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Development and Validation of HPLC Method for Estimation of Linagliptin Drug in Solid Dosage Form

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Abstract: ***Background:*** *Linagliptin is a xanthine-derived, highly selective dipeptidyl peptidase-4 (DPP-4) inhibitor widely utilized in the therapeutic management of Type 2 diabetes mellitus. Structurally distinct from other therapeutic agents in its class, it is primarily cleared unchanged via the biliary-fecal pathway. Consequently, it features a clinical advantage as it requires no active dosage corrections in patients with renal or hepatic impairment. Currently, there is an explicit absence of an official compendial method for its estimation in major pharmacopoeias such as the United States Pharmacopeia (USP), necessitating the development of precise and cost-effective regulatory methodologies.*

Objective: *To develop and validate a simple, rapid, precise, and accurate High-Performance Liquid Chromatography (HPLC) method for the quantitative determination of Linagliptin in pharmaceutical solid dosage forms.*

Methods: *Chromatographic separation was achieved using an Agilent Technologies 1100 Series HPLC system equipped with a Hypersil C18 column (250 mm × 4.6 mm i.d., 5 µm particle size). The optimized mobile phase consisted of a mixture of Methanol and 0.01M Sodium Acetate buffer (with pH adjusted to 3.5 using dilute glacial acetic acid) in a ratio of 85:15 v/v. The flow rate was set isocratically at 1.0 mL/min, using an injection volume of 20 µL under ambient temperature conditions. UV detection was executed at the optimized wavelength of 296 nm. Method validation was thoroughly executed in compliance with International Council for Harmonisation (ICH) Q2(R1) regulatory guidelines.*

Keywords: Linagliptin, HPLC, Method Validation, ICH Guidelines,

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) represents a progressive, metabolic healthcare challenge globally, with increasing prevalence among elderly populations and individuals with chronic comorbidities. Achieving and maintaining glycemic control is often a dynamic battle, necessitating advanced combination therapies as pancreatic beta-cell function deteriorates over time. Incretins are gut-derived hormones secreted in response to nutrient intake, augmenting glucose-dependent insulin release beyond typical homeostatic thresholds. The two primary incretins are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). Dipeptidyl peptidase-4 (DPP-4) inhibitors represent a powerful therapeutic class that acts by blocking the systemic degradation of these hormones. This prolongs incretin activity, effectively increasing glucose-dependent insulin release from pancreatic cells while actively reducing glucagon secretion.

Linagliptin—chemically designated as 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione—is a highly selective, xanthine-based oral DPP-4 inhibitor. Structurally independent from other agents in its class, Linagliptin features a unique non-renal elimination profile; it is excreted unchanged via the biliary-fecal pathway. Pharmacokinetic investigations confirm that no dosage adjustment is required in patients suffering from renal or hepatic impairment, presenting a major advantage over alternative anti

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diabetic agents. It is a white to pale-yellow solid with a molecular weight of 472 g/mol, a melting point of 133–135°C, and is freely soluble in methanol.

A comprehensive literature survey reveals that despite its widespread clinical usage, there is no official compendial method established in the United States Pharmacopeia (USP) for its individual evaluation in pharmaceutical dosage forms. Analytical separation techniques such as High-Performance Liquid Chromatography (HPLC) are the backbone of regulatory quality assurance due to their unmatched speed, resolution, and reproducibility. Developing simple, rapid, and economical analytical techniques utilizing cost-effective buffers is vital for manufacturing and routine testing facilities. The present study describes the development, optimization, and validation of a rapid HPLC technique using an accessible Methanol and Sodium Acetate buffer system, fully validated against international regulatory thresholds (ICH Q2(R1)).

II. MATERIALS AND REAGENTS

2.1 Procurement of Chemicals and Standards

An authenticated reference standard of Linagliptin was generously provided by CTX Lifescience Pvt. Ltd. (Surat, Gujarat, India). Commercial tablets of Linagliptin (**Linapil tablets, Alkem Healthcare Science, Label Claim: 5 mg per tablet, Batch No: 25443991**) were purchased from a local commercial pharmacy. All chemical solvents used throughout the experimentation—including HPLC-grade Methanol, Glacial Acetic Acid, and Sodium Acetate—were procured from Merck Ltd. and Fisher Scientific Pvt. Ltd. (India). High-purity deionized water was prepared using an in-house water purification setup.

2.2 Instrumental Architecture and Software

Chromatographic investigations were carried out on an **Agilent Technologies 1100 Series HPLC system** consisting of an isocratic pump and a high-sensitivity UV detector. Chromatographic data processing and peak integrations were managed via **EZ Chrome Elite software**. System adjustments were handled via an analytical microbalance (ESSAE DS-852J Series) , a Digisun Electronics pH system 7007 , and a Servewell Instruments ultrasonicator (RC-SYSTEM 1700) for degassing mobile phases.

III. EXPERIMENTAL METHODOLOGY

3.1 Chromatographic Conditions Optimization

The optimized, finalized chromatographic conditions utilized for method validation are systematically detailed in **Table 1**:

Table 1: Finalized Chromatographic Conditions for Linagliptin Analysis

Parameter	Optimized Operational Specification
HPLC Instrument System	Agilent Technologies 1100 Series
Stationary Phase (Column)	Hypersil C18 (250mm 4.6 mm i.d,5 um particle size)
Mobile Phase Composition	Methanol : 0.01M Sodium Acetate Buffer (pH 3.5 via Glacial Acetic Acid) (85:15 v/v)
Isocratic Flow Rate	1.0 mL/min
Detection Target Wavelength	296 nm
Injection Sampling Volume	20 uL
Operational Temperature	Ambient
Diluent System Matrix	Mobile Phase Composition

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3.2 Preparation of Buffer and Mobile Phase

0.01 M Sodium Acetate Buffer: Accurately weighed quantities of analytical grade Sodium Acetate were dissolved in HPLC-grade water to prepare a 0.01M solution. The pH of the solution was monitored and carefully adjusted to 3.5 using dilute glacial acetic acid.

Mobile Phase Assembly: Methanol and the adjusted 0.01M Sodium Acetate buffer were thoroughly mixed in a precise volume ratio of **85:15 v/v**. The combined mobile phase was filtered through a 0.45um nylon membrane filter and subjected to 10 minutes of active degassing via ultrasonication to prevent micro-bubble baseline interference.

3.3 Standard Solution Engineering

Primary Standard Stock Solution (100 ug/mL): Exactly 10 mg of Linagliptin reference standard was weighed and quantitatively transferred into a clean 100 mL volumetric flask. Approximately 50 mL of HPLC-grade Methanol was introduced to dissolve the drug matrix, and the solution was sonicated for 10 minutes. The volume was then brought up to the 100 mL calibration mark with Methanol to deliver a primary stock concentration of 100 ppm (100 ug/mL).

Working Standard Dilutions: Appropriate aliquots were mathematically drawn from the standard stock solution and diluted with the specific diluent matrix (85% Methanol: 15% Buffer) to yield working target concentrations spanning 25, 37.5, 50, 62.5, and 75 ug/mL for calibration and validation evaluations.

3.4 Application to Marketed Formulation (Assay Preparation)

Twenty commercial **Linapil tablets (5 mg Linagliptin)** were weighed independently to deduce average batch tablet weight. The tablets were crushed into a fine, homogeneous powder matrix. A portion of powder equivalent to exactly 10 mg of active Linagliptin API was weighed and transferred into a 100 mL volumetric flask. The sample was dissolved using Methanol, sonicated for 15 minutes to facilitate total matrix extraction, filtered through a standard filter medium to remove insoluble tablet excipients, and diluted to volume to achieve a final sample working concentration profile.

Fig 1: UV-Visible Absorption Spectrum of Linagliptin Standard

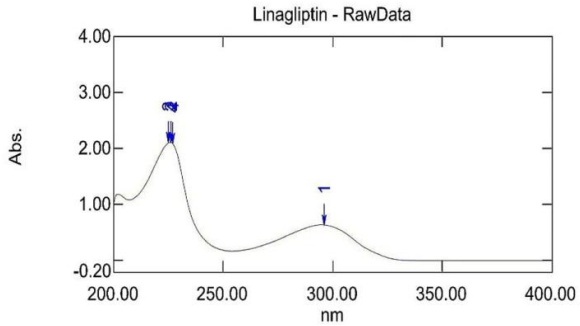

Figure 1: UV-Visible absorption spectrum scanning showing maximum absorbance wavelength lambda max of Linagliptin at 296 nm.

IV. RESULTS AND DISCUSSION

4.1 System Suitability Validation

System suitability testing was performed by making replicate injections of the standard Linagliptin solution to verify that the operational system hardware functions within strict acceptable regulatory performance criteria.

Fig 2:Optimized Standard HPLC Chromatogram of Linagliptin

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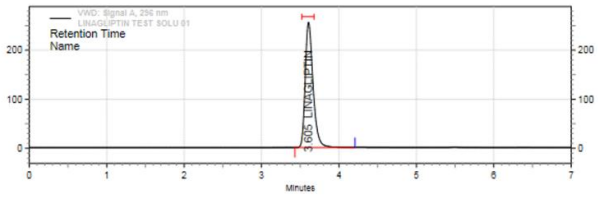

Figure 2: Standard HPLC chromatogram showing a sharp, well-resolved symmetric peak of Linagliptin at an optimized retention time of approximately 3.58 to 3.60 minutes.

Table 2: System Suitability Parameters (n=6)

Parameter Criterion Evaluated	Found Method Value	Required Regulatory Threshold
Retention Time	3.58 min	Information Parameter
Theoretical Plates	5894	> 2000 plates
Tailing Factor	1.12	< 2.0
Peak Area %RSD	0.43%	2.0%

4.2 Specificity

Specificity was examined by injecting blank diluent alongside the prepared tablet formulation matrix. No interfering peaks from blank reagents or sample tablet excipients were observed at the retention window of Linagliptin (3.58 minutes), demonstrating that the developed method is highly selective for the active pharmaceutical ingredient.

Fig 3: Chromatographic Trace of Mobile Phase Matrix Baseline (Blank Target)

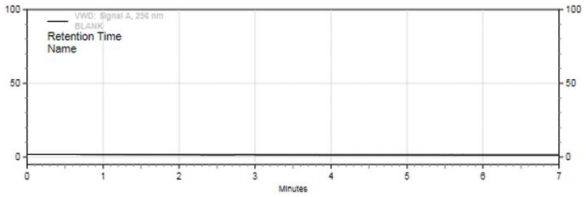

Figure 3: Representative HPLC chromatogram of blank diluent matrix displaying clear baseline resolution and an absolute absence of matrix component interference at the drug retention window

4.3 Linearity and Range

Linearity was calculated across five distinct concentration points ranging from **25 to 75 ug/mL** (50% to 150% of target concentration metrics). Peak area values were plotted against concentrations to generate a standard regression equation line.

Fig 4: Standard Linear Regression Calibration Curve of Linagliptin

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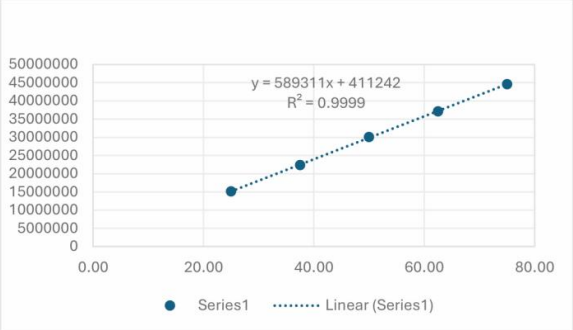

Figure 4: Calibration curve plot exhibiting an excellent linear regression correlation profile (R^2 = 0.998) across working target concentration values spanning 25 to 75 ug/mL (y = 589,311x + 156,813).

Table 3: Linearity Calibration Data Matrix

Linear Solution Point	Concentration (µg/mL)	Mean Peak Area Output
L1	25.0	14,732,951
L2	37.5	22,098,426
L3	50.0	29,465,112
L4	62.5	36,831,059
L5	75.0	44,198,720

- **Linear Regression Equation:** y = 589,311x + 156,813
- **Correlation Coefficient (R^2): 0.998**

The correlation coefficient value (R^2 = 0.998) indicates excellent linear correlation between target drug concentration and analytical detector peak area response.

4.4 Method Accuracy (Recovery Studies)

Method accuracy was verified by standard addition techniques at 80%, 100%, and 120% spikes relative to pre-analyzed formulation baselines.

Table 4: Accuracy Recovery Matrix Findings (n=3)

Level Spike %	Amount Added (µg/mL)	Amount Recovered (µg/mL)	Mean % Recovery	% RSD
80%	40.0	39.94	99.96%	1.11%
100%	50.0	49.90	98.73%	1.05%
120%	60.0	59.85	99.28%	1.17%

The recovery results ranging from 98% to 102% demonstrate that the method is highly accurate and free from systemic formulation matrix bias.

4.5 Method Precision

Precision parameter checking was split into Repeatability (Intraday Precision) and Intermediate Precision (Interday Precision performed across distinct operational calendar dates).

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Table 5: Intraday and Interday Precision Metrics

Precision Type	Sample Test Number	Calculated Mean % Assay	% RSD Outcomes
Intraday Precision	n=6	99.12%	**0.54%**
Interday Precision	n=6	98.78%	**0.89%**

All statistical evaluation parameters for precision generated %RSD scores under the strict 2.0\% standard limit, validating the methodology as highly reproducible.

4.6 Sensitivity Metrics (LOD and LOQ)

Sensitivity parameters were mathematically determined using the standard deviation of responses and the calibrated analytical calibration curve slope:

The detection limit (LOD) of the method was determined by the following equation:

LOD= 3.3 × Avg. SD / Slope

LOD = 156813× 3.3 / 589311

LOD = 0.88 µg/ml

Were, SD = Standard Deviation = 156813,

S = Slope of the curve = 589311.

The Limit of Detection (LOD) was found to be 0.88µg/ml.

The quantification limit (LOQ) of the method was determined by the following equation:

LOQ=10 × Avg. SD / Slope

LOQ = 156813× 10 / 589311

LOQ = 2.66µg/ml

Were, SD = Standard Deviation = 156813,

S = Slope of the curve = 589311.

The Limit of Quantification (LOQ) was found to be 2.66 µg/ml.

The low LOD and LOQ metrics confirm that the method is sensitive enough for routine microscale quality control screening.

4.7 Robustness Testing

Robustness was assessed by introducing deliberate minor changes to critical operational parameters, including flow rate and detection wavelength.

Change in flow rate standard solution 0.9

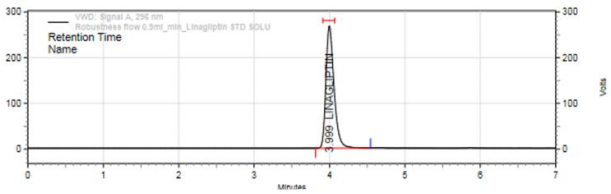

Fig 5: Chromatogram of Robustness of Change in Flow Rate (0.9 std)

Change in flow rate test solution 0.9

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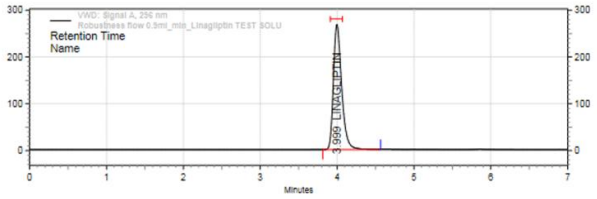

Fig 6: Chromatogram of Robustness of Change in Flow Rate (0.9 test)

Change in flow rate standard solution1.1

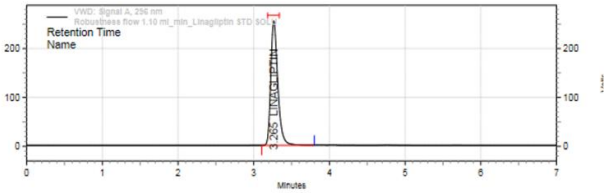

Fig 7: Chromatogram of Robustness of Change in Flow Rate (1.10 std)

Change in flow rate test solution 1.1

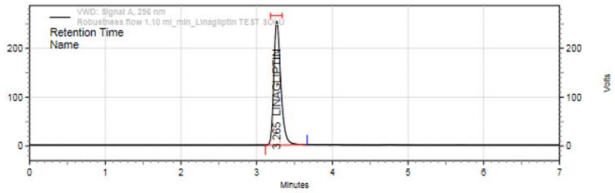

Fig 8: Chromatogram of Robustness of Change in Flow Rate (1.10 test)

Change in wavelength standard solution 294

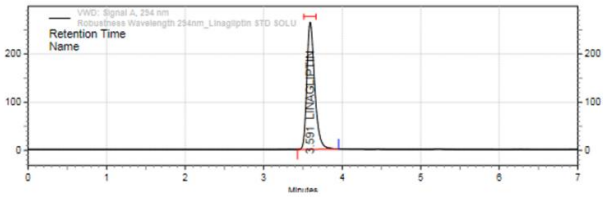

Fig 9: Chromatogram of Robustness of Change in Wavelength 294 std

Change in wavelength test solution 294

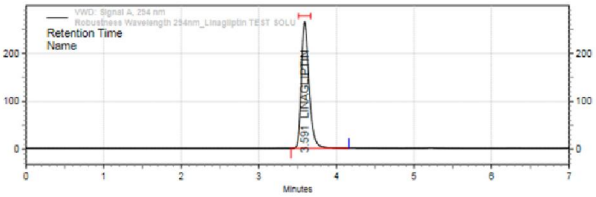

Fig 10: Chromatogram of Robustness of Change in Wavelength 294 test

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Change in wavelength standard solution 298

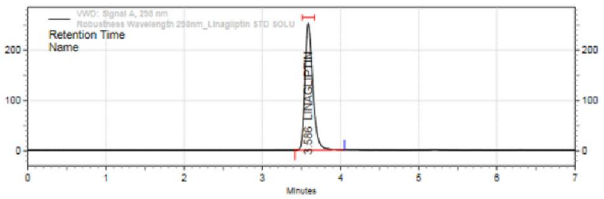

Fig 11: Chromatogram of Robustness of Change in Wavelength 298 std

Change in wavelength test solution 298

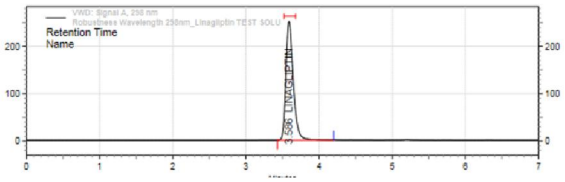

Fig 12: Chromatogram of Robustness of Change in Wavelength 298 test

Table 6: Robustness Parameter Matrix Findings

Robust Parameter Variant	Adjusted Operational Specification	Found Peak Area Value	Found Formulation % Assay
Standard Baseline	1.0 mL/min at 296 nm	29,465,112	99.12%
Flow Rate Low	0.90 mL/min	33,307,595	98.78%
Flow Rate High	1.10 mL/min	27,324,596	97.89%
Wavelength Low	294 nm	30,847,732	99.92%
Wavelength High	298 nm	29,297,990	98.27%
System % RSD	**Acceptance threshold** The % RSD should be not more than 2.0%	—	**0.74%**

The %RSD remained within acceptable limits (The % RSD should be not more than 2.0%), confirming the robustness of the analytical method against small experimental variations.

4.8 Formulation Analysis (Assay)

The validated method was applied for the quantitative determination of Linagliptin in commercial Linapil 5 mg tablets.

Figure 13: Chromatogram from the quantitative determination of Linagliptin in commercial solid oral tablets (Linapil 5 mg), showing clean separation from common pharmaceutical excipients.

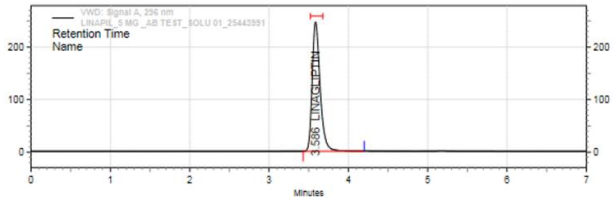

Fig 13: Chromatogram of Market Formulation Test 1

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The validated method was applied for the quantitative determination of Linagliptin in commercial Linapil 5 mg tablets. The assay results of the marketed formulation were found to be 96.88% and 97.32%, which are slightly below the specified acceptance limits. This indicates a minor deviation from the labeled amount of the drug. However, the % assay values obtained were consistent and reproducible, demonstrating good precision of the method. The developed analytical method is therefore suitable for routine quality control analysis, although the formulation may require further evaluation..

V. CONCLUSION

A simple, rapid, precise, accurate and rugged HPLC method was developed and validated for the estimation of Linagliptin in pharmaceutical solid oral dosage forms. The method utilizes a straightforward Methanol and Sodium Acetate mobile phase system, providing sharp resolution and an efficient run time with a retention time of approximately 3.58 minutes. Validation evaluations performed in compliance with ICH Q2(R1) guidelines confirmed excellent linearity, precision, sensitivity, and accuracy. Because the method uses readily accessible reagents and features a short runtime, it is highly suitable for routine quality control, batch release, and assay testing of Linagliptin formulations in analytical manufacturing environments.

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