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Spectrophotometric Method Development and Validation for the Estimation of Trelagliptin Succinate in Bulk and Pharmaceutical Dosage Form

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Abstract **Original Research Article**

A Simple, precise and sensitive ultraviolet spectrophotometric method was developed for the estimation of Trelagliptin succinate in bulk and pharmaceutical dosage form, method was run through LABINDIA UV-Visible spectrophotometer-3200 with solvent of acetonitrile was used. In this method working wavelength was selected at 276 nm. The developed method was validated as per the international conference on harmonization (ICH Q2(R1)) guidelines. The drug was obeying Beer-Lambert's law revealed good correlation in the concentration range of 10 - 60 µg/ml with correlation coefficient (r^2) of 0.9993. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 99.97 % for Trelagliptin succinate. The absorbance was found to be 0.0365 - 0.0506 with %RSD for both inter-day precision & intraday precision. The limit of detection and quantification were calculated and was found to be 0.0563 µg/mL and 0.1689 µg/mL respectively. The developed method was successfully applied to the determination of Trelagliptin in commercially available dosage form with percentage assay of 98.52. The proposed method is recommended for routine analysis of Trelagliptin in bulk and dosage forms in quality control testing laboratories. Since it is rapid, simple, accurate and sensitive.

Keywords: Trelagliptin succinate, UV- Spectrophotometry, Beer-Lambert's law, Quantitative Analysis, Validation and ICH.

INTRODUCTION

Trelagliptin succinate is a novel Dipeptidyl peptidase- 4 inhibitors (DPP-4) with chemical name IUPAC: (-[[6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxopyrimidin-1-yl] methyl]-4-fluorobenzonitrile; butane dioic acid. Its molecular weight is 475.5 g/mol with molecular formula $C_{22}H_{26}FN_5O_6$. It is used for the treatment of type 2 diabetes. It acts by inhibiting dipeptidyl peptidase- 4 to prevent the inactivation of incretin hormone (release of insulin) and increases the concentration of GLP-1(glucagon like peptide) (Liu Z *et al.*, 2020)

The literature survey revealed that two UV methods (Shereen Mowaka *et al.*, 2028, Zaghary WA *et al.*, 2017) and five RP-HPLC methods (Anerao Ajit *et al.*, 2016, Zhiqiang Luo *et al.*, 2017, Malleswar KD *et al.*, 2019, Luo Z *et al.*, 2018, Qi Wang· Xiuli Chen *et al.*, 2015) and two LC-MS methods (Shereen Mowaka, *et al.*, 2021, Li Zhou, *et al.*, 2020) are reported for the determination of Trelagliptin succinate individually and with other drugs till date. Present study involves the development and validation of a new UV Spectrophotometric method for the determination of Trelagliptin succinate in bulk and its pharmaceutical formulations with good accuracy and economy. The structure of Trelagliptin succinate is shown in (Figure 1). The developed analytical method was validated according to ICH validation guidelines (ICH guidelines 2005, Lavanya G, *et al.*, 2013).

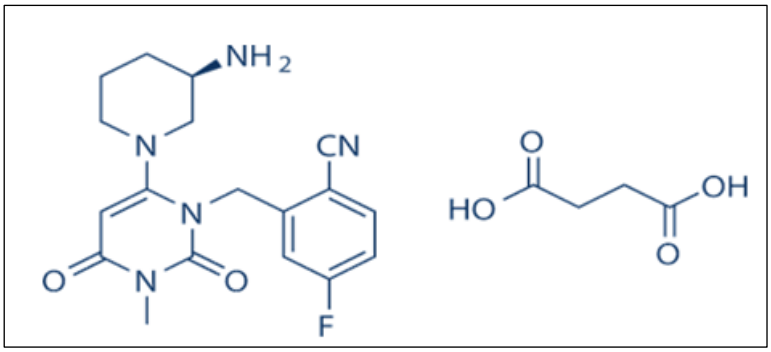

Figure 1: Chemical structure of Trelagliptin succinate

MATERIALS AND METHODS

Chemicals: Trelagliptin succinate (≥99%), was brought as a gift sample from Hetero Labs, Hyderabad, India. HPLC grade methanol, acetonitrile, and water was purchased from Thermo Fisher Scientific India Pvt. limited, Mumbai, India. The rest of the chemicals and reagents were procured from standard commercial supplier.

Equipments: LABINDIA (3200) Double beam UV-Visible spectrophotometer with 1cm matched quartz cells was used for the measurement of absorbance. Shimadzu-AX-200 electronic balance was used for weighing the samples. Citizen-Ultrasonicator and Class 'A' volumetric glassware's were used for the study.

Experimental Procedure:

Selection of Solvent for Analysis: In the present study the UV spectra of Trelagliptin was obtained from different solutions (Methanol, Acetonitrile, Distilled water, Formic acid, Dimethyl sulfoxide,) were studied. The drug was freely soluble in methanol, acetonitrile, formic acid, dimethyl sulfoxide. At the end of these studies, acetonitrile was chosen as solvent for completed the study.

Preparation of standard stock solution: Primary stock solution of Trelagliptin was prepared by dissolving accurately weighed 10 mg of drug and transferred in to a clean and dry 100 mL volumetric flask and dissolved in a few mL acetonitrile made the volume up to the mark using same solvent.

Preparation of working solutions of analyte:

From stock solution 1.0 mL, 2.0mL, 3.0 mL, 4.0 mL, 5.0 mL, 6.0 mL was taken into a series of different 10 mL volumetric flasks and made up to the mark with diluent i.e., with distilled water.

Selection of detection wavelength for the estimation:

In the present study the drug solution of Trelagliptin (30 µg/mL) were prepared and scanned over a range of 200-400 nm. It was observed that the drugs showed maximum absorbance at 276 nm. The spectra of Trelagliptin (30 µg/mL) were shown in Figure 2. which was chosen as the detection wavelength for the determination of drug.

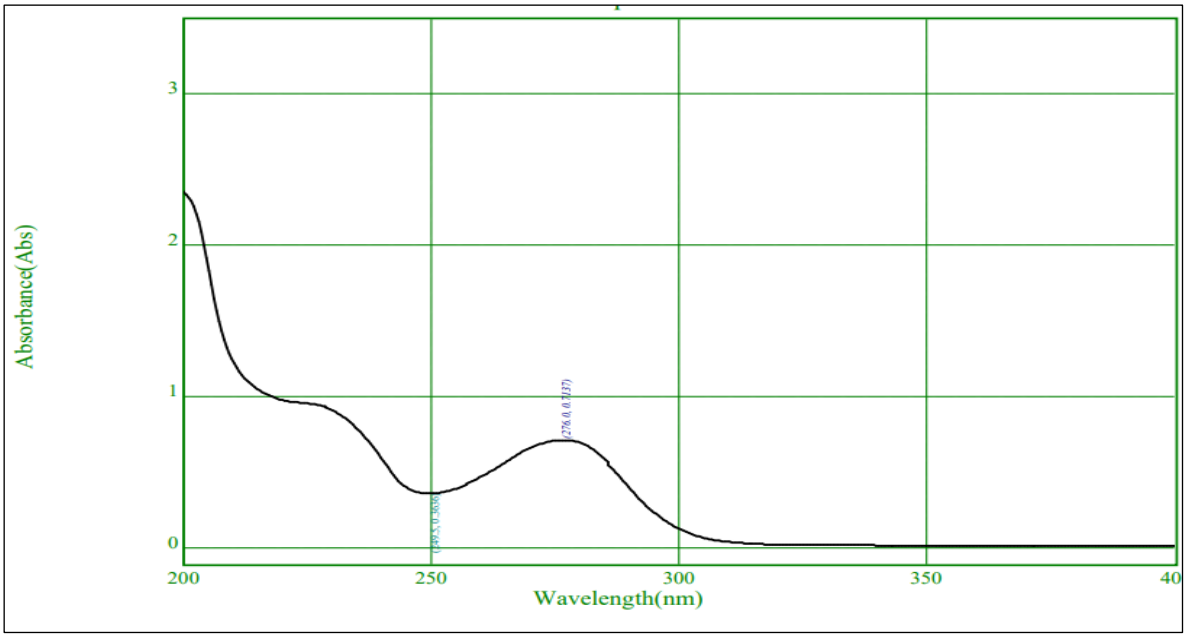

Figure 2: Spectra of Trelagliptin (30 µg/mL)

RESULTS

Method validation was performed by following the International Conference on Harmonization guidelines (ICHQ2R1).

Linearity:

Appropriate aliquot s (1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mL) of prepared working standard solutions of Trelagliptin were transferred into series of 10 mL volumetric flasks and diluted and made up to the mark with distilled water to obtain final concentration of 10-60 µg/mL of Trelagliptin. The above solutions were scanned over the range of 200 to 400 nm against acetonitrile as a blank. The absorbance of each solution was measured at 276 nm against as acetonitrile blank. A calibration curve was prepared by plotting absorbance versus concentration.

The drug was obeying Beer-Lambert's law revealed good correlation in the concentration range of 10-60 µg/ml with correlation coefficient (r^2) of 0. 9993. Results of linearity study were shown in Figure 3, Figure 4 and Table 1.

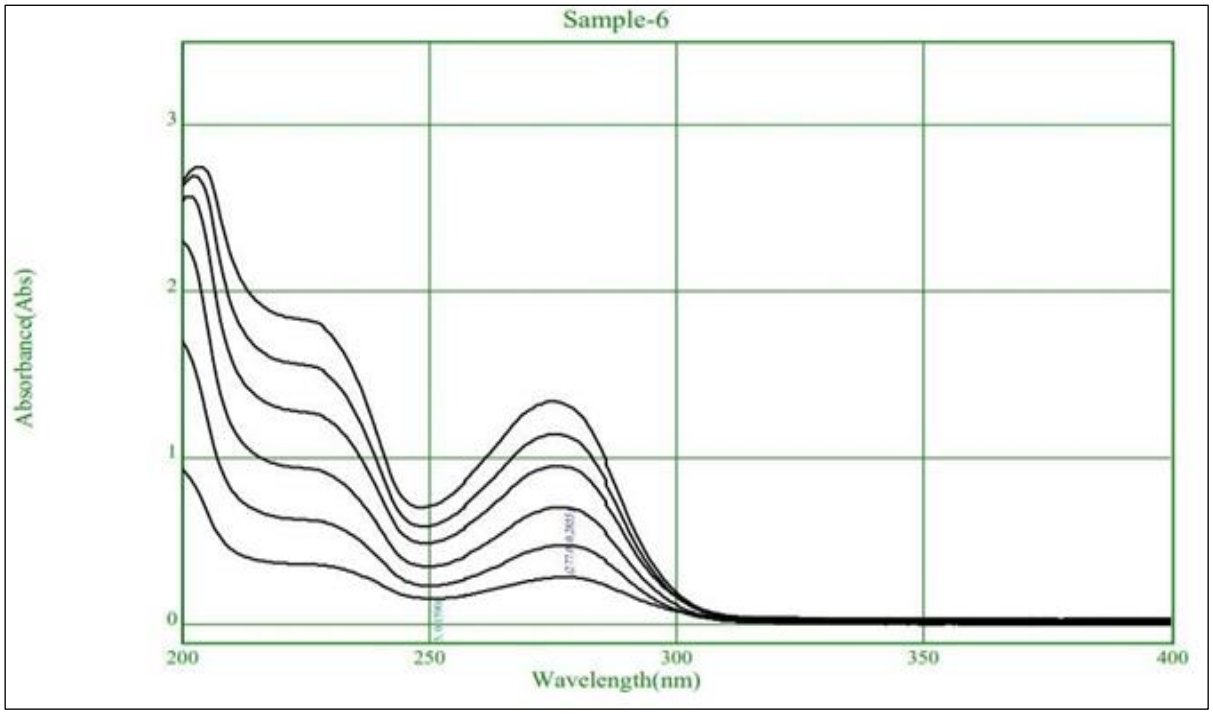

Figure 3: Overlay spectra of Trelagliptin

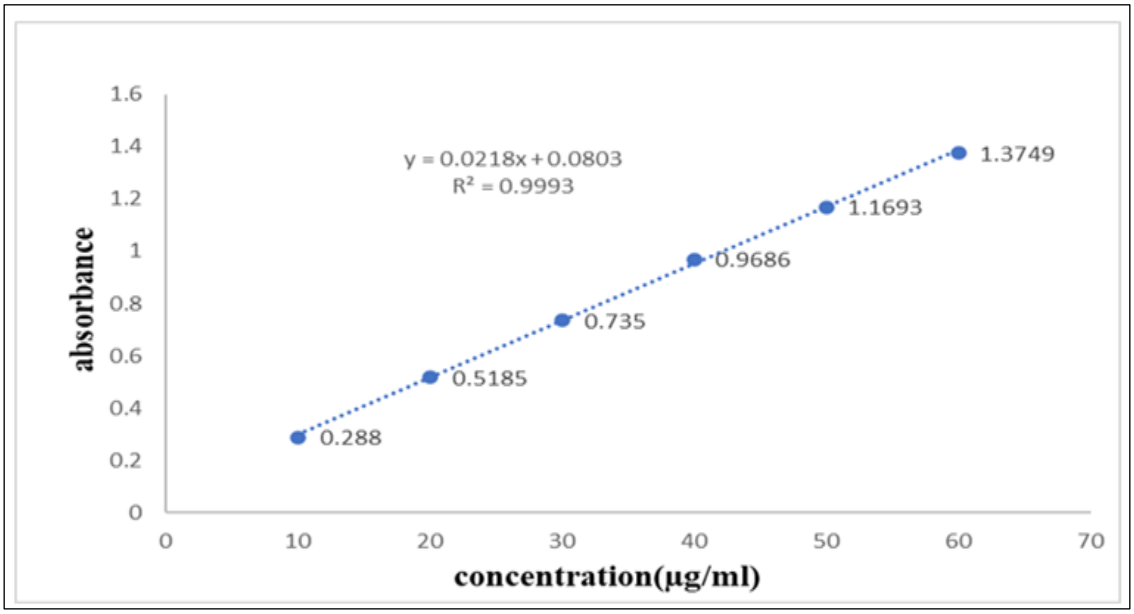

Figure 4: Linearity curve of Trelagliptin

Table 1: Linearity results of Trelagliptin

S. No	Concentration (µg/mL)	Absorbance (at 276 nm)
1	10	0.2880
2	20	0.5185
3	30	0.7350
4	40	0.9686
5	50	1.1693
6	60	1.3749

Precision: In intraday study, concentration of replicates of drug was calculated on the same day for three times. In inter-day study the concentration of drug were calculated on three successive days which expresses the laboratory variation in different days. In both intra and inter day precision study for the methods %RSD was calculated and results are shown in Table 2 and Table 3.

Table 2: Intra-day precision (Repeatability) data of proposed method

S. No	Concentration (µg/mL)	Trelagliptin		
		Absorbance at 276 nm		
		Forenoon	Afternoon	Evening
1	30	0.7346	0.7344	0.7341
2	30	0.7351	0.7346	0.7344
3	30	0.7349	0.7350	0.7349
4	30	0.7350	0.7349	0.7346
5	30	0.7352	0.7344	0.7351
6	30	0.7346	0.7350	0.7349
Mean		0.6929	0.7347	0.7346
S. D		0.000253	0.000286	0.000372
%RSD		0.03651	0.0389	0.0506

Table 3: Inter-day precision (Reproducibility) data of proposed method

S. No	Concentration (µg/mL)	Trelagliptin		
		Absorbance at 276 nm		
		Day 1	Day 2	Day 3
1	30	0.7346	0.7344	0.7341
2	30	0.7351	0.7346	0.7344
3	30	0.7349	0.7350	0.7349
4	30	0.7350	0.7349	0.7346
5	30	0.7352	0.7344	0.7351
6	30	0.7346	0.7350	0.7349
Mean		0.6929	0.7347	0.7346
S. D		0.000253	0.000286	0.000372
%RSD		0.03651	0.0389	0.0506

Accuracy: Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 25%, 50%, 100% each one in triplicate. From the recovery studies it was clear that the method is very accurate for quantitative estimation of tablet as the statistical results were within the acceptance range. Results were shown in Table 4.

Table 4: Recovery studies data of proposed method

S. No	Drug added (µg/ml)	Drug Found (µg/ml)	% Recovery	Mean% recovery
1	20	19.98	99.92	99.90
2	20	19.96	99.99	
3	20	19.96	99.80	
4	40	39.98	99.92	99.96
5	40	39.97	99.92	
6	40	39.98	99.99	
7	60	59.99	99.99	99.97
8	60	59.97	99.99	
9	60	59.96	99.94	

Limit of Detection and Limit of Quantification: The limit of detection and limit of quantification of Trelagliptin by proposed method were determined using calibration curve. LOQ and LOD were calculated as LOD = 3.3 X S.D/S and LOQ = 10 X S.D/S. Where S is the slope of the calibration curve and SD is the standard deviation of response of least concentration of calibration curve in three replicates. Results were of LOD and LOQ was found to be 0.0563 µg/mL and 0.1689 µg/mL respectively.

Robustness: Robustness of the method was determined by carrying out the analysis at three different wavelengths (±2 nm). The respective absorbance was noted and the result was indicated by %RSD. The standard deviations, relative standard deviation was calculated and the results are summarized in Table 5. Results of the study are acceptable and can be considered to be very reasonable.

Table 5: Robustness studies data of proposed method

S. No	Concentration (µg/mL)	Trelagliptin		
		274 nm	276 nm	278 nm
1	30	0.7051	0.7349	0.7543
2	30	0.6995	0.7346	0.7549
3	30	0.7152	0.7351	0.7552
4	30	0.6985	0.7352	0.7547
5	30	0.6991	0.7347	0.7550
6	30	0.6974	0.7350	0.7547
Average		0.626	0.631	0.641
SD		0.0014	0.0018	0.0010
%RSD		0.0225	0.0294	0.0161

Application of the proposed method to tablet dosage form: The proposed method was applied to the quantification of Trelagliptin tablet dosage form. Suggested that the method is suitable for the determination of Trelagliptin with good accuracy and precision. The excipients in the dosage form do not interfere in the assay procedure. The results of the assay were shown in Table 6.

Table 6: Assay results of Trelagliptin succinate

Formulation	Label claim	Amount found	% Assay
In house preparation	50 mg / tablet	49.26 mg/tablet	98.52

Forced degradation studies:

To verify the method for its stability indicating potential, Trelagliptin was forcefully degraded using 0.1N HCl, 3% v/v H_2O_2, 0.1N NaOH, and dry heat at 80°C. The degraded solutions were scanned against control.

Acid hydrolysis: Acid hydrolysis was performed by adding 1mL of 0.1N HCl to 3ml of Trelagliptin working stock solution (30 µg/mL).

Base hydrolysis: Base hydrolysis was performed by adding 1ml of 0.1N NaOH to 3ml of Trelagliptin working stock solution (30 µg/mL).

Oxidative hydrolysis: Oxidative hydrolysis was performed by adding 1ml of 3% v/v H_2O_2 to Trelagliptin working stock solution (30 µg/mL).

Thermal degradation: Thermal degradation study was performed by adding 3ml of working stock solution (30 µg/mL) and vials are sealed and was kept in hot air oven at fixed temperature 80ºC for a period of 2 hours. Results of stability study was shown in Figure 5. (Shailesh W, *et al*., 2017).

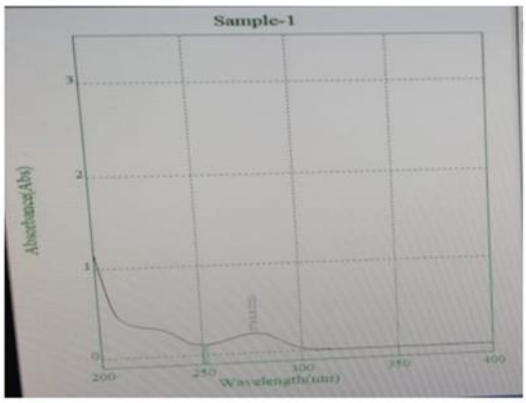

Figure 4 a : UV Spectrum of Trelagliptin in 0.1N HCl

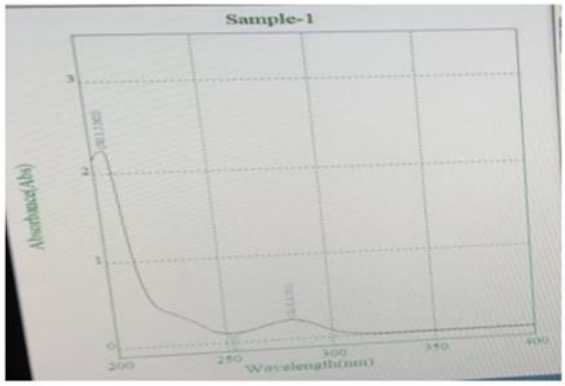

Figure 5 b: UV Spectrum of Trelagliptin in 0.1 N NaOH

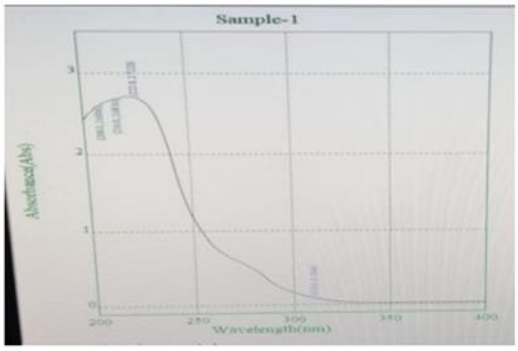

Figure 5 c : UV Spectrum of Trelagliptin in 3% H_2O_2

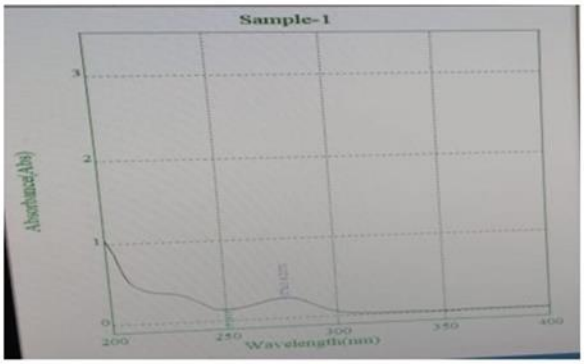

Figure 5 d: UV Spectrum of Trelagliptin in Dry heat at 80°C

Figure 5: Forced degradation study results of Trelagliptin

DISCUSSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.90% to 99.97%) of the drug were obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in sub-microgram level, which indicates the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (98.52%).

CONCLUSION

The proposed method was simple, sensitive, and cost-effective. Validated in terms of precision, linearity and accuracy. The results are reproducible, and can be used successfully for the estimation of Trelagliptin in bulk and its pharmaceutical formulations.

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