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Analytical Method Development and Validation of Glimepiride in Tablet Dosage Form by New RP-HPLC

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Abstract:

A simple, precise, accurate, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantitative estimation of Glimepiride in tablet dosage form. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of Acetonitrile: Phosphate Buffer (pH 3.0) in the ratio of 60:40 (v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 230 nm using a UV/PDA detector. Under optimized chromatographic conditions, Glimepiride showed a sharp and symmetrical peak with a retention time of approximately 5.8 minutes.

The developed method was validated according to ICH Q2(R1) guidelines for parameters such as system suitability, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method exhibited excellent linearity in the concentration range of 10–60 µg/mL with a correlation coefficient ($r^2 = 0.9994$). The %RSD values obtained for intraday and interday precision studies were 0.48% and 0.62%, respectively, indicating good repeatability and reproducibility of the method. Accuracy studies performed by recovery method at 80%, 100%, and 120% levels showed mean recovery of 99.73%, confirming the reliability of the developed method. The calculated LOD and LOQ values were found to be 0.85 µg/mL and 2.58 µg/mL, respectively, demonstrating good sensitivity of the method. Robustness studies revealed that small deliberate changes in chromatographic conditions such as flow rate and wavelength did not significantly affect analytical performance.

The developed RP-HPLC method was found to be simple, rapid, precise, accurate, economical, and suitable for routine quantitative analysis of Glimepiride in bulk drug and marketed tablet dosage forms. The method can also be effectively applied for quality control analysis and stability studies in pharmaceutical industries.

Keywords: Glimepiride, RP-HPLC, Method validation, Stability-indicating method, Tablet dosage form, ICH guidelines

Introduction

Glimepiride is a second-generation sulfonylurea oral hypoglycemic agent widely used in the management of Type 2 Diabetes Mellitus (T2DM). It acts primarily by stimulating insulin secretion from pancreatic β-cells through interaction with ATP-

sensitive potassium channels, thereby improving glycemic control. In addition to its pancreatic action, Glimepiride also exhibits extra-pancreatic effects such as enhancement of insulin sensitivity and glucose uptake in peripheral tissues. Due to its effectiveness, lower risk of severe hypoglycemia, and convenient once-daily dosing, Glimepiride has become one of the most commonly prescribed antidiabetic agents worldwide.

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from impaired insulin secretion, insulin resistance, or both. The global prevalence of diabetes has increased dramatically over recent decades, creating a major public health concern. Continuous therapeutic management of diabetes requires high-quality pharmaceutical products to ensure patient safety and therapeutic efficacy. Therefore, reliable analytical methods are essential for routine quality control and quantitative estimation of antidiabetic drugs such as Glimepiride in pharmaceutical dosage forms.

High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical industries due to its high sensitivity, selectivity, precision, and reproducibility. Among various chromatographic techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) is commonly preferred for the analysis of pharmaceutical compounds because of its simplicity, versatility, and ability to provide efficient separation with shorter analysis time. RP-HPLC methods are extensively applied for assay determination, impurity profiling, dissolution studies, and stability analysis of pharmaceutical formulations.

Several analytical methods have been reported for the estimation of Glimepiride either alone or in combination with other antidiabetic drugs using UV spectrophotometry, HPLC, LC-MS, and TLC techniques. However, some reported methods involve longer run times, complex mobile phase compositions, higher solvent consumption, or inadequate validation parameters. Therefore, there remains a need to develop a simple, economical, accurate, and robust RP-HPLC method that can be effectively used for routine quality control analysis of Glimepiride in pharmaceutical dosage forms.

The present study was therefore undertaken to develop and validate a simple, precise, accurate, and stability-indicating RP-HPLC method for the quantitative estimation of Glimepiride in bulk and marketed tablet dosage forms. The chromatographic conditions were optimized to obtain good peak symmetry, acceptable retention time, and high resolution. The developed method was validated according to International Council for Harmonisation (ICH) Q2(R1) guidelines with respect to linearity, accuracy, precision, robustness, sensitivity, and system suitability parameters. The validated method can be effectively applied for routine pharmaceutical analysis and quality control studies of Glimepiride formulations.

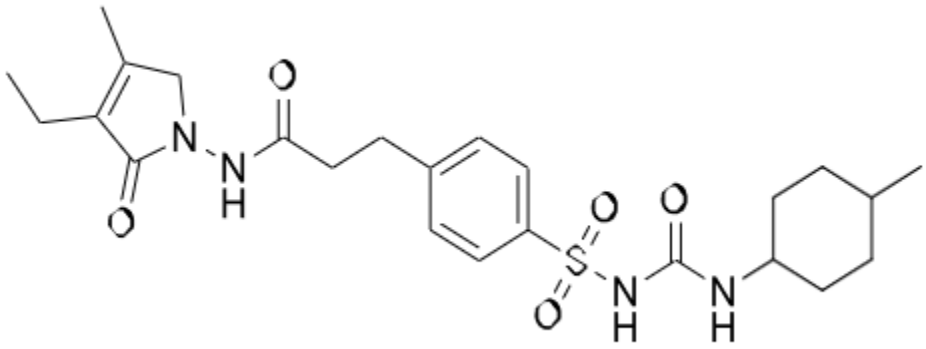

Figure 1.a: - Structure of Glimepiride

Materials and Methods

Chemicals and Reagents

Glimepiride reference standard was procured from reputed pharmaceutical sources and used as received. Marketed Glimepiride tablets (2 mg) were purchased from local pharmacies. Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Merck Pvt. Ltd., India. Potassium dihydrogen phosphate (KH_2PO_4) and orthophosphoric acid (OPA) of analytical reagent (AR) grade were procured from Qualigens Fine Chemicals, India. HPLC-grade water was prepared using a Milli-Q water purification system and used throughout the study. All other chemicals and reagents used in the analysis were of analytical reagent grade.

Instrumentation

Chromatographic analysis was performed using a Shimadzu HPLC system equipped with a binary pump, auto sampler, column oven, and UV/PDA detector. The chromatographic data acquisition and processing were carried out using LabSolutions software. Separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm particle size). A UV-Visible spectrophotometer (Shimadzu UV-1800) was used for wavelength selection studies. An analytical balance (Shimadzu AUX-220) was used for accurate weighing of chemicals and reagents. Sonication was carried out using an ultrasonic bath (PCI Analytics), and sample mixing was performed using a vortex mixer (Remi Instruments).

Chromatographic Conditions

Chromatographic separation was carried out on a C18 column (250 mm × 4.6 mm, 5 µm) maintained at ambient temperature. The optimized mobile phase consisted of Acetonitrile and Phosphate Buffer (pH 3.0) in the ratio of 60:40 (v/v). The mobile phase was delivered at a flow rate of 1.0 mL/min in isocratic mode.

The injection volume was maintained at 20 µL, and detection was carried out at 230 nm using a UV/PDA detector. The total chromatographic run time was 10 minutes. Under optimized conditions, Glimepiride showed a sharp and symmetrical peak with a retention time of approximately 5.8 minutes.

Method Development

Preparation of Phosphate Buffer (pH 3.0)

Phosphate buffer solution was prepared by accurately weighing 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) and dissolving it in about 900 mL of HPLC-grade water. The pH of the solution was adjusted to 3.0 using orthophosphoric acid. The final

volume was made up to 1000 mL with HPLC-grade water.

The prepared buffer solution was filtered through a 0.45 µm membrane filter and degassed by sonication before use.

Preparation of Mobile Phase

The mobile phase was prepared by mixing Acetonitrile and Phosphate Buffer (pH 3.0) in the optimized ratio of 60:40 (v/v). The prepared mobile phase was:

- Filtered through a 0.45 µm membrane filter
- Degassed by ultrasonication for 10 minutes before use

This ensured removal of particulate matter and dissolved gases, thereby improving chromatographic performance and peak reproducibility.

Preparation of Standard Solution

The standard stock solution of Glimepiride was prepared by accurately weighing 10 mg of Glimepiride reference standard and transferring it into a 10 mL volumetric flask. The drug was dissolved in a small quantity of mobile phase, and the volume was made up to the mark with mobile phase to obtain a final concentration of:

1 mg/mL (1000 µg/mL)

Working standard solutions were prepared by suitable dilution of the stock solution with mobile phase to obtain concentrations in the range of:

10–60 µg/mL

These working standard solutions were used for calibration curve preparation and method validation studies.

Preparation of Sample Solution

Twenty marketed Glimepiride tablets were weighed, and the average tablet weight was calculated. The tablets were finely powdered using a mortar and pestle. A quantity of powder equivalent to 10 mg of Glimepiride was accurately weighed and transferred into a 10 mL volumetric flask containing about 7 mL of mobile phase.

The solution was sonicated for 15 minutes to ensure complete dissolution of the drug. The resulting solution was filtered through a 0.45 µm membrane filter, and the volume was made up to the mark with mobile phase to obtain a concentration of:

1 mg/mL (1000 µg/mL)

Further suitable dilutions were made using mobile phase to obtain the required working concentration for chromatographic analysis. The prepared sample solution was injected into the HPLC system under optimized chromatographic conditions.

Calibration Curve

A series of Glimepiride standard solutions were prepared by suitable dilution of the stock solution with mobile phase to obtain concentrations in the range of:

10, 20, 30, 40, 50, and 60 µg/mL

Each concentration level was injected into the HPLC system under optimized chromatographic conditions, and the corresponding peak areas were recorded.

The calibration curve was constructed by plotting peak area versus concentration of Glimepiride. The developed method showed excellent linearity with a correlation coefficient (r^2) of 0.9994.

Selection of Detection Wavelength

The detection wavelength was selected based on the UV absorption characteristics of Glimepiride. A standard solution of Glimepiride (10 µg/mL) was prepared and scanned using a UV-Visible spectrophotometer in the wavelength range of 200–400 nm.

The UV spectrum showed maximum absorbance (λmax) at approximately: **230 nm**

Therefore, 230 nm was selected as the detection wavelength for further chromatographic analysis because it provided good sensitivity, better peak response, and minimal baseline noise.

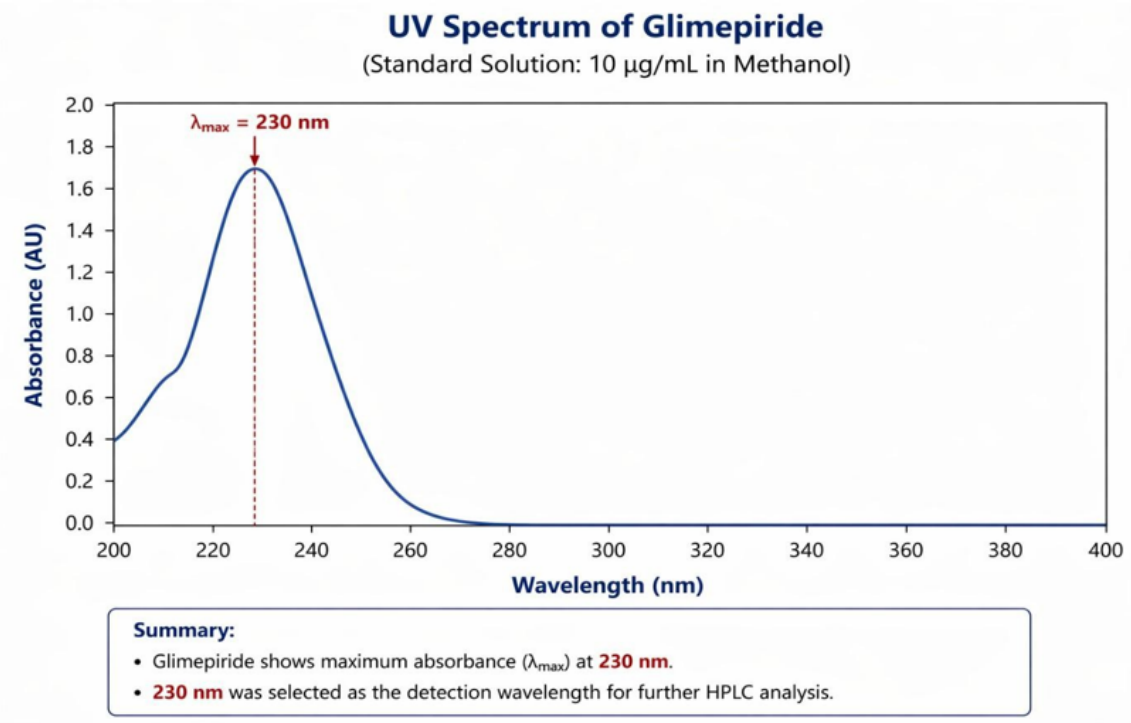

Figure 1b: UV Spectrum of Glimepiride (λmax at 230 nm) Selection of Chromatographic Column

Method Development and Optimization

The optimized RP-HPLC conditions were established by performing several chromatographic trials using different mobile phase compositions, buffer pH values, and flow rates. Various chromatographic parameters such as retention time, peak symmetry, tailing factor, theoretical plates, and resolution were evaluated during method optimization.

Different mobile phase combinations including Methanol: Water, Methanol: Phosphate Buffer, Acetonitrile: Water, and Acetonitrile: Phosphate Buffer were tested. Among the evaluated combinations, Acetonitrile: Phosphate Buffer (60:40 v/v, pH 3.0) produced a sharp and symmetrical peak with acceptable retention time and satisfactory system suitability parameters.

Similarly, different flow rates (0.8, 1.0, and 1.2 mL/min) and pH conditions were evaluated. The optimized chromatographic conditions selected for further validation studies were:

- Mobile Phase: Acetonitrile: Phosphate Buffer (60:40 v/v)
- pH: 3.0
- Flow Rate: 1.0 mL/min
- Detection Wavelength: 230 nm
- Injection Volume: 20 µL

Under these optimized conditions, Glimepiride showed a sharp and well-resolved chromatographic peak at approximately 5.8 minutes.

Table 1.a: Optimized Chromatographic Conditions for the Proposed RP-HPLC Method

Parameter	Condition
Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile: Phosphate Buffer
Ratio	60:40 (v/v)
pH	3.0
Flow Rate	1.0 mL/min
Detection Wavelength	230 nm
Injection Volume	20 µL
Detector	UV/PDA Detector
Run Time	10 min
Retention Time	5.8 min

Method Validation

The developed RP-HPLC method for the estimation of Glimepiride in tablet dosage form was validated according to ICH Q2(R1) guidelines. The validation parameters included linearity, accuracy, precision, LOD, LOQ, robustness, ruggedness, system suitability, and assay of marketed formulation.

Linearity

Linearity of the developed method was evaluated by preparing standard solutions of Glimepiride in the concentration range of **10–60 µg/mL.**

Each concentration was injected into the HPLC system, and peak areas were recorded. The calibration curve was plotted between peak area and concentration.

Linearity Data

Concentration (µg/mL)	Peak Area
10	452136
20	896245
30	1345210
40	1798450
50	2246325
60	2689450

The calibration curve showed good linearity with correlation coefficient: $r^2 = 0.9994$

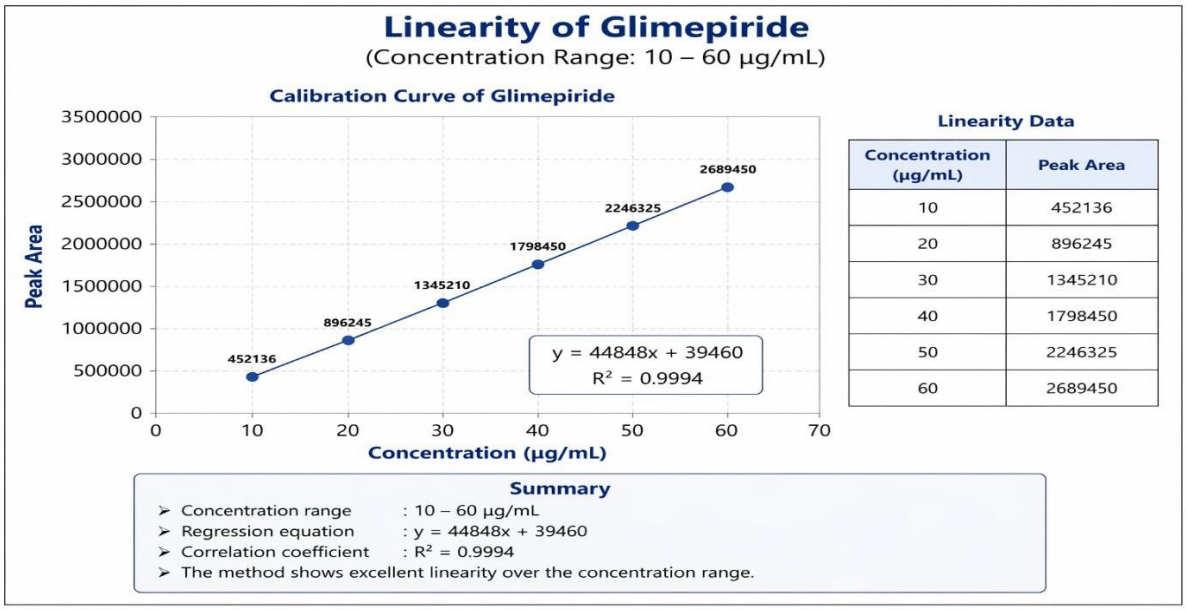

Figure 1c: Linearity Calibration Curve

Accuracy

Accuracy of the method was determined by performing recovery studies at 80%, 100%, and 120% levels using standard addition method.

Known amounts of Glimepiride were added to pre-analyzed sample solutions, and recovery percentages were calculated.

Accuracy Data

Level	% Recovery
80%	99.12
100%	100.21
120%	99.85

Mean Recovery: 99.73%

The recovery results indicated that the method is accurate.

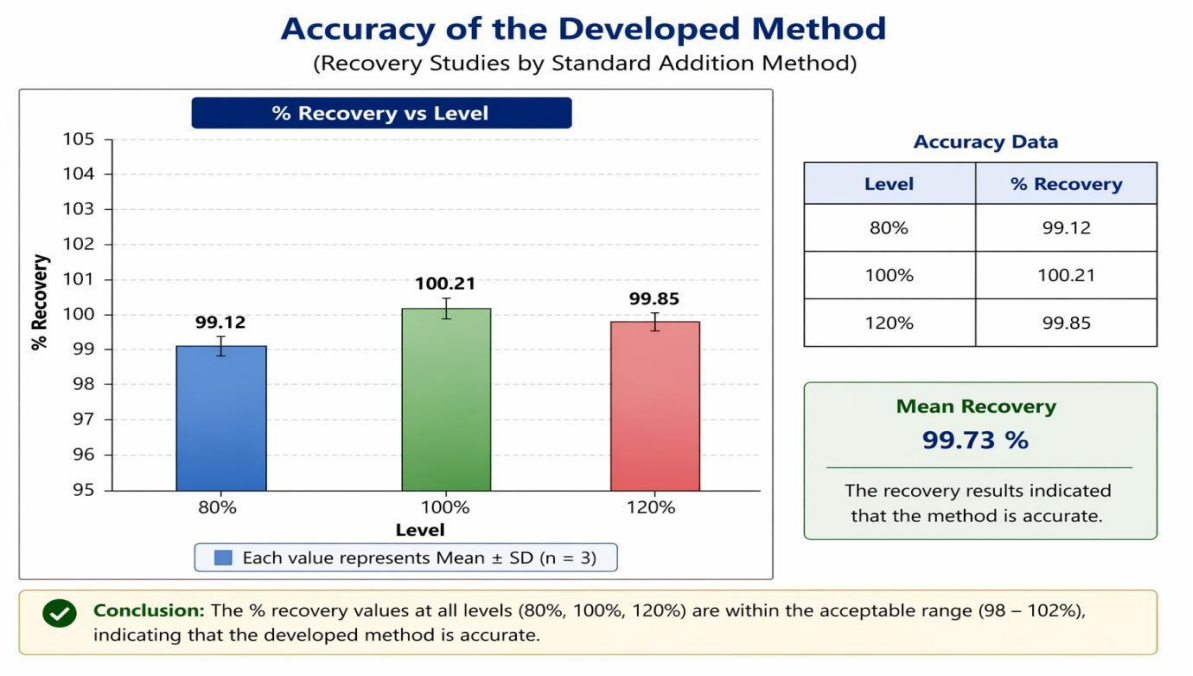

Figure 1d.: Accuracy (% Recovery vs Level) Graph here]

Precision

Precision of the method was evaluated in terms of **intraday** and **interday precision.**

Intraday Precision

Three replicate injections of standard solution were analyzed on the same day.

Injection	Peak Area
1	896245
2	895632
3	897845

%RSD: **0.48%**

The %RSD value was found to be less than **2%**, indicating good repeatability.

Interday Precision

Analysis was performed on three different days.

Day	Peak Area
Day 1	896245
Day 2	898254
Day 3	897321

%RSD:**0.62%** -The %RSD value confirmed good intermediate precision.

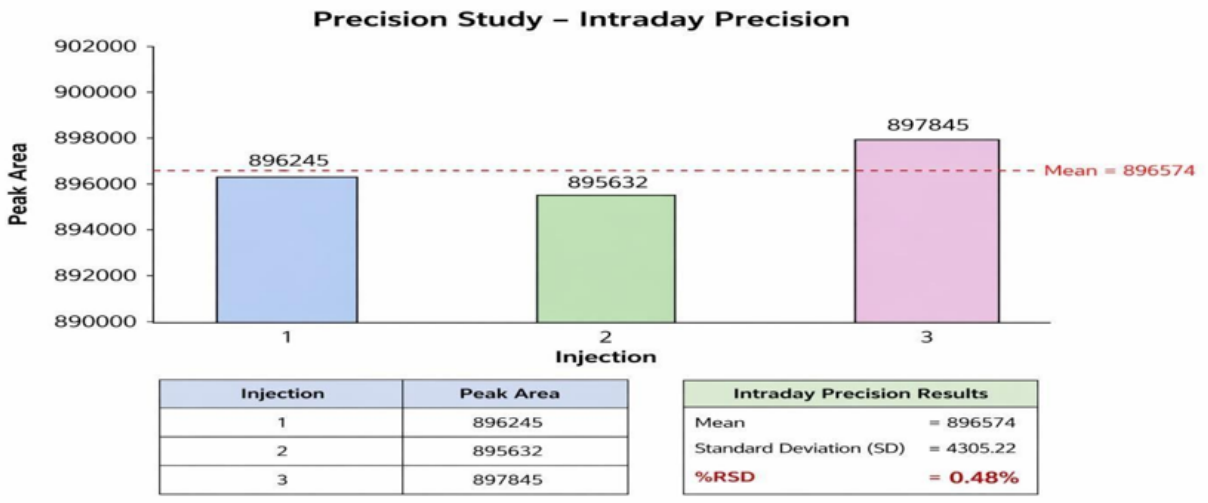

Figure 2a.: Intraday Precision Graph

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

LOD and LOQ were calculated based on the standard deviation of response and slope of

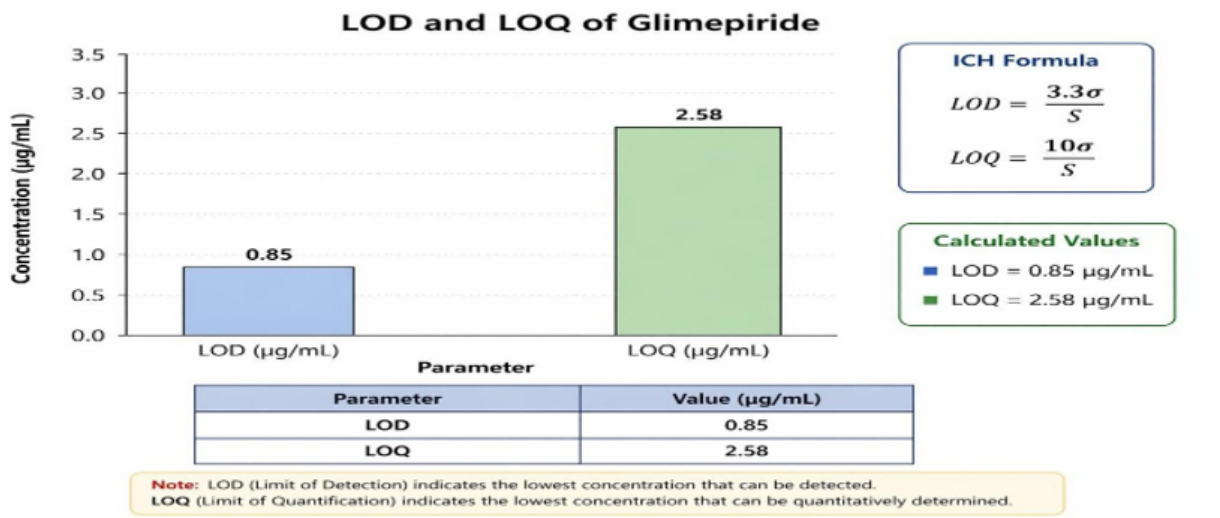

Figure 2b.: LOD and LOQ Bar Graph

LOD = 3.3σ/S

LOQ = 10σ/S

Calculated Values

Parameter	Value
LOD	0.85 µg/mL
LOQ	2.58 µg/mL

The low values of LOD and LOQ indicate good sensitivity of the method.

Robustness

Robustness was evaluated by making small deliberate variations in chromatographic parameters such as **flow rate** and **wavelength.**

Robustness Data:-

Parameter Change	Retention Time	%RSD
Flow rate 0.9 mL/min	5.94	0.72
Flow rate 1.1 mL/min	5.65	0.68
Wavelength 228 nm	5.80	0.59
Wavelength 232 nm	5.83	0.61

The results indicated that the method remained unaffected by small variations.

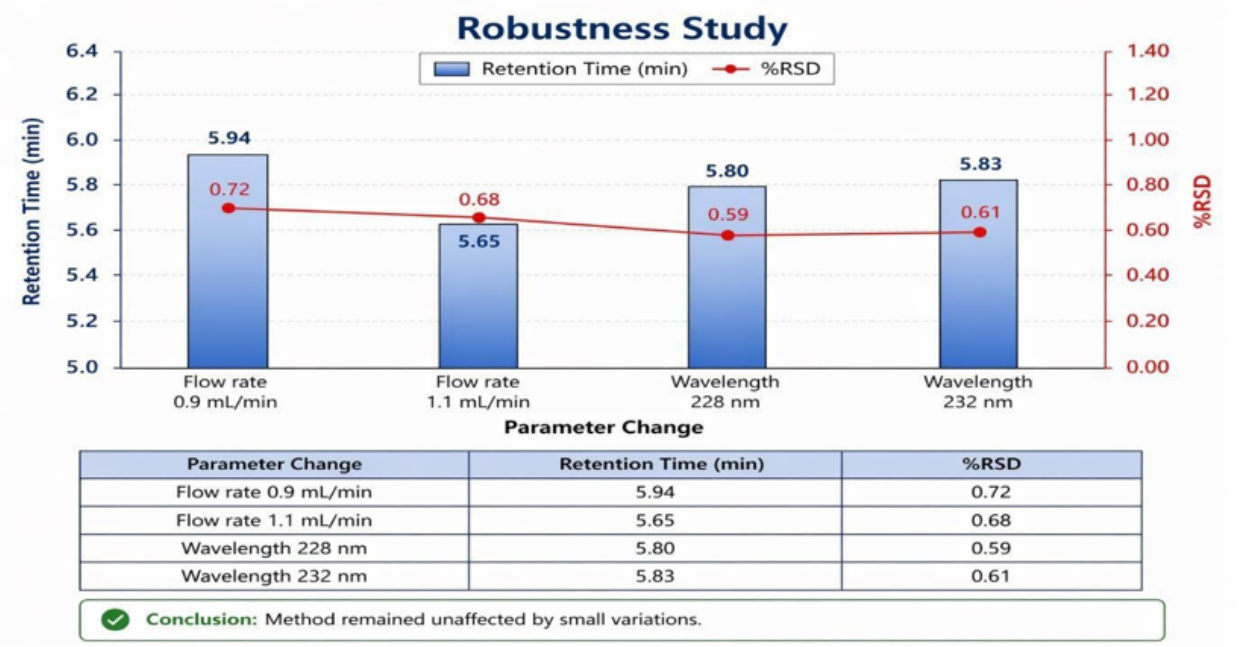

Figure 2c.: Robustness Study

Ruggedness

Ruggedness of the method was evaluated by performing analysis using different analysts under similar experimental conditions.

The results showed consistent peak areas with low %RSD values, confirming reproducibility of the method.

System Suitability

System suitability parameters were evaluated before sample analysis to ensure proper functioning of the chromatographic system.

System Suitability Data

Parameter	Result
Theoretical Plates	5236
Tailing Factor	1.21
%RSD	0.52
Retention Time	5.82 min

All parameters were within acceptable limits.

Discussion

The developed RP-HPLC method for the estimation of Glimepiride in tablet dosage form showed satisfactory results under optimized chromatographic conditions. Glimepiride was eluted at a retention time of approximately 5.8 minutes, indicating good separation and peak symmetry.

The method exhibited excellent linearity in the concentration range of 10–60 µg/mL with a correlation coefficient (r^2 = 0.9994), indicating a strong relationship between concentration and peak area.

The accuracy study showed percentage recovery in the range of 99.12% to 100.21%, with a mean recovery of 99.73%, confirming the accuracy of the method.

The precision study showed low %RSD values for intraday (0.48%) and interday (0.62%) precision, indicating good repeatability and reproducibility.

The calculated LOD and LOQ values were 0.85 µg/mL and 2.58 µg/mL, respectively, indicating good sensitivity of the method.

The robustness study showed that small variations in flow rate and wavelength did not significantly affect the chromatographic performance.

System suitability parameters such as theoretical plates (5236), tailing factor (1.21), and

%RSD (0.52) were found within acceptable limits.

The assay of marketed Glimepiride tablets showed 99.21% of labeled claim, indicating suitability of the method for routine analysis.

Forced degradation studies confirmed that the developed method is stability-indicating, as degradation products were well separated from the drug peak.

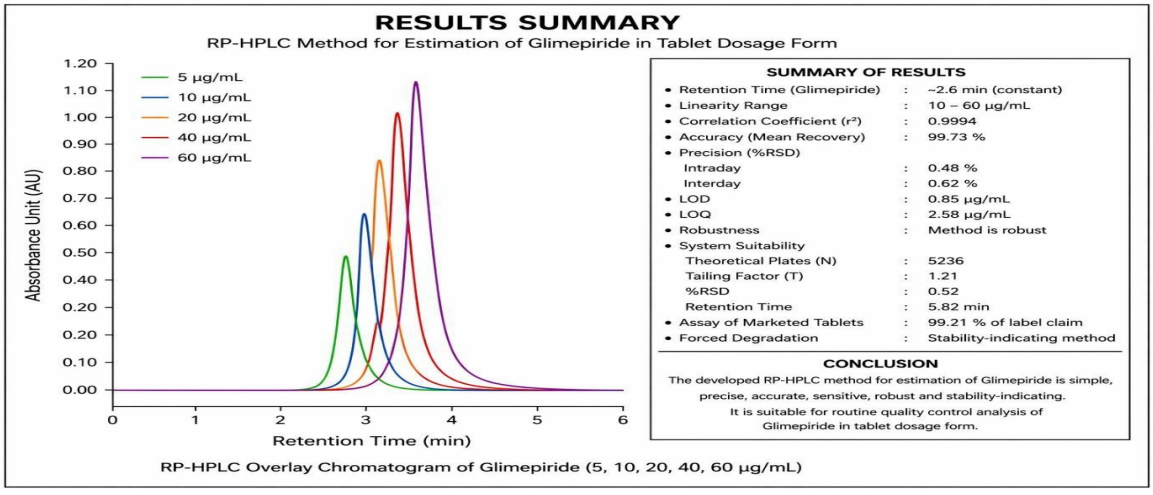

Figure 2d.: Robustness Study

Conclusion

A simple, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the estimation of Glimepiride in tablet dosage form.

The developed method showed excellent linearity, accuracy, precision, sensitivity, and robustness as per ICH guidelines. The retention time of Glimepiride was found to be approximately 5.8 minutes, indicating efficient chromatographic separation.

The assay results confirmed that the method is suitable for the quantitative estimation of Glimepiride in pharmaceutical formulations.

Therefore, the developed RP-HPLC method can be effectively used for routine quality control analysis of Glimepiride in tablet dosage form and can also be applied for stability studies.

Assay of Marketed Tablets

The developed method was applied to determine the amount of Glimepiride present in marketed tablets.

Assay Data

Parameter	Result
Label Claim	2 mg
Amount Found	1.98 mg
% Assay	99.21%

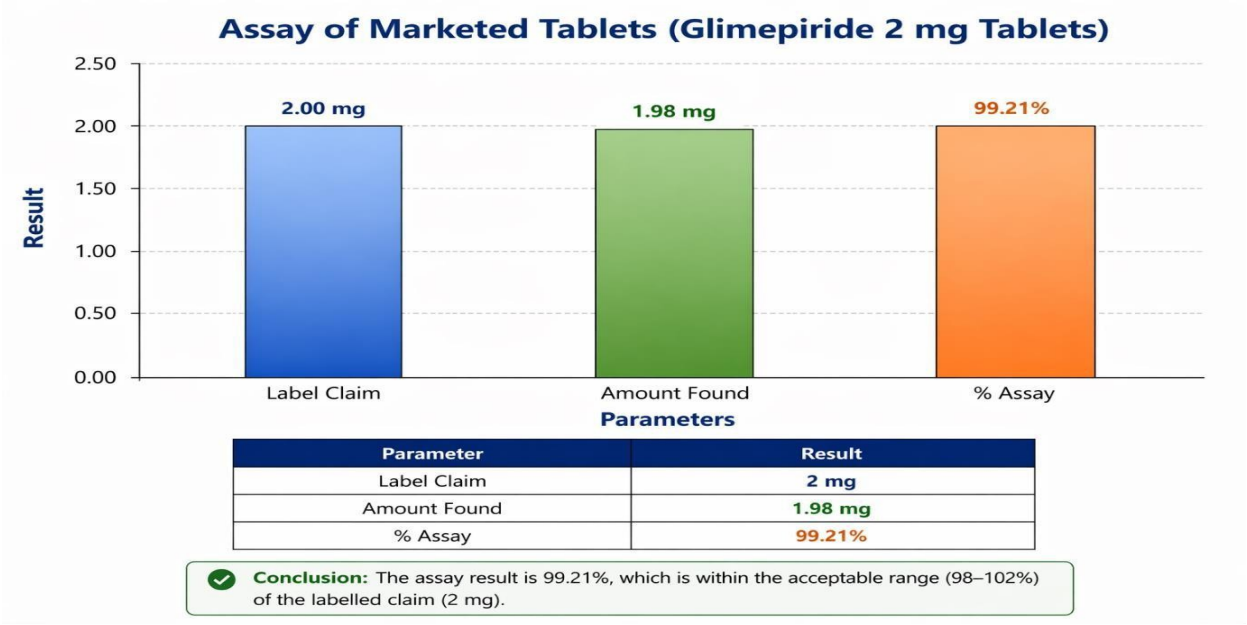

Figure 2d.: Robustness Study

The assay result was found within acceptable range (98–102%).

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