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DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR DETERMINATION OF ANTIDIABETIC DRUG TRELAGLIPTIN IN BULK AND MARKETED PHARMACEUTICAL DOSAGE FORMS

K. Jyothi[1]*, Sravani[2], Veera raghava[2], Likitha Jangam[2], Sufiyan[2], Anil Rathod[2]

[1] Assistant Director, Department of Pharmaceutical Chemistry, St. Mary's College of Pharmacy, Address: # 9/1/248, St. Francis Street, Hyderabad, Telangana - 500025, India.

[2]Students, St.Mary's College of Pharmacy, Address: # 9/1/248, St. Francis Street, Hyderabad, Telangana - 500025, India.

Abstract:

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Trelagliptin in bulk form and marketed formulations. Separation of Trelagliptin was successfully achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol : Phosphate buffer (0.02M, pH-3.6) in the ratio of 45:55% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Trelagliptin. The correlation coefficient was found to be 0.9995 for Trelagliptin. The LOD and LOQ for Trelagliptin were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Trelagliptin, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: *Trelagliptin, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.*

Corresponding author:

K. Jyothi,

Department of Pharmaceutical Chemistry, St. Mary's College of Pharmacy, Hyderabad, Telangana - 500025, India.

Mobile:

Email Id:

QR CODE

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INTRODUCTION:

Trelagliptin is a dipeptidyl peptidase-4 inhibitor indicated in the control of diabetes mellitus. Trelagliptin is under investigation in clinical trial NCT03555591 (Specified Drug-Use Survey of Trelagliptin Tablets "Survey on Long-term Use in Patients with Type 2 Diabetes Mellitus")[1]. Trelagliptin is a once-weekly DPP-4 inhibitor indicated for the treatment of Type 2 Diabetes Mellitus (T2DM) in adults. It improves glycemic control by extending the activity of incretin hormones, which stimulate insulin release and reduce glucagon secretion. Trelagliptin is a highly potent, selective, and long-acting dipeptidyl peptidase-4 (DPP-4) inhibitor used in the management of Type 2 Diabetes Mellitus[2]. By inhibiting the DPP-4 enzyme, it prevents the degradation of incretin hormones, thereby increasing insulin secretion and reducing glucagon release in a glucose-dependent manner. Trelagliptin is a once-weekly dipeptidyl peptidase-4 (DPP-4) inhibitor prescribed for the management of Type 2 Diabetes Mellitus in adults. It helps control blood sugar levels by blocking the DPP-4 enzyme, which increases incretin hormones to enhance insulin secretion and reduce glucose production by the liver[3]. The IUPAC Name of Trelagliptin is 2-[[6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxopyrimidin-1-yl] methyl]-4-fluorobenzonitrile. The Chemical Structure of Trelagliptin is as following

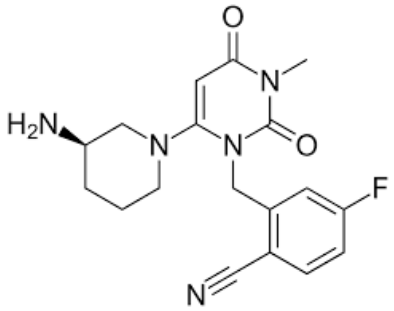

Fig-1: Chemical Structure of Trelagliptin

After performing an extensive literature review[31-32], an attempt was made to develop a smooth plain sailing, unambiguous, valid, speedy, and decisive strategy for estimating Trelagliptin in bulk form and marketed pharmaceutical dosage form by using RP-HPLC.

MATERIALS AND METHODS:

Table-1: Instruments Used

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Trelagliptin	Synpharma Research Lab, Hyderabad
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Ethanol	Sd fine-Chem ltd; Mumbai

5	DMSO	Sd fine-Chem ltd; Mumbai
6	DMF	Sd fine-Chem ltd; Mumbai
7	Orthophosphoric Acid	Sd fine-Chem ltd; Mumbai

HPLC METHOD DEVELOPMENT:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Trelagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Twenty capsules were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Trelagliptin equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Procedure:

Inject the samples by changing the chromatographic conditions[4] and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[13-14].

Mobile Phase Optimization:

Initially the mobile phase[5] tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (0.02M, pH-3.6) in proportion 45:55% v/v.

Optimization of Column:

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

Preparation of Buffer and Mobile Phase:

Preparation of Potassium Dihydrogen Phosphate (KH2PO4) Buffer (0.02M) (pH-3.6):

Dissolve 2.72172g of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration[6] and ultra-sonication.

Preparation of Mobile Phase:

Accurately measured 450 ml (45%) of Methanol and 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

METHOD VALIDATION PARAMETERS

System Suitability

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Trelagliptin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Trelagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Trelagliptin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Trelagliptin above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay[7] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range:

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (12ppm of Trelagliptin):

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (16ppm of Trelagliptin):

Take 0.16ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (20ppm of Trelagliptin):

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (24ppm of Trelagliptin):

Take 0.24ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (28ppm of Trelagliptin):

Take 0.28ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Trelagliptin Product Solution for Precision:

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Trelagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For Preparation of 80% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.08ml of the above Trelagliptin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Trelagliptin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 120% Standard Stock Solution:
Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.12ml of the above Trelagliptin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Trelagliptin and calculate the individual recovery[8] and mean recovery values.

Limit of Detection and Limit of Quantification (LOD & LOQ):
Preparation of 1.165µg/ml Solution (For LOD):
Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1165ml of the above Trelagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 3.53µg/ml Solution (For LOQ):
Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.353ml of the above Trelagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For Preparation of Standard Solution:
Accurately weigh and transfer 10 mg of Trelagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Trelagliptin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Forced Degradation Studies
The purpose of stability testing[9-12] is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug.

RESULTS AND DISCUSSION:

Selection of Wavelength:
The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum[15] of Trelagliptin was obtained and the Trelagliptin showed absorbance's maxima at 255nm. The UV spectra of drug are follows:

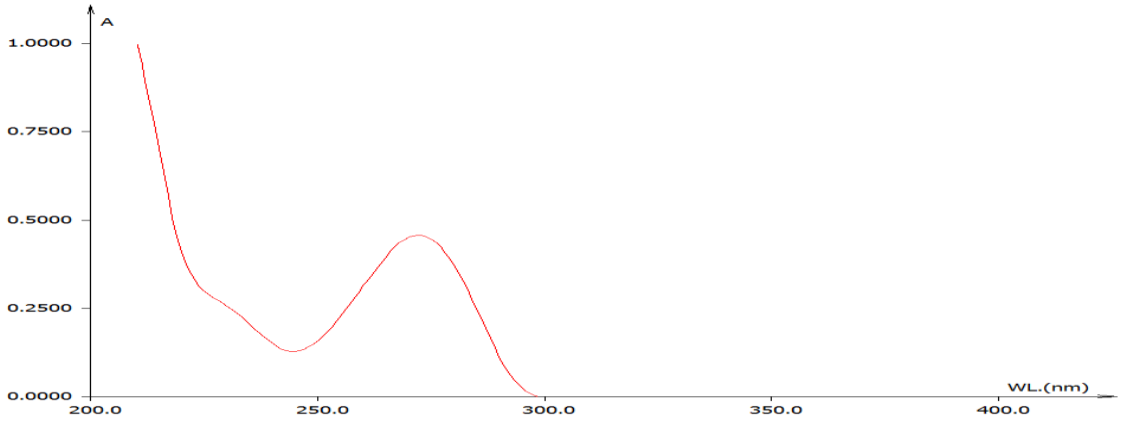

Fig-2: UV Spectrum of Trelagliptin (255nm)

Observation: While scanning the Trelagliptin solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Method Development

Several concurrent trails developed the proposed method to establish the preferred chromatographic conditions, which would be helpful to conduct a complete validation study. The optimal conditions considered for mobile phase were 45:55% v/v ratio of Methanol and 0.02M potassium dihydrogen orthophosphate buffer at pH 3.6 and Develosil ODS HG-5 RP C18, 5□m, 15cmx4.6mm i.d. Column[16] were used, which gave a sharp symmetric peak, minimum tailing factor with short run time for Trelagliptin at a flow rate of 1.0 mL/min and detection wavelength 255 nm was optimized. Sample of 20µL was injected in to HPLC System[17] and the different optimization of chromatographic parameters is depicted in Table 3. The retention time for Trelagliptin was found to be 3.210 minutes (Figure 3).

Optimized Chromatographic Method:

Table-3: Optimized Chromatographic Conditions

Mobile phase	Methanol : Phosphate buffer (0.02M, pH-3.6) = 45:55 v/v
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic

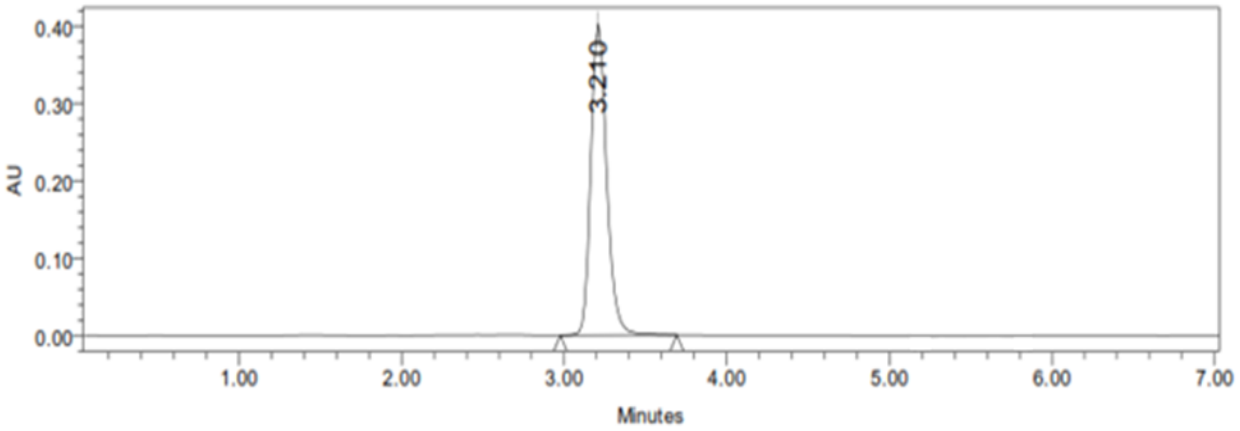

Fig-3: Optimized Chromatographic Condition

Conclusion: This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So, it's optimized chromatogram.

Validation of Analytical Method

System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters[18] were established. The data are shown in Table-4 & 5.

Table-4: Data of System Suitability Test

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	3.253	284568	7368	1.26
2	Injection 2	3.254	285684	7295	1.25
3	Injection 3	3.215	283659	7346	1.27
4	Injection 4	3.297	284754	7394	1.29
5	Injection 5	3.253	283695	7425	1.25
6	Injection 6	3.213	284578	7385	1.27
Mean			**284489.7**	**7368.833**	**1.265**
S.D			**752.5617**		
%RSD			**0.26453**		

Table-5: System Suitability Results for Trelagliptin (Flow rate)

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Trelagliptin = 0.12
2	Theoretical plate	N > 2000	Trelagliptin = 7258
3	Tailing Factor	(Tf) < 2	Trelagliptin = 1.25

Specificity:

Specificity[19] can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing three drugs was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific. The chromatograms representing the peaks of blank, Trelagliptin and the

sample containing the three drugs were shown in following figures respectively.

Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

Linearity: To evaluate the linearity[20], serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Trelagliptin. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve[21] was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Plotting of Calibration Graphs: The resultant areas of linearity peaks are plotted against Concentration.

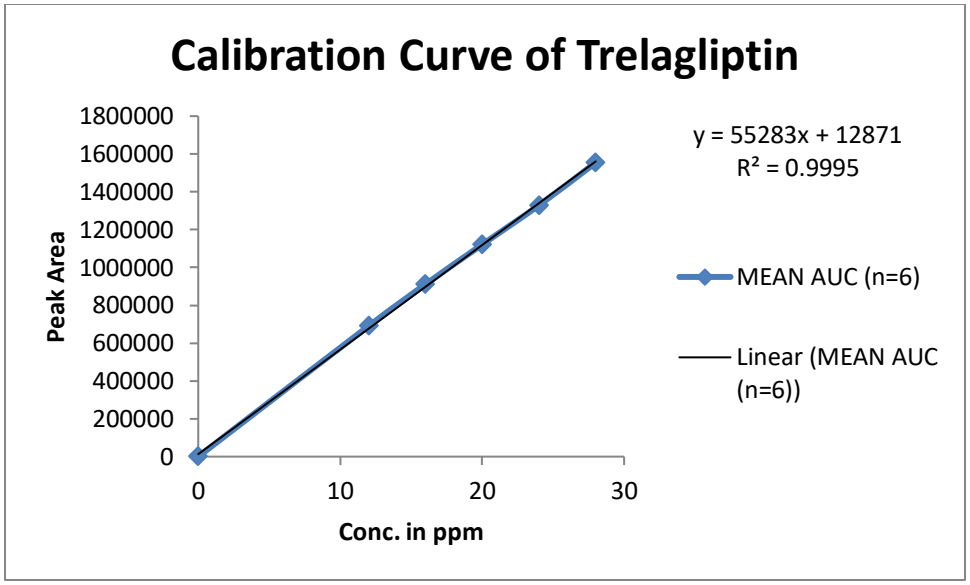

Fig-4: Standard Curve for Trelagliptin

Observation: Linearity range[22] was found to be 0-28µg/ml for Trelagliptin. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Trelagliptin.

Table-6: Linearity Readings for Trelagliptin

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Trelagliptin is a straight line.

Y = mx + c
Slope (m) = 55283
Intercept (c) = 12871
Correlation Coefficient (r) = 0.9995

Acceptance/Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 12871. These values meet the validation criteria.
Accuracy:
Inject the three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Trelagliptin and calculate the individual recovery and mean recovery values[23]. Accuracy at different concentrations (80%, 100%, and 120%) was prepared and the % recovery was calculated.

Table-7: Accuracy Results of Trelagliptin

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113% S.D. = 0.473694346 % R.S.D.= 0.471753
S_2 : 80 %	8	7.843532	446485	100.637	
S_3 : 80 %	8	8.19449	465887	100.73	
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S_5 : 100 %	10	9.978655	564521	100.868	
S_6 : 100 %	10	10.19623	576549	101.716	
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S_8 : 120 %	12	12.16785	685546	100.69	
S_9 : 120 %	12	12.18644	686574	100.823	

Observation: The mean recoveries were found to be 100.411, 100.664 and 100.463% for Trelagliptin. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision: The precision[24] of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Trelagliptin. The percent relative standard deviations were calculated for Trelagliptin are presented in the Table-8.

i) Repeatability
Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-8: Repeatability Results of Trelagliptin

HPLC Injection Replicates	AUC for Trelagliptin
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**
Standard Deviation	**1318.666**
% RSD	**0.462558**

Observation: The repeatability study which was conducted on the solution having the concentration of

about 20µg/ml for Trelagliptin (n=6) showed a RSD of 0.462558% for Trelagliptin. It was concluded that the analytical technique showed good repeatability.

ii) Intermediate Precision / Ruggedness

To evaluate the intermediate precision[25] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[29-30].

Intra Day (Day-1)/Analyst-1:

Table-9: Results of Ruggedness for Trelagliptin (Analyst-1)

S.No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Trelagliptin	3.253	284568	7368	1.26
2	Trelagliptin	3.254	285684	7295	1.25
3	Trelagliptin	3.215	283659	7346	1.27
4	Trelagliptin	3.204	286598	7457	1.22
5	Trelagliptin	3.202	287965	7635	1.29
6	Trelagliptin	3.297	285698	7459	1.28
Mean			**285695.3**		
Std. Dev.			**1508.898**		
% RSD			**0.528149**		

Inter Day (Day -2/Analyst-2)

Table-10: Results of Ruggedness for Trelagliptin (Analyst-2)

S.No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Trelagliptin	3.297	294754	7394	1.29
2	Trelagliptin	3.253	293695	7425	1.25
3	Trelagliptin	3.213	294578	7385	1.27
4	Trelagliptin	3.297	296534	7584	1.23
5	Trelagliptin	3.210	296571	7745	1.24
6	Trelagliptin	3.254	298698	7658	1.25
Mean			**295805**		
Std. Dev.			**1819.334**		
% RSD			**0.615045**		

Robustness: Robustness[26] is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Table-11: Result of Method Robustness Test for Trelagliptin

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	283261	3.254	7258	1.25
Less Flow rate of 0.9 mL/min	315864	3.297	7569	1.29
More Flow rate of 1.1 mL/min	298542	3.212	7841	1.41
Less organic phase	279856	3.253	7965	1.27
More organic phase	306985	3.215	7458	1.28

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD[27] is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Table-12: Results of LOD

	LOD
SD of Intercept	19518.16286
Slope	55283

Observation: The LOD was found to be 1.165µg/ml for Trelagliptin.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ[28].

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Table-13: Results of LOQ

	LOQ
SD of Intercept	19518.16286
Slope	55283

Observation: The LOQ was found to be 3.53µg/ml for Trelagliptin.

Assay of Pharmaceutical Dosage form

Twenty tablets/Capsules were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system). From this above stock arrangement (1 ml) was exchanged to five distinctive 10 ml volumetric flagons and volume was made up to 10 ml with same dissolvable framework (Mobile stage).

The readied arrangements were infused in five repeats into the HPLC framework and the perceptions were recorded.

A duplicate injection (Blank Solution) of the standard arrangement likewise infused into the HPLC framework and the chromatograms and peak zones were recorded and figured.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

The assay was performed as explained in the previous chapter. The results which are obtained are following:

Table-14: Recovery Data for Estimation Trelagliptin in Trelaglip-100 Tablets

Brand Name of Trelagliptin	Labelled Amount of Drug (mg)	Amount (mg) Found by the Proposed Method (n=3)	Assay %
Trelaglip-100 Tablet (Zuventus)	100mg	99.247mg	99.563%

Observation: The amount of drug in Trelaglip-100 Tablet was found to be 99.247 (± 0.378) mg/tab for Trelagliptin & % Purity was 99.563 (± 0.487)%.

SUMMARY AND CONCLUSION:

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Trelagliptin, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Trelagliptin it is evident that most of the HPLC work can be accomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Trelagliptin in different formulations.

REFERENCES:

1. https://go.drugbank.com/drugs/DB15323
2. https://pubchem.ncbi.nlm.nih.gov/compound/Trelagliptin
3. https://en.wikipedia.org/wiki/Trelagliptin
4. R. Snyder, J. Kirkland, L. Glajch, Practical HPLC Method Development, john Wiley and sons International publication, II Edn., 2011.
5. S. Ashutoshkar, Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers, 452-474, 2005.
6. H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors', New Delhi. 1-9, 157-167.
7. H.H. Williard, L.L. Merit, F.A. Dean, F.A. Settle, Instrumental Methods Of Analysis, 6th Edn, C.B.S. Publishers and Distributors, New Delhi.: 430-440, 495-504,529-545.
8. B.K. Sharma, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut: 286-300.
9. Instant notes on analytical chemistry by D. Kealey and P.J. Haines, © BIOS Scientific Publishers Limited, UK, 6-7, 2002.
10. Gurdeep R. Chatwal, Sham K. Anand, Instrumental methods of Chemical Analysis,5th edition, Himalaya Publishing House(Mumbai), P-2.566, 2005,.

11. M. E. Swartz, Journal of liquid chromatography, 28(7/8), 1253-1263(2005).
12. Journal of Chromatography .B, Analytical Technologies in the Biomedical and life Sciences. 2008 March 1; 863(2): 258-265. Published on Jan 18 2008.
13. International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1). November 2005.
14. International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A. 1994.
15. J. M. Green, a practical guide to analytical method validation, anal. Chem. News & features, pp. 305a–309a, 1 May 1996.
16. P. A. Winslow and r. F. Meyer, defining a master plan for the validation of analytical methods, j. Validation technology, pp. 361–367, 1997.
17. Aoac peer-verified methods program, manual on policies and procedures, Arlington, Va., USA (1998).
18. R. Patil: J of Chromatographia, 67, 575, (2008).
19. Baht and Leena: J of Liq. Chrom., 30, 309, (2007).
20. H.H. Williard, L.L. Merit, F.A. Dean and F.A. Settle, Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi, 2002.
21. GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).
22. Food and Drug Administration (FDA), "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices), Vol: 65 (169), 52776–52777, 2000.
23. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
24. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
25. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
26. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
27. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
28. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application-A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
29. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
30. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
31. Madhavi Arram, Srinivas Medidi and Gupta Niraj, Development and validation of simplified RP-HPLC method for quantification of Trelagliptin in tablet dosage form: Greenness analysis using AGREE penalties, International Journal of Zoological Investigations, Vol. 10, No. 2, 425-436 (2024).
32. Shaik Naazneen, RP-HPLC Method Development and Validation for the Estimation of Trelagliptin in Pure and Pharmaceutical Dosage Form, International Journal of Science and Research (IJSR), Volume 13 Issue 7, July 2024, Pages: 1400-1404.