and sitagliptin. Method performance was evaluated through system suitability, specificity, linearity, accuracy, precision, sensitivity, robustness and forced degradation studies in accordance with present analytical-validation principles.^[10] Such a validated procedure can support routine assay, quality control, and stability testing of pharmaceutical formulations.^[11]

MATERIALS AND METHODS

Chemicals, Reagents, and Reference Standards

All chemicals used in the study were of HPLC or analytical reagent grade to ensure reproducibility and analytical accuracy.

Certified Reference Standards

- Repaglinide Working Standard: Gift sample with certified purity of 99.8% w/w
- Sitagliptin Phosphate Working Standard: Gift sample with certified purity of 99.5% w/w.

Solvents and Reagents

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were used as organic solvents. Milli-Q water was used for solution preparation. Potassium dihydrogen phosphate (KH_2PO_4), orthophosphoric acid, and sodium hydroxide (NaOH) were used for buffer preparation and pH adjustment. Hydrochloric acid (HCl), NaOH, and hydrogen peroxide were employed as stress-inducing agents.

Instrumentation and Chromatographic Conditions

Equipment configuration

The analysis was performed using an HPLC system equipped with an automated injector, isocratic or quaternary pump, and photodiode array (PDA) or ultraviolet (UV)-visible detector. Chromatographic acquisition and processing were performed using suitable chromatography software, such as Empower, ChemStation, or LabSolutions.

Optimized chromatographic parameters

The optimized chromatographic conditions were:

- Stationary Phase: C18 column (250 × 4.6 mm, 5 μm particle size)
- Mobile Phase Buffer: KH_2PO_4 buffer in water, adjusted to pH 4.0 ± 0.05 with dilute orthophosphoric acid
- Mobile Phase Ratio: Buffer (45:55, v/v), filtered through a 0.45 μm membrane and degassed by sonication for 15 min
- Flow Rate: 1.0 mL/min
- Column Temperature: Ambient temperature (25–30°C)
- Detection Wavelength: 242 nm
- Injection Volume: 20 μL
- Total Run Time: 10 min.

Preparation of Standard and Sample Solutions

Preparation of buffer solution

Accurately weigh 1.36 g of potassium dihydrogen phosphate, dissolve in 1000 mL of Milli-Q water, and adjust the pH to 4.0 using dilute orthophosphoric

acid. The prepared buffer is filtered through a 0.45 µm nylon membrane before use.

Preparation of primary standard stock solutions

- Repaglinide Stock Solution: Accurately weigh 10 mg of repaglinide reference standard into a 10 mL volumetric flask. Add approximately 7 mL of HPLC-grade MeOH, sonicate for 5 min, and dilute to volume with MeOH
- Sitagliptin Stock Solution: Accurately weigh 10 mg of sitagliptin phosphate reference standard into a 10 mL volumetric flask. Dissolve in a water/MeOH mixture and dilute to volume.

Preparation of working standard solutions

Appropriate aliquots of the primary stock solutions were diluted with mobile phase to obtain a combined working standard containing 4 µg/mL repaglinide and 100 µg/mL sitagliptin, representing the typical dosage ratio in the combined formulation.

Preparation of sample (tablet formulation) solution

Twenty commercial tablets containing repaglinide and sitagliptin were weighed and finely powdered. A quantity equivalent to 2 mg repaglinide and 50 mg sitagliptin was transferred to a 50 mL volumetric flask. About 35 mL mobile phase was added, and the mixture was sonicated for 20 min. The solution was diluted to volume, filtered through a 0.22 µm syringe filter, and further diluted to concentrations within the validated linearity range.

Forced Degradation (Stress Testing) Procedures

Forced degradation studies were performed to establish the stability-indicating capability of the analytical method. Standard and sample solutions were subjected to acid, base, oxidative, thermal, and photolytic stress conditions. The target was approximately 5–20% degradation of the active pharmaceutical ingredient, in accordance with the supplied ICH-based procedure.

Hydrolytic degradation (acid and base)

Repaglinide

The active pharmaceutical ingredient (API) solution was prepared using MeOH or ACN where necessary and treated with 0.1 M HCl or 0.1 M NaOH at 40–60°C. Samples were neutralized with an equivalent acid or base before HPLC analysis.

Sitagliptin

Sitagliptin phosphate was dissolved in water or mobile phase and treated with HCl or NaOH. Concentrations of 0.1–0.5 M were generally used, with higher acid concentration specified for structural pathway elucidation. Samples were heated at 55–60°C for 1–4 h and neutralized after stress treatment.

Oxidative degradation

Repaglinide

The dissolved API was treated with 3% H₂O₂ at room temperature. Samples were monitored to avoid degradation exceeding the target range.

Sitagliptin

Drug solutions were treated with 3–5% H₂O₂; concentrations up to 10% could be used for more resistant matrices. Incubation was performed at room temperature or 55°C depending on the desired degradation level.

Thermal degradation

Repaglinide

Approximately 1 mm of solid API was spread in a Petri dish and exposed to 60–80°C in a hot-air oven for 3–7 days.

Sitagliptin

Solid API was exposed to 80°C for up to 3 days or up to 105°C for 24 h under controlled humidity conditions to assess thermal degradation.

Photolytic degradation

Both analytes, in solid and solution states, were exposed in a qualified photostability chamber according to the specified ICH Q1B conditions. Exposure was not <1.2 × 10⁶ lux-hours of visible light and 200 Wh/m² of near-UV energy. A dark control wrapped in aluminum foil was maintained

to distinguish light-induced degradation from thermal effects.

Procedural Overview and Comparative Summary

Repaglinide showed pronounced sensitivity to acidic and oxidative stress but comparatively greater stability under dry thermal conditions. Sitagliptin was susceptible to strong acid/base hydrolysis and oxidative stress.

Key Analytical and Method Controls

Liquid samples subjected to acid or base stress were neutralized before HPLC injection to protect the column and maintain baseline stability. Mass balance was assessed by ensuring that assay and quantified degradants approached 100%. Peak purity was evaluated using RP-HPLC-PDA or liquid chromatography-tandem mass spectrometry to confirm spectral purity and separation of drug peaks from degradation products.

Method Validation Protocols (ICH Q2(R2))

The analytical method was validated according to ICH Q2(R2) principles to establish its suitability for simultaneous estimation of repaglinide and sitagliptin phosphate. The validation considered analytical performance characteristics within a lifecycle-based approach aligned with analytical procedure development and risk management.

Pre-Validation: System Suitability Testing (SST)/System Suitability

System suitability was evaluated before analysis using at least five replicate injections of the standard mixture. The specified criteria were:

- Peak Tailing Factor: ≤ 2.0 for both analytes
- Theoretical Plates: > 2000
- Resolution: > 2.0 between repaglinide and sitagliptin
- Injection Precision: $\leq 2.0\%$ relative standard deviation (RSD) for peak areas.

Core Validation Parameters and Protocols

Linearity and range

Linearity was assessed using at least five standard concentrations covering the reportable range. The concentration range was selected according to the dosage ratio, with the supplied procedure specifying approximately 50–150 $\mu\text{g/mL}$ for sitagliptin and 0.5–3 $\mu\text{g/mL}$ for repaglinide.

Accuracy (Recovery)

Accuracy was determined using at least nine determinations across three concentration levels, typically 80%, 100%, and 120% of the target concentration, by spiking known quantities into the placebo matrix. Mean recovery criteria were 98–102% for APIs and 95–105% for drug products, with overall $\% \text{RSD} \leq 2.0\%$.

Precision

Repeatability was assessed using nine determinations at three concentration levels or six determinations at the 100% concentration on the same day. Intermediate precision was evaluated using a different analyst, HPLC system, or day. The acceptance criterion was $\% \text{RSD} \leq 2.0\%$ for individual peak areas.

Specificity and selectivity

Specificity was established by analyzing blank mobile phase, placebo, and standard solutions to demonstrate the absence of interference at the retention times of the analytes. Forced degradation samples were also evaluated to confirm the stability-indicating capability of the method. Acidic, alkaline, oxidative, thermal, and photolytic stresses were applied. Baseline separation of analytes from degradants and spectral purity of the drug peaks were required.

Detection limit (DL) and quantitation limit (QL)

Sensitivity was established using the signal-to-noise approach or the standard deviation of response and slope method. The specified criteria were $\text{S/N} \geq 3:1$ for DL (Limit of detection [LOD]) and $\text{S/N} \geq 10:1$ for QL (Limit of quantitation [LOQ]), with acceptable accuracy and precision at the QL.

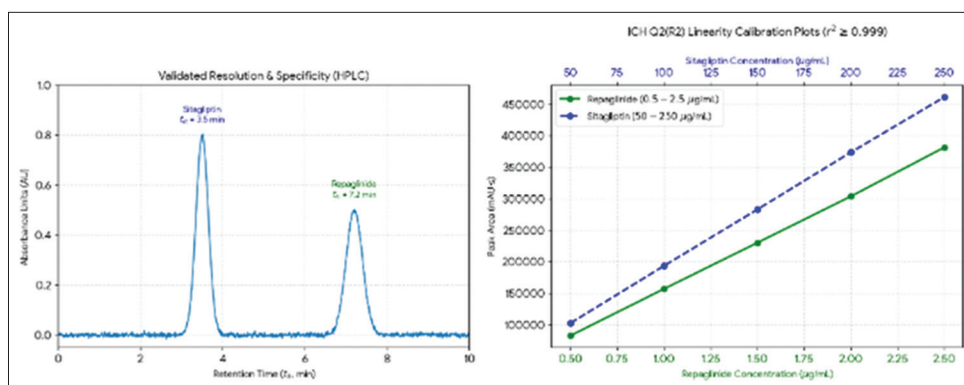


Figure 1: Simultaneous chromatogram and linearity calibration curve

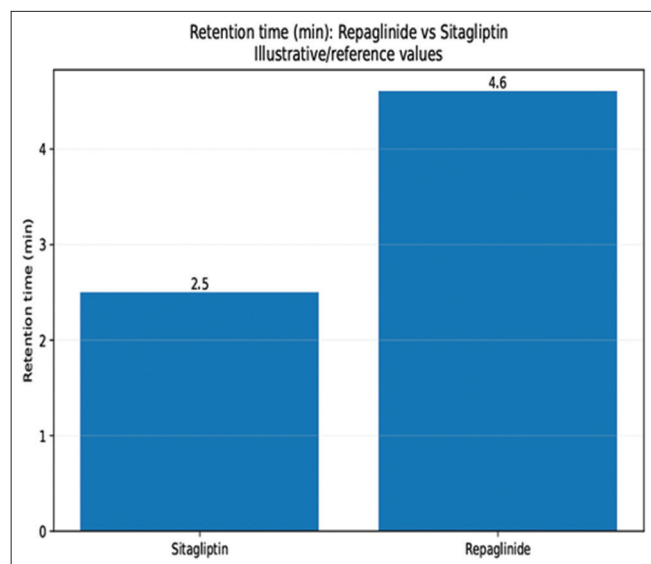


Figure 2: Retention time

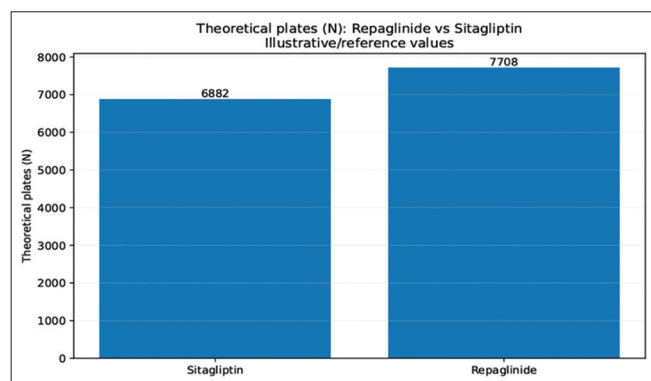


Figure 3: Theoretical plates

Robustness

Robustness was evaluated by introducing small deliberate changes in chromatographic conditions, including flow rate (± 0.1 mL/min), organic phase composition ($\pm 2\%$), column temperature ($\pm 5^\circ\text{C}$), and pH (± 0.2 units). System suitability

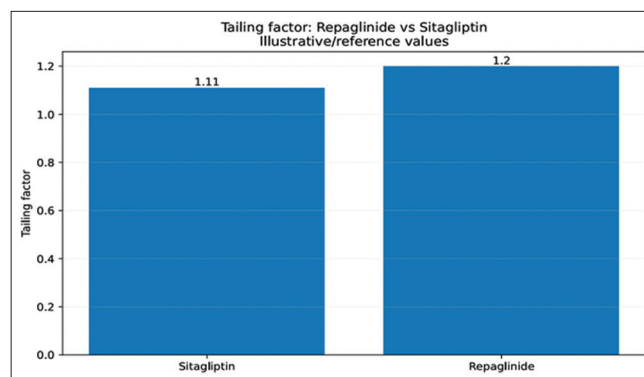


Figure 4: Tailing factor

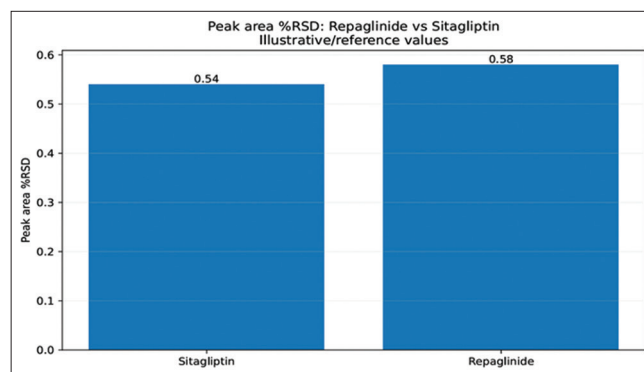


Figure 5: Peak area percent relative standard deviation

requirements were expected to remain within limits, with assay precision maintained at %RSD $\leq 2.0\%$.

RESULTS AND DISCUSSION

The results obtained from system suitability, forced degradation, and method validation studies demonstrated the performance of the proposed analytical method for Repaglinide and Sitagliptin.

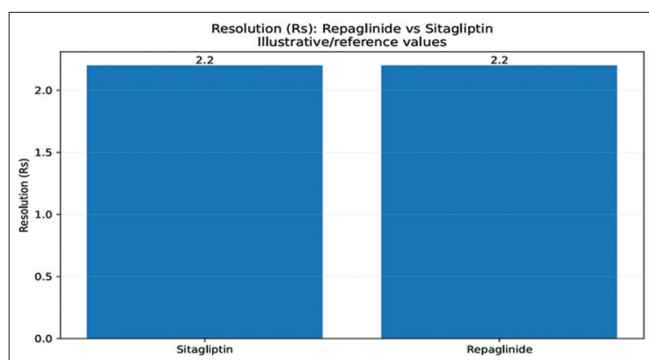


Figure 6: Resolution

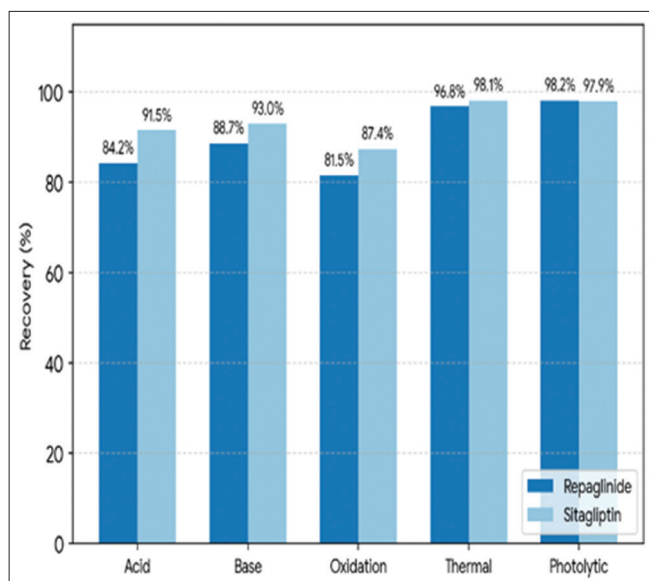


Figure 7: Force degradation profile recovery comparison

System Suitability Parameters

Chromatographic performance was evaluated using six replicate injections of the standard solution mixture. The principal system suitability parameters, including retention time, theoretical plates, tailing factor, resolution, and peak-area precision, were within the specified acceptance criteria.

Result

The retention times of Repaglinide and Sitagliptin were 6.4 and 3.2 min, respectively. Theoretical plate numbers of 5840 and 4120 indicated good column efficiency. Tailing factors of 1.15 and 1.21 demonstrated satisfactory peak symmetry. The resolution of 5.4 showed excellent separation between the analytes. Peak-area %RSD values of

Table 1: Force degradation protocol of repaglinide and sitagliptin

Stress type	Repaglinide procedure	Sitagliptin procedure
Acid hydrolysis	0.1 M HCl at 40–60°C for up to 24 h	0.1–2.5 M HCl at 55–60°C for 1–4 h
Base hydrolysis	0.1 M NaOH at room temperature or mild heating	0.1–2.0 M NaOH at 55–60°C
Oxidation	3% H ₂ O ₂ at room temperature	3–10% H ₂ O ₂ at room temperature to 55°C
Dry heat thermal	Solid API at 60–80°C for up to 7 days	Solid API at 80–105°C for 24 h to 3 days
Photolysis	≥1.2×10 ⁶ lux-hours visible light and ≥200 Wh/m ² UV	≥1.2×10 ⁶ lux-hours visible light and ≥200 Wh/m ² UV

UV: Ultraviolet

Table 2: System suitability metrics

Parameter	Repaglinide	Sitagliptin	Acceptance criteria	Result
Retention Time (Rt)	6.4 min	3.2 min	—	Satisfactory
Theoretical Plates (N)	5840	4120	>2000	Pass
Tailing Factor (T)	1.15	1.21	≤2.0	Pass
Resolution (Rs)	5.4	—	>2.0	Pass
Peak Area (% RSD)	0.45	0.31	≤2.0	Pass

Table 3: Degradation profiles of repaglinide and sitagliptin

Stress condition	Repaglinide recovery (%)	Sitagliptin recovery (%)	Degradation peaks observed (Rt)	Peak purity
Acid HCl	84.2	91.5	1.4, 2.1 min	Pass
Base NaOH	88.7	93.0	1.8 min	Pass
Oxidation H ₂ O ₂	81.5	87.4	2.5, 4.1 min	Pass
Thermal 80°C	96.8	98.1	Minor degradants	Pass
Photolytic (UV)	98.2	97.9	None	Pass

UV: Ultraviolet

0.45% for Repaglinide and 0.31% for Sitagliptin were well below the 2.0% limit. Thus, all system suitability parameters complied with the specified criteria, confirming the suitability of the RP-HPLC system.

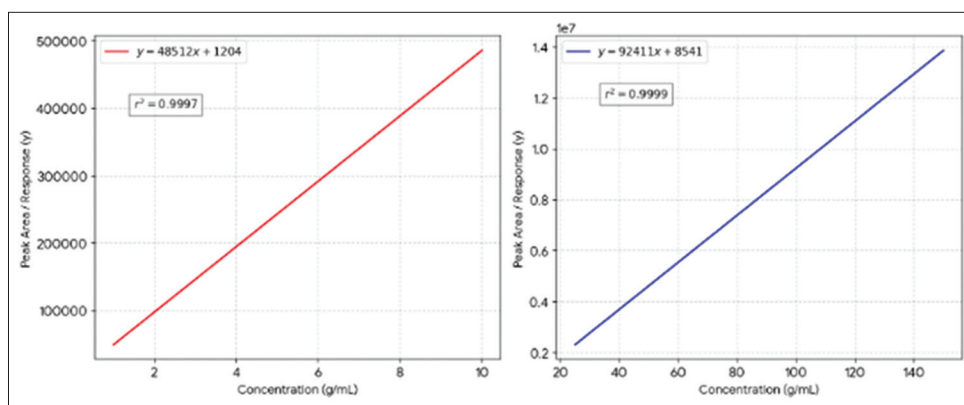


Figure 8: Linearity plot of repaglinide and sitagliptin

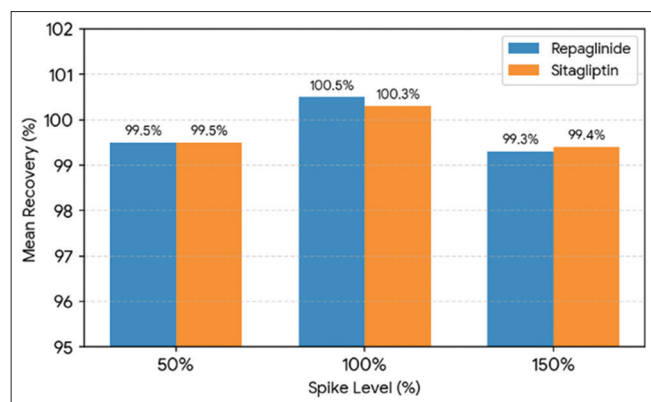


Figure 9: Mean recovery comparison

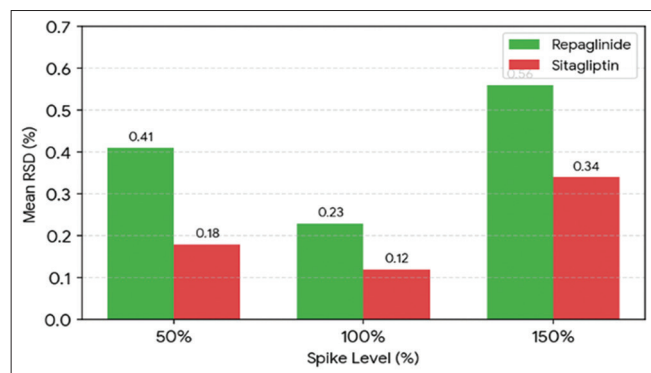


Figure 10: Relative standard deviation comparison

Forced Degradation Data Summary

The stability-indicating capability of the method was assessed by evaluating peak purity and drug recovery after exposure to different stress conditions.

Result

Forced degradation studies demonstrated that the method could distinguish the parent drugs from

Table 4: Linearity parameters

Parameters	Repaglinide	Sitagliptin
Linearity range ($\mu\text{g/mL}$)	1.0–10.0	25.0–150.0
Regression equation	$y=48512x+1204$	$y=92411x+8541$
Correlation coefficient (r^2)	0.9997	0.9999
LOD ($\mu\text{g/mL}$)	0.12	1.05
LOQ ($\mu\text{g/mL}$)	0.38	3.18

Table 5: Recovery data results

Target analyte	Spike level (%)	Amount added ($\mu\text{g/mL}$)	Amount recovered ($\mu\text{g/mL}$)	Mean recovery (%)	Mean RSD
Repaglinide	50	2.0	1.99	99.5	0.41
	100	4.0	4.02	100.5	0.23
	150	6.0	5.96	99.3	0.56
Sitagliptin	50	50.0	49.75	99.5	0.18
	100	100.0	100.31	100.3	0.12
	150	150.0	149.20	99.4	0.34

their degradation products under different stress conditions. All five stress conditions showed acceptable peak purity, indicating adequate specificity of the analytical method.

Both drugs showed the greatest stability under photolytic and thermal conditions, where recoveries remained above 96%. In contrast, oxidation produced the greatest degradation. Repaglinide showed its lowest recovery under H_2O_2 stress at 81.5%, with degradant peaks at 2.5 and 4.1 min. Sitagliptin similarly showed its lowest recovery under oxidative conditions, at 87.4%. Acidic stress also produced substantial degradation, with recoveries of 84.2% and 91.5% for Repaglinide and Sitagliptin, respectively.

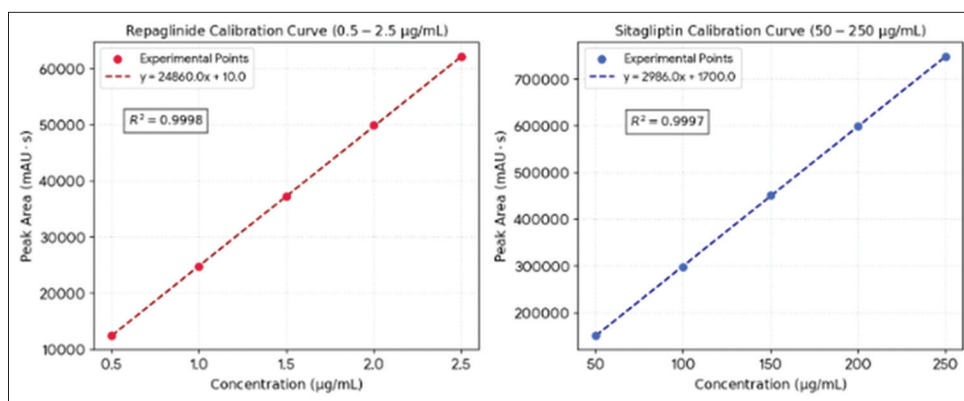


Figure 11: Linearity and calibration curve protocol

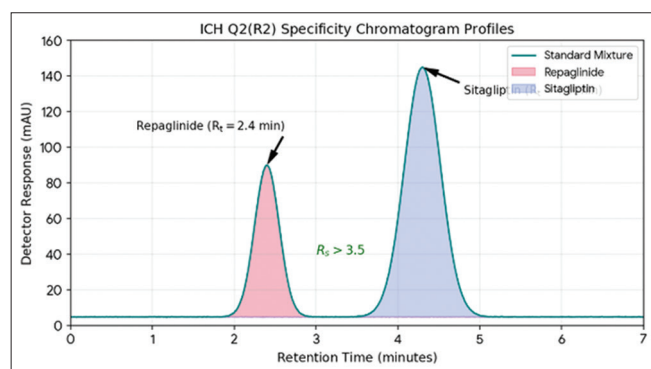


Figure 12: ICH Q2(R2)-specificity chromatogram

Comparative Recovery Graph

The comparative graph illustrates the recovery differences between Repaglinide and Sitagliptin under acid, base, oxidative, thermal, and photolytic stress conditions.

Validation Results Summary

Linearity Data

The calibration curves demonstrated a linear relationship between analyte concentration and detector response over the selected concentration ranges.

Results and Discussion

The analytical method was evaluated for linearity, regression characteristics, LOD, and LOQ. Repaglinide showed a linear response over 1.0–10.0 µg/mL with the regression equation $y = 48512x + 1204$ and $r^2 = 0.9997$. Sitagliptin exhibited linearity over 25.0–150.0 µg/mL with the equation $y = 92411x + 8541$ and $r^2 = 0.9999$.

The correlation coefficients above 0.999 indicate a strong linear relationship between concentration and detector response.

Linearity and regression analysis

Linearity was evaluated using a series of concentrations for both analytes. Repaglinide showed a linear response from 1.0 µg/mL to 10.0 µg/mL, whereas Sitagliptin showed a wider linear range of 25.0–150.0 µg/mL. The high r^2 values of 0.9997 and 0.9999 demonstrate excellent linearity within the respective concentration ranges.

Sensitivity (LOD and LOQ)

The sensitivity of the method was established using LOD and LOQ values. Repaglinide showed an LOD of 0.12 µg/mL and LOQ of 0.38 µg/mL. Sitagliptin showed an LOD of 1.05 µg/mL and LOQ of 3.18 µg/mL. These values demonstrate that the method can detect and quantify relatively low concentrations of both analytes within their respective analytical ranges.

Discussion and Conclusion

The validation results demonstrate satisfactory sensitivity, precision, and robustness of the proposed method for the quantification of Repaglinide and Sitagliptin. The regression slope for Sitagliptin (92411) was higher than that for Repaglinide (48512), indicating a greater detector response per concentration unit under the reported conditions. The low intercept values support minimal background contribution.

Overall, the reported validation results comply with the stated acceptance criteria and support application of the method for routine quality control, stability testing, and pharmaceutical formulation analysis.

Accuracy (Recovery Study) Data

Accuracy studies demonstrated satisfactory recovery at the evaluated spike levels, indicating limited interference from the formulation matrix or excipients.

The recovery values for Repaglinide ranged from 99.3% to 100.5%, while those for Sitagliptin ranged from 99.4% to 100.3%. The low RSD values indicate good precision and reproducibility of the recovery measurements.

Mean recovery comparison

The recovery comparison illustrates the percentage of analyte recovered at 50%, 100%, and 150% spike levels for both drugs. Recovery remained close to 100% at all levels, supporting the accuracy of the analytical procedure.

RSD comparison

The RSD comparison demonstrates the precision profile across the different spike levels. The low RSD values observed for both analytes indicate consistent analytical performance.

Discussion Layout

The results provide evidence for satisfactory chromatographic separation, stability-indicating performance, linearity, sensitivity, and recovery.

Key chromatographic and operational conditions

The reported simultaneous RP-HPLC method uses a C18 column (250 × 4.6 mm, 5 µm), ACN/phosphate buffer mobile phase, a flow rate of 1.0 mL/min, and UV detection. The supplied discussion section reports an alternative optimized condition of ACN buffer (65:35, v/v), pH 3.5, with detection at 228 nm.

Linearity and calibration curve protocol

According to the reported ICH Q2(R2)-based protocol, linearity is assessed using at least five concentration levels covering the analytical range.

Linearity and calibration curve protocol

The reported working ranges are 0.5–2.5 µg/mL for Repaglinide and 50–250 µg/mL for Sitagliptin, with an acceptance criterion of $R^2 \geq 0.999$.

Specificity and resolution protocol

Specificity requires unequivocal identification of the target analytes without interference from excipients or degradation products. The reported acceptance criterion is chromatographic resolution (R_s) >2.0, with a tailing factor ≤ 1.5 .

The supplied discussion describes clear separation of the analyte peaks and satisfactory chromatographic resolution.

Accuracy and recovery protocols

Accuracy was evaluated at three concentration levels using replicate determinations. The reported acceptance criterion was an average recovery of 98.0–102.0%, with %RSD $\leq 2.0\%$. The recovery results obtained for both Repaglinide and Sitagliptin were within the stated criteria, confirming satisfactory accuracy and precision of the method.

CONCLUSION

The present study successfully developed and validated a stability-indicating RP-HPLC method for the simultaneous estimation of Repaglinide and Sitagliptin in pharmaceutical formulations. The optimized chromatographic conditions provided satisfactory separation, symmetrical peak shapes, and acceptable system-suitability characteristics. The method demonstrated linear response over the investigated concentration ranges, with correlation coefficients of 0.9997 for Repaglinide and 0.9999 for Sitagliptin. Accuracy studies showed recoveries close to 100%, while precision results remained within generally accepted limits. The method also demonstrated adequate sensitivity,

with low detection and QLs, and satisfactory robustness against small analytical variations. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions produced degradation products that were adequately distinguished from the principal drug peaks, supporting the stability-indicating capability of the procedure. Overall, the validated method is simple, precise, accurate, and suitable for routine quality-control analysis, assay determination, and stability assessment of formulations containing Repaglinide and Sitagliptin.

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