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Optimization and validation of RP HPLC Method Development on Trelagliptin Succinate

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Abstract

A simple, precise, accurate and stability-indicating RP-HPLC method was developed and validated for the estimation of Trelagliptin Succinate from tablet. In the present study RP - HPLC method, Agilent (Autosampler) Gradient System DAD Detector and C18 (Agilent) column with 250mm x4.6 mm i.d and 5µm particle size was used. Acetonitrile: Water 0.1 % Acetic acid (65:35v/v) pH 3.0 was used as the mobile phase for the method. The analysis of Trelagliptein succinate in methanol using UV spectrophotometric analysis yielded a well-defined peak absorption maximum (λ max) which showed that it could be detected analytically. The detection wavelength was 278 nm and flow rate was 0.8 ml/min. The chromatographic conditions were optimized based on systematic trials on varying mobile phase compositions.In the developed method, the retention time of Trelagliptin Succinate were found to be 3.9 min. The method had a high linearity in 5-25 µg/mL concentration range with a regression equation of y = 49.78x – 43.516 correlation coefficient (R 2) of 0.999 indicating a good linear relationship between concentration and peak area. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. The developed method was found to be simple, accurate, precise, economic and reproducible. Intraday and interday precision studies also demonstrated a very low level of the values of %RSD, below 2%, indicating outstanding repeatability and reproducibility of the method. The LOD of Trelagliptin succinate was found to be 0.1246 (µg/mL) indicate the analytical methods was concluded.The LOQ of Trelagliptin succinate was found to be 0.3776(µg/mL) analytical method that validated.Analysis of marketed formulation % Lable Claim was found to be 98-101% ,satisfactory and are accepted. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Trelagliptin Succinate in bulk drug as well as in formulations.

Keywords: RP-HPLC; Analytical method validation; Trelagliptin Succinate; ICH guidelines

1. Introduction

Trelagliptin Succinate is a highly potent, selective, and long-acting **dipeptidylpeptidase-4 (DPP-4) inhibitor** used to manage type 2 diabetes mellitus. It works by inhibiting the enzyme responsible for degrading incretin hormones, thereby increasing insulin secretion and reducing glucagon release in a glucose-dependent manner.

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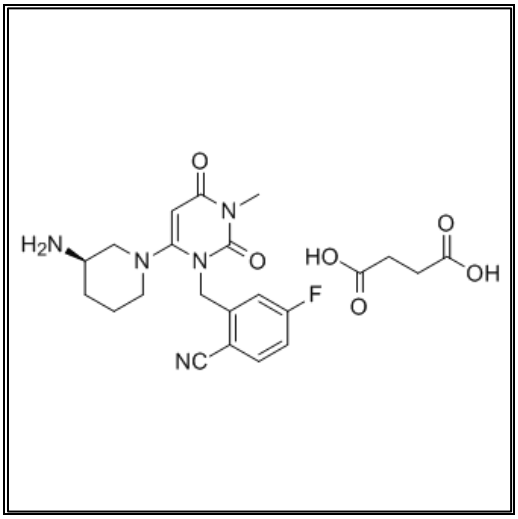

Figure 1 Structure of Trelagliptin succinate

It works by inhibiting the enzyme responsible for degrading incretin hormones, thereby increasing insulin secretion and reducing glucagon release in a glucose-dependent manner Chemically speaking (2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4- tetrahydropyrimidin-1-yl}methyl)-4-fluorobenzonitrile.,its molecular formula is C18H20FN5O2 and molecular weight is 357.389 g/mol .It works by inhibiting the enzyme responsible for degrading incretin hormones, thereby increasing insulin secretion and reducing glucagon release in a glucose-dependent.[1-4]

2. Materials and methods [5-7]

2.1. Chemicals

The pure drug samples of Trelagliptin succinate were received as a gift sample from Reliable's Shree Industrial Training Centre and Laboratory, Jalgaon. The marketed tablet formulation Trelagliptin was purchased from local medical. HPLC grade methanol, acetic acid, and purified water were purchased from Merck India Ltd. All solvents and reagents were filtered through a 0.45 µm membrane filter and then degassed prior of using for removal of any particulate matter and dissolved gases. The chemicals and reagents used in the study were of analytical reagent grade or HPLC grade for ensuring accuracy, reliability, and reproducibility of the analytical method.

Instrumentation and analytical conditions: The chromatographic analysis was carried out using an Agilent 1100 HPLC system equipped with a diode array detector (DAD, G1314B) and Chem Station software for data acquisition and processing. The system was capable of operating at a maximum pressure of 400 bar with a discharge flow rate range of 0.001 to 5 mL/min. The pressure display accuracy was maintained at ±5%, and the system could accommodate up to four mobile phases with a mixing ratio range of 0 to 100%. The pump unit was a high-performance reciprocating pump (HP-1100), which ensured precise and consistent solvent delivery during analysis.

2.2. Wavelength selection

Standard solutions were scanned in the range of 200-400nm ,against 10 ml Acetonitrile and volume make with water solvent system as reference Trelagliptin were showed absorbance maxima (λ max) at 278 nm.

2.3. Preparation of standard solution

2.3.1. Trelagliptin standard stock solution: (Stock I)

An accurately weighed quantity, 5 mg of Trelagliptin (TLG) was dissolved in Acetonitrile and water in a 10ml volumetric flask and volume made up to 10.0 ml to produce a solution of 10µg/ml.

Preparation of Sample solution: Accurately weight and transfer 5mgTrelagliptin working standard into 10 ml volumetric flask as about diluent Acetonitrile completely and make volume up to the mark with the same solvent to get 10µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.2ml was

transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Acetonitrile: Water (0.1%Acetic acid) Water, prepared in (6.5mlACN: 3.5ml 0.1% acetic acid v/v).

3. Result and Discussion

3.1. Method optimization

RP-HPLC method development for estimation of Trelagliptin succinate was performed with the evaluation of different compositions of Acetonitrile+0.1 % acetic acid), (65:35% water v/v), at different flow rates. The aim of the optimization was to achieve adequate separation, good peak shape, and suitable run time for both analytes. Several trial conditions were analysed, and the chromatographic performance was evaluated on the basis of retention time, symmetry, theoretical plate count, and resolution. The optimized condition was selected on the basis of the best overall chromatographic behaviour and was used for all subsequent validation experiments.

Table 1 Method Development Trials for Trelagliptin

Trial No.	Mobile Phase Composition (v/v)	Flow Rate (mL/min)	Injection Volume
Trial 01	Methanol+ 0.1% (acetic acid)Water, (90+10% v/v) 20 Mcg, C18 (Agilent) (4.6mm x 250mm)	1.0	20 µL
Trial 02	Methanol+ 0.1% (acetic acid)Water, (80+20% v/v) 20 Mcg, C18 (Agilent) (4.6mm x 250mm)	1.0	20 µL
Trial 03	Methanol + 0.1% (acetic acid)Water, (80+20% v/v) 0.7 20 Mcg, C18 (Agilent) (4.6mm x 250mm)	0.8	20 µL
Trial 04	Methanol+ 0.1% (acetic acid)Water, (70+30% v/v) 20Mcg C18 (Agilent) (4.6mm x 250mm)0.7ml/min	0.8	20 µL
Trial 05	Acetonitrile+ 0.1% (acetic acid)Water, (60+40% v/v) 0.7 20 Mcg, C18 (Agilent) (4.6mm x 250mm)	0.8	20 µL
Trial 06	Acetonitrile+ 0.1% (acetic acid)Water, (65+35% v/v) 20 Mcg, Flow 0.7 C18 (Agilent) (4.6mm x 250mm)	1.0	20 µL
Trial 07	Acetonitrile+ 0.1% (acetic acid)Water, (65+35% v/v) 20 Mcg, Flow 1 C18 (Agilent) (4.6mm x 250mm)	0.7	20 µL
Trial 08	Acetonitrile+ 0.1% (acetic acid)Water, (65+35% v/v) 20 Mcg, Flow 0.8 C18 (Agilent) (4.6mm x 250mm)	0.7	20 µL

3.2. Final chromatographic condition

The following chromatographic conditions were established by trial and error and were kept constant throughout the analysis.

Analytical column: Agilent C18 Column (250mm x 4.6mm)

- Partical size: 5µm
- Injection volume: 20µl
- Flow rate: 0.8 ml/min
- Detection: 278 nm
- Run Time: 15 min
- Mobile phase: Acetonitrile: Water (0.1% acetic acid) pH adjust 3.0 (65:35

Under these optimized conditions, both analytes provided well-defined peaks with satisfactory separation and acceptable retention times.

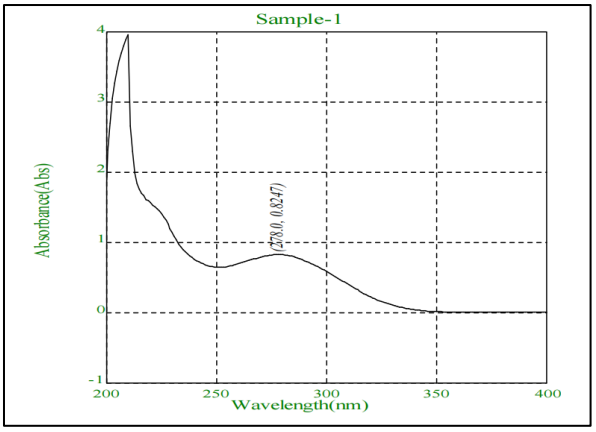

Figure 2 UV absorption spectrum of Trelagliptin succinate

3.3. Validation parameters

3.3.1. Linearity

From Trelagliptin succinate standard stock solution, different working standard solution (5-25µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using mixed volume loop injector. Chromatograms were recorded. The area for each concentration was recorded (**Table No. 2)** The Calibration curves are shown in Figure .Linearity of of Trelagliptin succinate was observed in the range of 5- 25 µg/ml Detection wavelength used was 278 nm.

The plot should be linear passing through the origin; Correlation Coefficient should not be less than 0.999 it is under acceptance limit indicate method was validated.

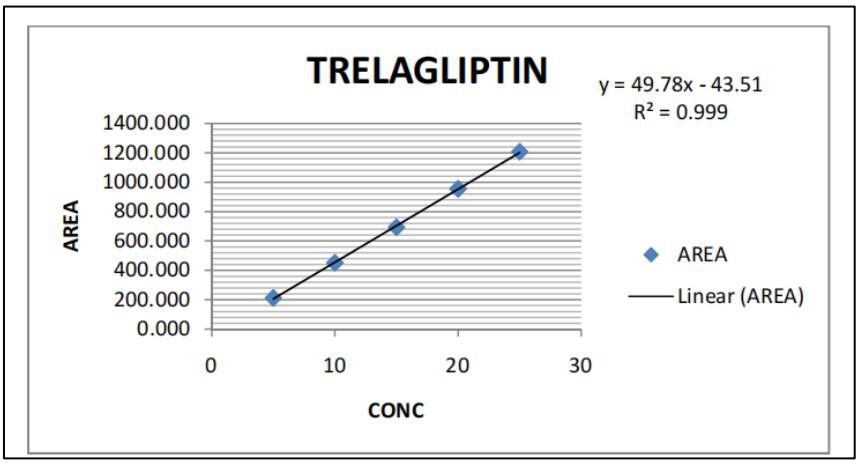

Figure 3 Calibration curve of Trelagliptin succinate (HPLC)

Table 2 Linearity Parameters Trelagliptin succinate

Sr no	Concentration	Peak area
1	5	212.3445
2	10	451.235
3	15	693.215
4	20	954.005
5	25	1205.630

3.3.2. Precision

Precision is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings.

Intraday and Inter day Precision studies on RP-HPLC method for Trelagliptin succinate which shows the high precision %amount in between 98% to 102% indicates to analytical method that concluded. The method was established by analyzing various replicates standards of Trelagliptin succinate. All the solution was analyzed thrice in order to record any intra- day & inter-day variation in the result that concluded. The result obtained for intraday is shown in **(Table No. 3)** respectively.Intraday and in terday precision studies demonstrated %RSD values <2% as per the acceptance criteria ,method was validated.

Table 3 Intraday Precision Parameters

Sr no	**Concentration**	**Intraday Precision**		**Interday Precision**	
	TLG	Mean+ %RSD	% Amount founded	%RSD	% Amount founded
1	10	452.35±1.92	99.61	453.10±2.64	99.76
2	15	714.67±0.78	101.54	710.47±1.57	100.97
3	20	955.69±1.04	100.36	954.46±0.96	100.24

3.3.3. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed. The % recovery was found to be within 98-102%, under the acceptable range.

Table 4 Assay Parameters of Trelagliptin succinate

Drug	**% level**	**Amt. taken (µg/m l**	**Amt. Added (µg/m**	**Amt found Mean* ± S.D.**	**Amt. recovered Mean *±S.D.**	**%Recovery Mean *± S.D.**
Trelagliptin succinate	80	5	4	9.00± 0.025	1.00**±** 0.025	99.88**±**0.62
	100	5	5	9.99±0.037	4.99±0.037	99.77±0.21
	120	5	6	10.97±0.03	5.97±0.039	99.52±0.65

3.3.4. Repeatability

- Repeatability studies on RP-HPLC for Trelagliptin succinatewas found to be 100.08% , The
- %RSD was less than 2%, which shows high percentage amount found in between 98% to
- 102% indicates the analytical method that concluded

Table 5 Repeatability Parameters

Injection	**Conc of Trelagliptine succinate**	**Amount found (mg)**	**%Amount found**
1	450.91	10.00	100.05
2	458.48	10.03	100.10
	Mean	10.01	100.08
	SD	5.35	
	%TRSD	1.18	

3.3.5. Ruggedness and Robustness

The robustness was evaluated by strategically modifying small analytical parameters including mobile phase composition and detection wavelength. When the mobile phase ratio was changed from 64:36 to 66:34, the chromatographic response showed consistency. Trelagliptin succinate revealed mean areas of 448.83±2.16 and 445.83±0.45, with %RSD values of 0.48% and 0.10%, respectively, when the detection wavelength was varied between 277 nm and 279 nm, Trelagliptin succinate revealed mean areas of 466.2±0.88 and 425.33±1.24, with low %RSD values 01.9 and 0.29.

Table 6 Robustness Parameters

Condition Change	Conc	Amount of detected(mean ±SD)	%RSD
Mobile phase composition-(64+36)	10	448.83±2.16	0.48
Mobile phase composition-(66+34)	10	445.83±0.45	0.10
Wavelength change277nm	10	466.2±0.88	0.19
Wavelength Change 279nm	10	425.33±1.24	0.29

3.3.6. LOD & LOQ

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD=10 (SD)/ S

10 X 1.88/ 49.78

= 0.3776

The **LOQ** of Trelagliptin succinate was found to be 0.3776 (µg/mL) analytical methods was concluded.

3.3.7. Assay

The developed RP-HPLC method was successfully applied for the assay of Trelagliptin in the marketed pharmaceutical formulation. The chromatograms revealed well resolved peaks for Trelagliptin with retention times of approximately 3.85 min. The percentage labeled claim was 100.56 % with acceptable range.

Table 7 Assay Parameters Trelagliptin succinate

Formulation	Label Claim (mg)	Amount found	% label claim	%RSD
Trelaglip	20	20.0928	100.46	0.069
Trelaglip	20	20.111	100.56	0.066

The developed method was validated as per the International Conference on Harmonization (ICH) 14, 15 guidelines with respect to linearity and range, specificity, precision, accuracy, robustness, limit of detection and limit of quantification.

4. Conclusion

Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Trelagliptin Succinate in API and tablet dosage forms. Both methods are suitable for the determination of Trelagliptin Succinate in Single-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of

standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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