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Analytical Method Development and Validation of Trelagliptin by RP-HPLC

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Abstract:

Aim: The aim of the present study was to develop and validate a simple, rapid, precise, and robust RP-HPLC method for the quantitative estimation of Trelagliptin in pharmaceutical analysis. Materials and Methods: Chromatographic separation was achieved using RP-HPLC under optimized isocratic conditions with UV detection. The developed method was validated according to ICH Q2 (R2) guidelines for various analytical parameters including linearity, accuracy, precision, robustness, Limit of Detection (LOD), and Limit of Quantification (LOQ).

Results: The method showed excellent linearity over the selected concentration range with a correlation coefficient (r^2) of 0.999. The intra-day and inter-day precision studies showed low %RSD values of 0.12% and 0.26%, respectively, indicating good repeatability and reproducibility. Accuracy studies demonstrated satisfactory recovery within acceptable limits. Robustness testing showed a %RSD value of 1.41%, confirming that minor variations in analytical conditions did not significantly affect the method performance. The LOD and LOQ were found to be 1.53 µg/mL and 4.63 µg/mL, respectively. System suitability parameters were within acceptable limits. Conclusion: The developed RP-HPLC method was found to be sensitive, accurate, precise, reliable, and suitable for routine quality control and quantitative estimation of Trelagliptin in pharmaceutical analysis.

Keywords: Trelagliptin, RP-HPLC, Chromatographic Conditions, Anti-diabetic Drug, Method Validation.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia arising from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and represents a significant public health challenge due to its increasing incidence and associated complications. Prolonged hyperglycemia is responsible for long-term damage and dysfunction of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels, thereby increasing the risk of serious complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy [1,2].The pathophysiology of diabetes involves a complex interplay of hormonal and metabolic disturbances that disrupt normal glucose homeostasis. Insulin and glucagon play a central role in maintaining blood glucose levels, and any imbalance in their secretion or action results in hyperglycemia. In diabetic conditions, either insulin is not produced in sufficient quantity or the body's cells fail to respond effectively to insulin, leading to impaired glucose uptake and elevated blood glucose levels [3]. Over time, this metabolic imbalance contributes to progressive cellular damage and organ dysfunction. Diabetes mellitus is broadly classified into Type 1 and Type 2 diabetes. Type 1 diabetes is an autoimmune condition characterized by the destruction of pancreatic β-cells, resulting in absolute insulin deficiency. In contrast, Type 2 diabetes mellitus, which accounts for the majority of cases, is primarily associated

with insulin resistance and a relative decline in insulin secretion. The global prevalence of diabetes has increased significantly over recent years and is expected to rise further, thereby increasing the burden on healthcare systems worldwide [4, 5].Effective management of diabetes requires a comprehensive approach that includes both non-pharmacological and pharmacological interventions. Lifestyle modifications such as dietary regulation, physical activity, weight management, and smoking cessation play a crucial role in improving glycemic control and reducing complications. In addition to these measures, various classes of antidiabetic drugs are widely used in clinical practice, including biguanides, sulfonylureas, thiazolidinedione's, meglitinides, α-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists, and sodium-glucose cotransporter-2 inhibitors. These agents exert their effects through different mechanisms such as enhancing insulin secretion, improving insulin sensitivity, and reducing glucose production or absorption [6, 7, 8].With the increasing use of antidiabetic drugs, ensuring their quality, safety, and efficacy has become critically important. In this context, analytical method development and validation play a vital role in pharmaceutical analysis by providing accurate, precise, and reproducible results in accordance with regulatory guidelines. Various analytical techniques such as reverse-phase high-performance liquid chromatography (RP-HPLC), UV spectrophotometry, and liquid chromatography–mass spectrometry (LC-MS) are commonly employed for the estimation of pharmaceutical compounds in bulk and dosage forms [9, 10].

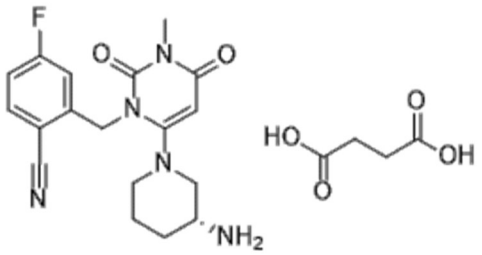

Fig 1: Chemical Structure of Trelagliptin Succinate

2. MATERIALS & METHODS

Chemicals:

Trelagliptin was procured from Swapnroop Drugs and Pharmaceuticals, Aurangabad. The marketed formulation, Trelagwk tablets (50 mg), was purchased from a local pharmacy. Methanol, 0.1% formic acid, and water were used during the study. All chemicals and reagents used were of analytical grade, and solvents were of suitable chromatographic grade.

Instruments:

The chromatographic analysis was carried out using an Agilent 1100 high-performance liquid chromatography (HPLC) system equipped with a UV detector. Separation was achieved on a C18 column (4.6 × 250 mm, 5 µm particle size). A VSI pH meter (VSI 1-B) was used for pH measurements. An analytical balance (WENSAR TM high-resolution balance) was used for accurate weighing of samples.

Chromatographic Conditions:

The chromatographic conditions were established by trial and error and were kept constant throughout the study. The analysis was carried out using an Agilent 1100 high-performance liquid chromatography (HPLC) system equipped with Chem Station software.

Separation was achieved on a C18 column (4.6 × 250 mm, 5 µm particle size). The mobile phase consisted of methanol and 0.1% formic acid in the ratio of 77:23 (v/v) and was delivered in isocratic mode. Detection was carried out at a wavelength of 278 nm using a UV detector.

The flow rate was maintained at 1.0 mL/min, and the analysis was performed at a temperature of 25°C. The injection volume was set at 20 µL.

3. SAMPLE PREPARATION:

Physical Form of the API

Trelagliptin was obtained as a white to off-white crystalline powder, soluble in organic solvents. The drug showed acceptable physicochemical properties, with loss on drying below 0.5%, residue on ignition below 0.1%, and heavy metal content within 10 ppm limits. The assay value was found to be 99.39%, indicating good purity of the drug.

Preparation of Stock Solution

An accurately weighed quantity of 10 mg of Trelagliptin was transferred into a 50 mL volumetric flask and dissolved in methanol. The volume was made up to the mark with methanol to obtain a stock solution having a concentration of 200 µg/mL (Stock I).

Preparation of Standard Solutions

Working standard solutions were prepared from Stock I by appropriate dilution with the mobile phase to obtain concentrations in the range of 2–10 µg/mL. Aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 mL were transferred into separate 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain concentrations of 2, 4, 6, 8, and 10 µg/mL, respectively.

Marketed Tablet Test Preparation

Twenty tablets of the marketed formulation (Trelagwk, 50 mg) were weighed and powdered. The average tablet weight was found to be 0.0635 g. A quantity of tablet powder equivalent to 10 mg of Trelagliptin (12.7 mg) was accurately weighed and transferred into a 50 mL volumetric flask. Methanol was added, and the solution was sonicated for 15 minutes to ensure complete extraction of the drug. The volume was then made up to the mark with methanol to obtain Stock II solution.

Assay Preparation

From Stock II, 0.2 mL was pipetted into a 10 mL volumetric flask and diluted with mobile phase to obtain a final concentration of 4 µg/mL, which was used for assay analysis.

4. METHOD VALIDATION (ICH)

The developed analytical method was validated as per ICH guidelines to ensure its reliability and suitability for analysis.

1. Linearity

Linearity was evaluated by analyzing standard solutions over a suitable concentration range, and a calibration curve was constructed by plotting concentration versus peak area. The method is considered linear if the correlation coefficient (R^2) is found to be not less than 0.999.

2. Accuracy

Accuracy was determined by performing recovery studies at different levels (80%, 100%, and 120%) by the standard addition method. The method is considered accurate if the percentage recovery is found within the range of 98–102%.

3. Precision

Precision of the method was assessed in terms of repeatability and intermediate precision by analyzing replicate samples. The method is considered precise if the percentage relative standard deviation (%RSD) is not more than 2%.

4. Limit of Detection (LOD)

The limit of detection was determined based on the standard deviation of the response and the slope of the calibration curve. The method is considered sensitive if the signal-to-noise ratio is approximately 3:1.

5. Limit of Quantitation (LOQ)

The limit of quantitation was determined using the standard deviation of the response and the slope of the calibration curve. The method is considered acceptable if the signal-to-noise ratio is approximately 10:1.

6. Specificity

Specificity was evaluated by comparing chromatograms of blank, standard, and sample solutions to ensure no interference at the retention time of the analyte. The method is considered specific if no interfering peaks are observed.

7. Robustness

Robustness was assessed by introducing small deliberate variations in chromatographic conditions such as flow rate, mobile phase composition, and wavelength. The method is considered robust if no significant change in results is observed and %RSD remains within acceptable limits (≤ 2%).

8. System Suitability

System suitability tests were performed to verify the performance of the chromatographic system before analysis. The system is considered suitable if parameters such as %RSD (≤ 2%), tailing factor (≤ 2), and theoretical plates (≥ 2000) are within acceptable limits.

5. RESULTS & DISCUSSION:

High Performance Liquid Chromatography:

A reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Trelagliptin. Various chromatographic parameters were systematically optimized to obtain a sharp, well-resolved peak with good symmetry and reproducibility. The standard solution was prepared and analyzed under optimized conditions, and the chromatograms were recorded. The developed method was found to be simple, precise, and suitable for the quantitative analysis of Trelagliptin.

Selection of Wavelength:

The selection of detection wavelength plays a significant role in determining the sensitivity of the analytical method. The UV spectrum of Trelagliptin was scanned over a suitable wavelength range to identify the wavelength of maximum absorbance. Based on the spectral analysis, 278 nm was selected as the detection wavelength, as the drug exhibited adequate absorbance at this wavelength. This ensured reliable detection and consistent analytical performance.

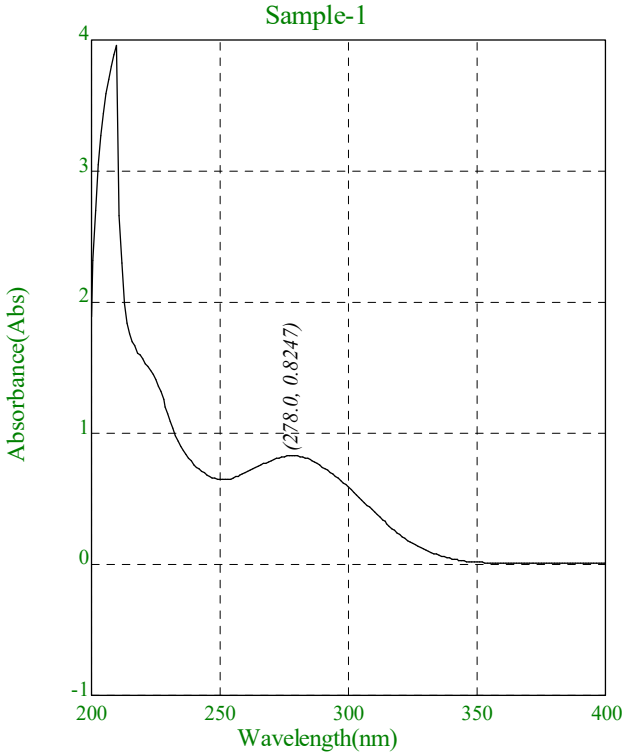

Fig 2: UV Spectrum of Trelagliptin

METHOD VALIDATION

Linearity

Linearity of the developed method was evaluated over the concentration range of 2–10 µg/mL by plotting peak area versus concentration. The method exhibited a linear response with the regression equation y =

441x + 448 and a correlation coefficient (R^2) of 0.999, which complies with the acceptance criteria ($R^2 \geq 0.999$).The calibration curve (Figure 2) showed a direct and proportional relationship between concentration and peak area. The %RSD values were found to be within acceptable limits (≤ 2%) for most of the concentration levels, indicating good precision of the method. Thus, the developed method was found to be linear and suitable for quantitative analysis within the selected concentration range.

Table 1: Summary of Results of Linearity

Conc (µg/mL)	Peak Area
2	1312.66
4	2246.60
6	3081.45
8	3970.35
10	4861.66

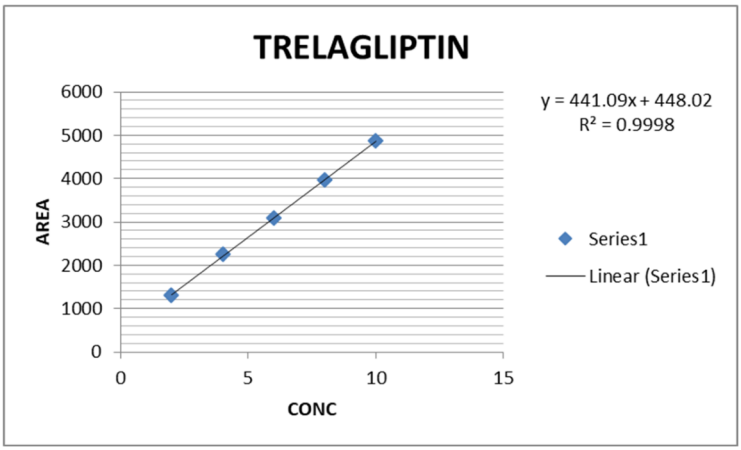

Fig 3: Linearity Curve of Trelagliptin

Accuracy:

The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Trelagliptin and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD, as demonstrated in Table 2

Table 2: Accuracy Data of Trelagliptin

Trelagliptin			Amount added	Amount Recovered	Recovery (97-103)
Name	Preparations	Area	µg/mL	µg/mL	%
Accuracy at 80%	Prep-1	2036.16	1.6	1.60	100.08
Accuracy at 80%	Prep-2	2036.15	1.6	1.60	100.08
Accuracy at 100%	Prep-1	2201.40	2.0	1.97	98.80
Accuracy at 100%	Prep-2	2200.20	2.0	1.97	98.66
Accuracy at 120%	Prep-1	2405.04	2.4	2.43	101.57
Accuracy at 120%	Prep-2	2404.82	2.4	2.43	101.55

Table 3: summary of Results of Accuracy

Accuracy Levels	Mean % Recovery	SD	% RSD (NMT2)
Accuracy at 80%	100.08	0.000	0.00
Accuracy at 100%	98.73	0.10	0.10
Accuracy at 120%	101.56	0.01	0.10

Precision:

Precision was evaluated in terms of intra-day and inter-day studies. The %RSD values were found to be within the acceptable limit of NMT 2.0%, indicating that the developed method is precise and reproducible.

Table 4: Intra-Day Precision Data of Trelagliptin

Name	Preparation	% Assay
Set-1	Prep-01	99.80
	Prep-02	99.68
Set-2	Prep-01	99.91
	Prep-02	99.80
Mean		99.80
SD		0.12
%RSD (NMT 2.0)		0.12

Table 5: Inter-Day Precision Data of Trelagliptin

Name	Preparation	% Assay
Day-1	Prep-01	100.01
	Prep-02	99.67
Day-2	Prep-01	100.24
	Prep-02	99.67
Mean		99.90
SD		0.26
%RSD (NMT 2.0)		0.26

Limit of Detection (LOD) & Limit of Quantification (LOQ):

The LOD and LOQ were calculated by the equations **LOD = 3.3×Std. Deviation / Slope** and **LOQ = 10×Std. Deviation / Slope** where, std. Deviation taken from accuracy and slope is from linearity.

Based on these equations, the calculated LOD and LOQ values for Trelagliptin were 1.53 µg/mL and 4.63 µg/mL, respectively.

Specificity:

Specificity of the developed method was evaluated by injecting blank, placebo, standard solution, and sample solution under the same chromatographic conditions.

Table 6: Specificity of Trelagliptin

Sr. No.	Solution	
	Blank	0.00
	Trelagliptin Standard Sample	2.632
	Trelagliptin Test Sample	2.637

The chromatograms were observed for any interference at the retention time of Trelagliptin. It was found that there was no interference from excipients or mobile phase at the analyte peak. The peak of Trelagliptin was well resolved, indicating that the method is specific for the estimation of Trelagliptin.

Robustness:

Robustness of the method reflects that the results are unaffected and reliable even with small changes in the method parameters. In the present study, the flow rate and wavelength were slightly changed on lower and higher sides of the optimized conditions to evaluate their effect on peak area. The results obtained under varied conditions are shown in Table 7. The %RSD was found to be within acceptable limits, indicating that the method is robust.

Table 7: Robustness Change in Parameter for Trelagliptin

Mobile phase 74+24		
Sr No.	**Concentration (µgm/mL)**	**Area**
1	4	2273.137
2	4	2270.694
	Mean	2271.916

Mobile phase 76+22		
Sr No.	**Concentration (µgm/mL)**	**Area**
1	4	2268.4633
2	4	2301.8610
	Mean	2285.1622

	SD	1.72737
	%RSD	0.076031
Wave length change		
277		
Sr No.	**Concentration (µgm/mL)**	**Area**
1	4	2269.718
2	4	2268.11
	Mean	2268.914
	SD	1.1366
	%RSD	0.0500

	SD	23.6157
	%RSD	1.03
Wave length change		
279		
Sr No.	**Concentration (µgm/mL)**	**Area**
1	4	2205.7812
2	4	2203.1911
	Mean	2204.4862
	SD	1.8314
	%RSD	0.0830

System Suitability:

These parameters were shown to be within specified limits. Column efficiency (theoretical plates), resolution factor, and peak asymmetry factor, tailing factor, and %RSD are the system suitability parameters. These parameters of the optimized method were found satisfactory. The system suitability was evaluated using trial injections of Trelagliptin, and the results indicated good repeatability and efficient chromatographic performance. The results of the system suitability studies are shown in Table (Table 8). These parameters were found to be within specified limits.

Table 8: System Suitability Studies of Trelagliptin

SR. NO.	Parameter	Injection 1	Injection 2	Mean	SD	%RSD	Acceptance Criteria
1	Retention Time (min)	2.635	2.636	2.636	-	-	Consistent
2	Peak Area (mAU*s)	2207.358	2205.894	2206.626	1.03	0.047%	NMT 2%
3	Peak Height (mAU)	427.692	422.377	-	-	-	-
4	Theoretical Plates (N)	5595	5387	-	-	-	NLT 2000
5	Tailing Factor (T)	0.90	0.91	0.905	-	-	NMT 2.0

MARKETED SAMPLE ANALYSIS:

The developed and validated RP-HPLC method was successfully applied for the quantitative estimation of Trelagliptin in marketed tablet formulation, and the assay results confirmed the suitability of the method for routine quality control analysis.

Table 9: Marketed Test Sample Results

Name	Area	RT (min)	Concentration	% Label Claim
Test Solution 1	2212.253	2.637	4.00	100.01
Test Solution 2	2226.167	2.637	4.00	100.80
Mean	2219.21			100.41
SD	9.84			0.558
% RSD	0.443			0.555

6. CONCLUSION

A simple, sensitive, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the estimation of Trelagliptin. The method was validated as per ICH Q2 guidelines and demonstrated satisfactory system suitability parameters, confirming adequate system performance. The method showed good linearity over the selected concentration range with a high degree of correlation. Precision studies revealed low %RSD values within acceptable limits, indicating excellent reproducibility. Accuracy studies confirmed good recovery, demonstrating the reliability of the method. Robustness studies showed that small deliberate variations in chromatographic conditions did not significantly affect the results. Hence, the developed method is reliable, reproducible, and suitable for routine quality control and analytical estimation of Trelagliptin.

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