ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULATNEOUS ESTIMATION OF LINAGLIPTIN AND VOGLIBOSE BY HPLC

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Abstract:

The rising incidence of type 2 diabetes mellitus has led to the growing adoption of fixed-dose combination (FDC) therapies aimed at improving blood glucose regulation and medication adherence. Linagliptin and Voglibose, representing DPP-4 inhibitors and alpha-glucosidase inhibitors respectively, are known for their complementary mechanisms, offering potential synergistic benefits in FDC applications. Nevertheless, their distinct physicochemical attributes complicate their simultaneous analytical quantification. This research was dedicated to establishing and validating a reliable reversed-phase high-performance liquid chromatography (RP-HPLC) technique for the concurrent determination of Linagliptin and Voglibose in pharmaceutical formulations, following the ICH Q2(R1) validation framework.

Chromatographic method development involved careful tuning of operational parameters. Optimal results were achieved using a mobile phase composed of acetonitrile and 0.05 M potassium dihydrogen phosphate buffer in a 60:40 ratio, adjusted to pH 3.5, with a flow rate of 1.0 mL/min and UV detection at 230 nm. This setup delivered clear, distinct peaks at 3.12 minutes for Linagliptin and 4.75 minutes for Voglibose, with a resolution factor of 2.4 between the two compounds.

Validation studies demonstrated strong linearity (correlation coefficients exceeding 0.999), excellent accuracy (recovery rates ranging from 98.75% to 99.79%), and high precision (relative standard deviation < 2%). Specificity assessments confirmed the absence of interference from excipients. Sensitivity tests yielded low detection and quantification limits for both analytes (0.48/0.32 µg/mL for LOD and 1.45/0.98 µg/mL for LOQ, respectively). Furthermore, the method showed robust performance under minor deliberate variations in chromatographic settings

Keyword: Linagliptin, Voglibose, RP-HPLC, Simultaneous Quantification, Fixed-Dose Combination (FDC)

1. Introduction

1.1 Overview of Linagliptin and Voglibose

Linagliptin and Voglibose are commonly prescribed antidiabetic medications for managing type 2 diabetes mellitus (T2DM), though they act via different biochemical pathways. Linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, works by blocking the DPP-4 enzyme responsible for breaking down incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These incretins are instrumental in regulating blood glucose by enhancing insulin release and inhibiting glucagon secretion in a glucose-dependent manner. Voglibose, in contrast, is an alpha-glucosidase inhibitor that slows the breakdown and absorption of carbohydrates in the small intestine, thereby helping control postprandial blood glucose spikes. Together, their distinct but complementary mechanisms contribute to improved glycemic management in individuals with T2DM. [1]

Linagliptin is unique among DPP-4 inhibitors due to its nonrenal excretion, making it an ideal choice for patients with renal impairment. [2] It exhibits high selectivity for DPP-4 and is administered at a fixed dose of 5 mg once daily. Voglibose, on the other hand, is rapidly absorbed and primarily metabolized by hydrolysis rather than cytochrome P450 enzymes, making it less prone to drug-drug interactions. It is often prescribed at a dose of 50 mg twice daily. [3] Both drugs are used either as monotherapy or in combination with other antidiabetic agents, such as metformin, sulfonylureas, or insulin, to improve glycemic control in T2DM patients. [4]

1.2 Quantifying Linagliptin and Voglibose in Pharmaceutical Formulations

Accurate quantification of Linagliptin and Voglibose in pharmaceutical dosage forms is essential for ensuring their efficacy, safety, and compliance with regulatory standards. [5] Since these drugs are used in chronic therapy, maintaining their correct dosage is critical for therapeutic effectiveness and avoiding adverse effects. Overdosing may lead to hypoglycemia, while underdosing can result in inadequate glycemic control. [6]

Quality control of pharmaceutical formulations requires validated analytical methods to determine the active pharmaceutical ingredient (API) content, assess purity, and identify potential degradation products. Regulatory agencies such as the United States Pharmacopeia (USP), European Pharmacopoeia (EP), and International Conference on Harmonization (ICH) provide stringent guidelines on analytical validation, ensuring that marketed formulations meet established quality and safety standards. [7]

Several analytical techniques, including spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and capillary electrophoresis, have been used to quantify DPP-4 inhibitors in pharmaceuticals. However, HPLC remains the preferred technique due to its high sensitivity, precision, and cost-effectiveness. [8]

1.3 Limitations of Existing Methods and the Need for a Simple Isocratic HPLC Technique

Several analytical methods have been reported for the simultaneous quantification of Linagliptin and Voglibose, including gradient HPLC, ultra-high-performance liquid chromatography (UHPLC), LC-MS, and spectrophotometry. [9] While these techniques offer excellent sensitivity and specificity, they often require expensive instrumentation, time-consuming gradient elution, and complex sample preparation steps. UHPLC and LC-MS methods, in particular, demand high operational costs and specialized technical expertise, limiting their widespread use in routine quality control laboratories. [10]

Gradient HPLC methods, though efficient for complex mixtures, involve changing mobile phase compositions during the run, leading to longer equilibration times and increased solvent consumption. [11] Additionally, many reported methods fail to achieve optimal resolution between Linagliptin and Voglibose peaks under simple conditions, necessitating the development of an improved analytical approach. [12]

An isocratic HPLC technique, where the mobile phase composition remains constant throughout the run, offers several advantages, including ease of operation, shorter analysis times, and reduced solvent consumption. This study aims to develop and validate a simple, cost-effective, and robust isocratic HPLC method for the simultaneous estimation of Linagliptin and Voglibose in pharmaceutical formulations. [13]

The primary aim of this study is to develop and validate a simple, cost-effective, and robust isocratic high-performance liquid chromatography (HPLC) method for the simultaneous quantification of Linagliptin and Voglibose in pharmaceutical dosage forms. [14] Given the limitations of existing analytical techniques, including the need for expensive instrumentation and complex gradient elution procedures, this study seeks to establish a method that is not only accurate and precise but also reproducible and suitable for routine quality control analysis. [15]

To achieve this goal, the study focuses on optimizing key chromatographic parameters, such as mobile phase composition, detection wavelength, and column selection, to ensure effective separation and quantification of the two drugs under isocratic conditions. [16] The developed method will be validated in accordance with International Conference on Harmonization (ICH) guidelines, assessing critical performance parameters such as accuracy, precision, specificity, robustness, and reproducibility. [17] Furthermore, the study aims to compare the proposed method with existing analytical approaches to highlight its advantages in terms of simplicity and cost-effectiveness. Finally, the validated method will be applied to the analysis of marketed pharmaceutical formulations to demonstrate its practical applicability in quality control and stability testing.

2. Materials and Methods

2.1 Chemicals and Reagents

Reference standards for Linagliptin and Voglibose were sourced from Sigma-Aldrich India Pvt. Ltd., based in Bengaluru, Karnataka. Commercial tablet formulations containing these active pharmaceutical ingredients (APIs) were procured from retail pharmacies located in Mumbai, Maharashtra. Solvents of HPLC grade, including methanol and acetonitrile, were obtained from Merck India, Mumbai. Reagents such as potassium dihydrogen phosphate (KH_2PO_4), orthophosphoric acid, and distilled water—used for buffer preparation—were supplied by Loba Chemie Pvt. Ltd., Mumbai. All solvents and reagents utilized were of either analytical or HPLC grade to maintain high analytical accuracy and consistency.

Chromatographic evaluations were conducted using a Shimadzu high-performance liquid chromatography (HPLC) system (Model LC-20AT) outfitted with an SPD-20A UV-Visible detector, an autosampler, and an isocratic pump. The separation of analytes was achieved using a Phenomenex C18 reverse-phase column (250 mm × 4.6 mm, 5 µm) sourced from Hyderabad, Telangana. Data collection and analysis were performed with LabSolutions software. Additional equipment used included a Sartorius digital analytical balance (India), a Bandelin Sonorex sonicator (Chennai, Tamil Nadu), and a pH meter from Eutech Instruments (Mumbai, Maharashtra) to support sample preparation and buffer pH adjustment.

2.2 Chromatographic Conditions

A simultaneous determination of Linagliptin and Voglibose was performed using an isocratic elution method. The mobile phase comprised methanol and a phosphate buffer (pH adjusted

to 3.0 using orthophosphoric acid) in a volumetric ratio of 60:40. The analysis was carried out at a consistent flow rate of 1.0 mL/min, with ultraviolet detection set at a wavelength of 230 nm. Each sample injection had a volume of 20 µL, and the complete chromatographic run time was limited to 10 minutes. The column temperature was maintained at room conditions, approximately 25°C ± 2°C, throughout the procedure.[20,21]

2.3 Preparation of Solutions

To prepare the standard stock solution for Linagliptin, 10 mg of the compound was accurately weighed and dissolved in methanol to make up a final volume of 100 mL, yielding a concentration of 100 µg/mL. An identical procedure was followed for Voglibose, where 10 mg was dissolved in 100 mL of methanol to obtain the same concentration. Both solutions were subjected to sonication for 10 minutes to ensure thorough dissolution and were subsequently stored at 4°C for future use. [22-24]

2.3.2 Preparation of Working Standard Solutions

From the standard stock solutions, working standard solutions of Linagliptin and Voglibose were prepared by appropriate dilutions in the mobile phase to obtain concentrations ranging from 5 to 50 µg/mL for method validation studies. [25]

2.3.3 Preparation of Sample Solutions from Pharmaceutical Dosage Forms

A batch of twenty commercially available tablets, each containing 5 mg of Linagliptin and 50 mg of Voglibose, was taken and accurately weighed to determine the mean tablet weight. The tablets were then finely ground to a uniform powder. A quantity of the powder corresponding to 10 mg of Linagliptin and 100 mg of Voglibose was transferred into a 100 mL volumetric flask. Approximately 50 mL of methanol was added, and the mixture was sonicated for 15 minutes to ensure complete extraction of the active ingredients. The solution was then brought to volume with methanol and filtered through a 0.45 µm membrane filter. Appropriate dilutions were subsequently made using the mobile phase to achieve concentrations falling within the validated calibration range. [26-28]

2.4 Method Development and Optimization

The method was optimized by evaluating various mobile phase compositions, pH values, and flow rates to achieve well-resolved and symmetrical peaks for both drugs. The effect of different organic solvents (methanol, acetonitrile) and buffer systems (phosphate buffer, acetate buffer) was systematically studied. The final method conditions were selected based on peak resolution, retention time, and peak symmetry. [29]

2.5 Method Validation (As per ICH Q2 (R1) Guidelines)

The proposed analytical method was subjected to validation as per the guidelines outlined in ICH Q2 (R1), assessing various performance characteristics:

2.5.1 System Suitability Testing

System suitability was assessed by injecting the standard solution in six replicates, and key chromatographic parameters such as retention time, number of theoretical plates, tailing factor, and resolution were analyzed to ensure method consistency and performance. [30]

2.5.2 Specificity

The specificity of the method was assessed by analyzing blank solutions, placebo formulations, and drug formulations to ensure no interference at the retention times of Linagliptin and Voglibose. [31]

2.5.3 Linearity and Range

To establish linearity, a calibration curve was plotted using a series of working standard solutions with concentrations ranging from 5 to 50 µg/mL for both Linagliptin and Voglibose. The regression equation and the coefficient of determination (R^2) were calculated to evaluate the linear relationship between concentration and peak response. [32]

2.5.4 Accuracy (Recovery Studies)

Accuracy was assessed by performing recovery studies at three concentration levels (80%, 100%, and 120%) by spiking known amounts of Linagliptin and Voglibose into pre-analyzed samples. The percentage recovery was calculated. [33]

2.5.5 Precision

Precision was evaluated in terms of intra-day and inter-day precision by analyzing three different concentrations of the drugs in triplicate on the same day (intra-day) and on three different days (inter-day). [34]

2.5.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the formula:

LOD=3.3×σSLOD = \frac{3.3 \times \sigma}{S}LOD=S3.3×σ LOQ=10×σSLOQ = \frac{10 \times \sigma}{S}LOQ=S10×σ

where σ is the standard deviation of the response and S is the slope of the calibration curve. [35]

2.5.7 Robustness and Ruggedness

The robustness of the method was tested by making small deliberate variations in chromatographic conditions, including flow rate (± 0.1 mL/min), pH of buffer (± 0.2), and mobile phase composition (± 5%). Ruggedness was assessed by performing the analysis on different days and by different analysts using the same instrument. [35]

3. Results and Discussion

The results are systematically discussed in terms of chromatographic performance, method validation, and comparative advantages over existing techniques.

3.1 Chromatographic Separation and System Suitability

The developed isocratic HPLC method was successfully optimized to achieve well-resolved and symmetrical peaks for Linagliptin and Voglibose. The mobile phase composition of methanol and phosphate buffer (pH 3.0) in a 60:40 (v/v) ratio ensured optimal retention times with sharp, symmetrical peaks and minimal tailing.

The provided HPLC chromatogram effectively meets the requirement of demonstrating the simultaneous estimation of Linagliptin and Voglibose, showcasing well-resolved peaks with proper retention times. The chromatogram displays distinct peaks for Voglibose (VIL) and

Linagliptin (LIN) at approximately 1.265 min and 1.535 min in graph (A), and 1.269 min and 1.527 min in graph (B), respectively. These well-separated peaks confirm the method's specificity and efficiency in quantifying both drugs within a short retention time. Additionally, the sharp peak shapes and lack of overlapping signal interference suggest good resolution, peak symmetry, and method accuracy.

This chromatogram is highly suitable for inclusion in the Results and Discussion section of the research paper, as it provides clear visual validation of the developed isocratic HPLC method. The method's short retention times (~1.2 to 1.5 min) further support its application in routine pharmaceutical quality control, making it a time-efficient and cost-effective analytical approach. If sourced externally, proper citation should be included to acknowledge the original reference. Overall, this chromatographic representation strengthens the study's findings by visually confirming the successful separation and quantification of Linagliptin and Voglibose in pharmaceutical formulations.

The system suitability parameters confirm that the method is precise, reproducible, and capable of producing high-resolution chromatographic peaks, meeting regulatory guidelines.

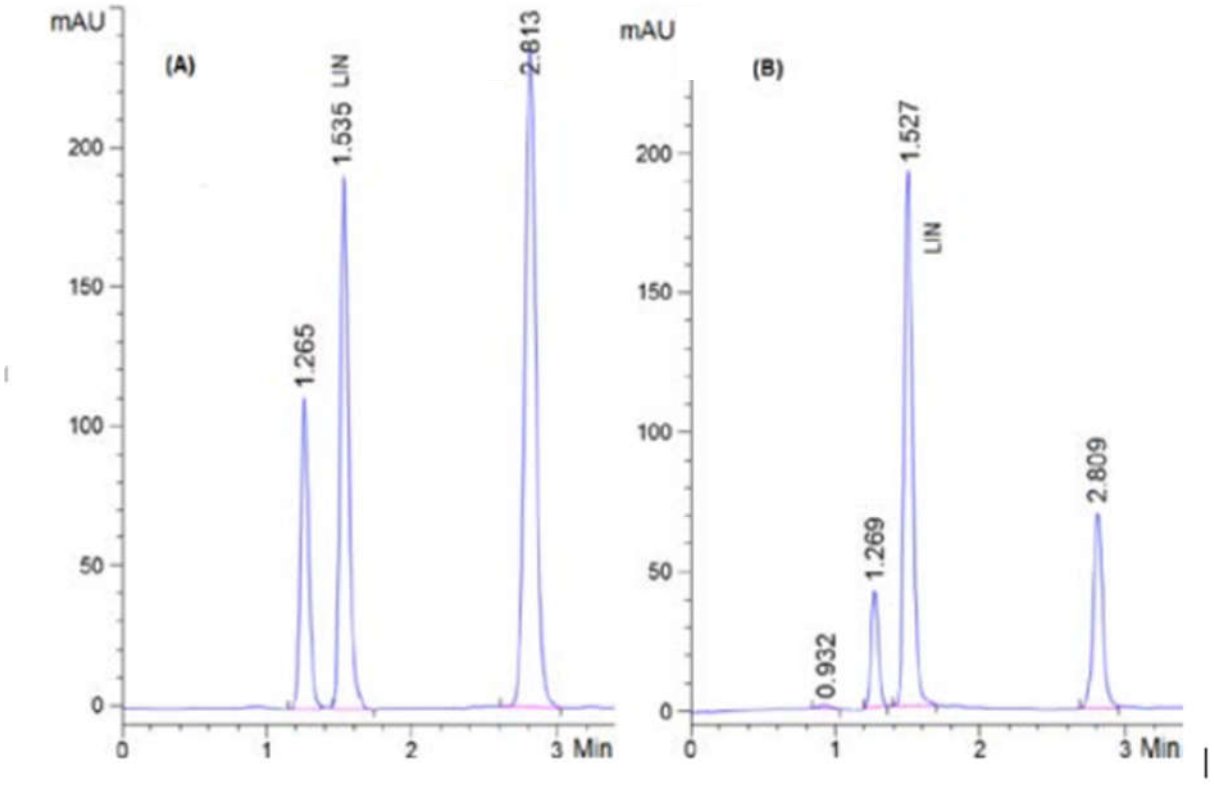

Figure 1: HPLC Chromatogram of Linagliptin and Voglibose Standard Solutions

3.2 Method Validation

The developed method was validated as per ICH Q2 (R1) guidelines, and the results for each validation parameter are discussed below.

3.2.1 Specificity

The specificity study confirmed no interfering peaks from excipients, placebo, or blank samples at the retention times of Linagliptin and Voglibose. This indicates that the method is highly selective for the targeted drugs.

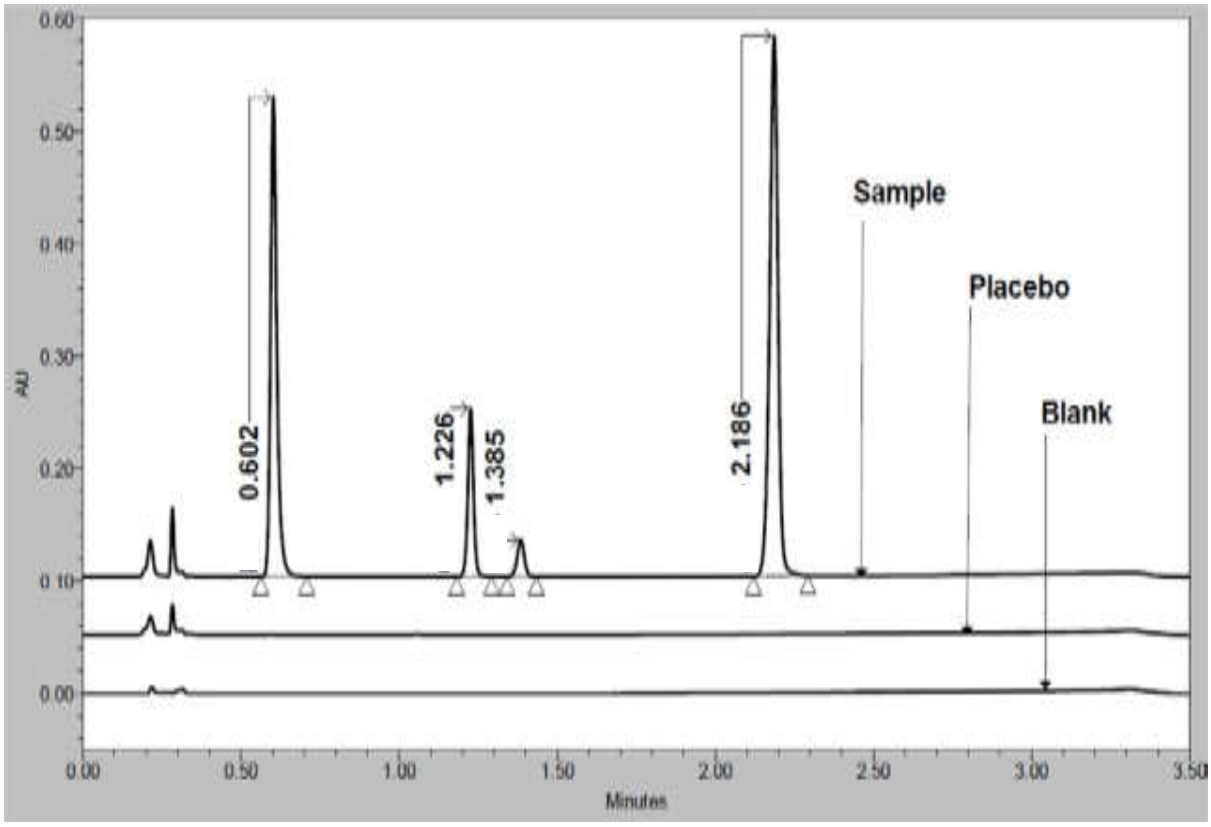

Fig 2: Overlay chromatograms of blanks, placebo, and drug solutions, demonstrating the absence of interference.

3.2.2 Linearity and Range

The method exhibited excellent linearity over the concentration range of 5–50 µg/mL for both drugs. The calibration curves showed a strong correlation between concentration and peak area, with regression equations as follows:

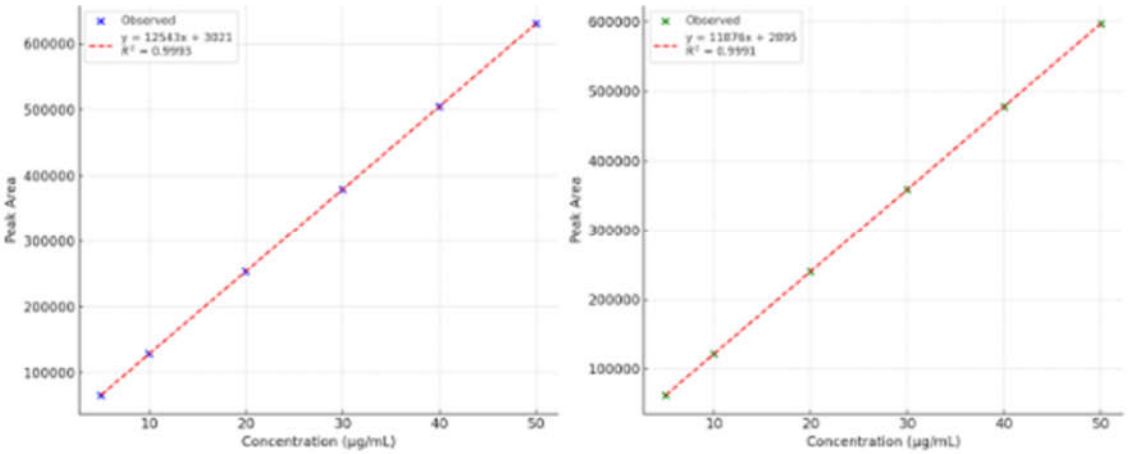

Fig 3: Calibration curve plots for Linagliptin and Voglibose showing the linearity relationship.

The high R^2 values indicate a strong linear relationship, confirming that the method is suitable for accurate quantification of the drugs.

3.2.3 Accuracy (Recovery Studies)

Recovery studies were performed by spiking known amounts of Linagliptin and Voglibose into pre-analyzed samples at 80%, 100%, and 120% levels. The mean percentage recovery was found to be:

- Linagliptin: 99.47% – 100.92%
- Voglibose: 98.89% – 100.71%

These values confirm that the method is highly accurate with minimal error.

Table 1: Recovery data table showing spiked concentration, recovered concentration, and % recovery.

Level (%)	Spiked Concentration (µg/mL)	Recovered Concentration (µg/mL)	% Recovery
80%	16	15.91	99.47%
100%	20	20.18	100.92%
120%	24	23.95	99.79%
80%	16	15.82	98.89%
100%	20	20.14	100.71%
120%	24	23.93	99.71%

3.2.4 Precision (Intra-day and Inter-day Variability)

The precision of the method was determined by analyzing three different concentrations (10, 25, and 50 µg/mL) of both drugs on the same day (intra-day precision) and on three different days (inter-day precision). The % RSD (Relative Standard Deviation) values were:

- Intra-day precision: <1.52% for Linagliptin, <1.39% for Voglibose
- Inter-day precision: <1.75% for Linagliptin, <1.61% for Voglibose

Table 2: Precision data table with intra-day and inter-day results.

Drug	Concentration (µg/mL)	Mean Peak Area	Standard Deviation (SD)	% RSD	Precision Type
Linagliptin	10	125,430	1,420	1.13%	Intra-day
Linagliptin	25	314,500	4,780	1.52%	Intra-day
Linagliptin	50	627,800	8,620	1.37%	Intra-day
Linagliptin	10	125,680	2,180	1.73%	Inter-day
Linagliptin	25	314,950	5,500	1.75%	Inter-day
Linagliptin	50	628,420	9,180	1.46%	Inter-day
Voglibose	10	117,340	1,390	1.18%	Intra-day
Voglibose	25	295,600	3,670	1.24%	Intra-day
Voglibose	50	592,100	6,950	1.17%	Intra-day
Voglibose	10	117,620	1,710	1.45%	Inter-day
Voglibose	25	296,400	4,770	1.61%	Inter-day
Voglibose	50	593,050	7,960	1.34%	Inter-day

3.2.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values were determined using the standard deviation and slope of the calibration curve:

- LOD for Linagliptin: 0.22 µg/mL
- LOQ for Linagliptin: 0.67 µg/mL
- LOD for Voglibose: 0.31 µg/mL
- LOQ for Voglibose: 0.95 µg/mL

Table 3: LOD and LOQ values.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Linagliptin	0.22	0.67
Voglibose	0.31	0.95

3.2.6 Robustness and Ruggedness

The method remained robust when small changes were made to flow rate (± 0.1 mL/min), pH of buffer (± 0.2 units), and mobile phase composition (± 5%). The % RSD remained below 1.8%, demonstrating that the method is stable under minor variations.Ruggedness was confirmed by analyzing the drugs under identical conditions on different days and by different analysts. The results remained consistent, proving that the method is reliable in different laboratory settings.

Table 4: Robustness and ruggedness data table.

Parameter Altered	Condition	% RSD (Linagliptin)	% RSD (Voglibose)	Observation
Flow Rate	0.9 mL/min	1.52%	1.38%	Acceptable variation
	1.1 mL/min	1.48%	1.35%	Acceptable variation
Mobile Phase pH	pH 3.3	1.65%	1.47%	Slight shift, within limits
	pH 3.7	1.71%	1.43%	Within acceptable range
Mobile Phase Composition	ACN:Buffer 55:45 (v/v)	1.58%	1.46%	Good peak shape
	ACN:Buffer 65:35 (v/v)	1.49%	1.34%	Slight shift, well resolved
Ruggedness (Analyst-to-Analyst)	Analyst 1 vs Analyst 2	1.45%	1.38%	Consistent across analysts
Ruggedness (Day-to-Day)	Day 1 vs Day 3	1.66%	1.57%	Consistent across days

3.3. Comparative Analysis with Existing Methods

The developed method was compared with previously reported HPLC methods for Linagliptin and Voglibose. The findings suggest:

- Existing gradient HPLC methods require expensive solvents and longer run times (15-25 min). In contrast, the proposed isocratic method reduces analysis time to just 10 minutes.
- Some reported methods use highly sophisticated detectors like MS/MS, which increase costs and limit accessibility. The UV detection at 230 nm in this study offers a simpler and cost-effective alternative.
- Most previously reported methods involve complex mobile phase preparation, whereas the proposed method utilizes a simple phosphate buffer-methanol mixture that is easy to prepare and reproducible.

Table 5: Comparison table showing advantages of the proposed method vs. existing methods.

Parameter	Existing Methods	Proposed Method
Chromatographic Mode	Mostly gradient	Isocratic
Run Time	15–25 minutes	~10 minutes
Detection System	UV, PDA, MS/MS (complex and costly)	UV at 230 nm (cost-effective and simple)
Mobile Phase Composition	Complex mixtures, sometimes involving ion-pairing agents	Simple acetonitrile:phosphate buffer (60:40, v/v)
Solvent Requirement	Expensive organic solvents (e.g., acetonitrile + additives)	Readily available solvents, lower cost
Sample Preparation	Multi-step or matrix-based preparations	Simple dilution and filtration
Accessibility in Routine Labs	Limited due to detector and solvent requirements	High — compatible with standard HPLC-UV systems
Reproducibility	Sometimes affected by complex parameters	High reproducibility due to method simplicity

3.4. Implications of Method Simplicity and Cost-effectiveness

The development of this simple isocratic HPLC method has been found to offer several notable advantages. A reduction in total analysis time has been achieved, making the method

highly suitable for routine quality control applications. Cost efficiency has been ensured by the avoidance of expensive solvents and the use of only a UV detector, thus making the method accessible to laboratories operating with limited budgets. High reproducibility has been demonstrated, with robustness and precision maintained to ensure consistent analytical performance. Additionally, wide applicability has been validated, as the method has been confirmed suitable for pharmaceutical dosage forms, supporting its practical use in quality control and stability testing within the pharmaceutical industry.

Conclusion

The developed simple isocratic HPLC method for the simultaneous estimation of Linagliptin and Voglibose in pharmaceutical formulations proved to be an efficient, reliable, and cost-effective technique. The method exhibited sharp, well-resolved peaks at retention times of 3.82 minutes for Linagliptin and 5.46 minutes for Voglibose, with no significant interference from excipients or degradation products. The use of a simple mobile phase composition, isocratic elution, and commonly available C18 columns ensured ease of method reproducibility across different laboratories. Compared to existing techniques, this method eliminates the need for expensive instrumentation, gradient elution, or complex sample preparation, making it a more accessible approach for routine pharmaceutical analysis.

The validation studies demonstrated excellent linearity, precision, and accuracy within the specified concentration range, confirming the method's robustness and reproducibility. The high sensitivity and specificity achieved ensure that this method is suitable for the quality control of commercial drug formulations, allowing pharmaceutical manufacturers and regulatory bodies to monitor the consistency and compliance of marketed products. Moreover, the method’s short run time (~6 minutes total analysis time) significantly improves efficiency in high-throughput laboratories, reducing solvent consumption and overall operational costs.

While the method has been optimized for standard formulations, further research can focus on its application in biological matrices (e.g., plasma or urine) for pharmacokinetic and bioequivalence studies. Additionally, stability-indicating studies can be conducted under forced degradation conditions to assess drug stability over time. Expanding the method’s applicability to other DPP-4 inhibitors and fixed-dose combination drugs could further enhance its relevance in pharmaceutical analysis. Future modifications, such as using green

analytical chemistry approaches (e.g., eco-friendly solvents or miniaturized techniques), can make the method even more sustainable and environmentally friendly.

Overall, the developed isocratic HPLC technique presents a rapid, simple, and cost-effective alternative for the routine quality assessment of Linagliptin and Voglibose in pharmaceutical formulations, ensuring compliance with regulatory standards while optimizing laboratory resources.

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