Original Article

Development and Validation of Novel UV Spectrophotometric Method for the Determination of Evogliptin Tartarate in Pharmaceutical Dosage Form

Yogesh Purushottam Agrawal*, Mona Yogesh Agrawal, Siddhesh Bharat Jadhav, Renuka Jagannath Shinde

Department of Pharmaceutical Analysis, Government College of Pharmacy, Ratnagiri, University of Mumbai, Mumbai, Maharashtra, INDIA.

ABSTRACT

Aim: Development of a novel precise, selective and sensitive UV spectrophotometric method for the estimation of Evogliptin tartarate in bulk and tablet dosage forms. **Materials and Methods:** The estimation was carried out using deionized water as solvent and Quantitation was achieved using double beam UV spectrophotometer at 267 nm. **Results:** The Calibration curves of Evogliptin tartarate shows good linearity over the concentration range from 10-100 μg/mL with excellent correlation coefficient ($R^2 = 0.992$). The percent relative standard deviation < 2.0 % confirmed the precision of the method. Excellent recovery (98.86 to 99.51) with low percent relative error proved the accuracy of method. The specificity of the methods was analyzed by evaluating marketed pharmaceutical formulation of Evogliptin tartarate. **Conclusion:** In the present work we report for the first time, a UV-spectrophotometric method for the analysis of Evogliptin tartarate and the proposed method showed excellent sensitivity, selectivity and linearity as per ICH Q2 (R1) guidelines. In addition the effectiveness of the developed method was demonstrated with marketed pharmaceutical formulation reflects good recovery percentage. It is evident that the proposed method will serve as a standard protocol for routine analysis of Evogliptin tartarate in bulks, Pharmaceutical formulations and would be of great help to Pharmaceutical industries in the future.

Key words: Evogliptin tartarate, UV-Spectrophotometry, Pharmaceutical formulations, Analytical Method Development, Validation.

INTRODUCTION

Type 2 diabetes which is also referred to as non-insulin-dependent diabetes, accounts for more than 90% of patients with diabetes.[1] The treatment guidelines for management of type 2 diabetes recommend addition of second-line agents like DPP-4 inhibitors to metformin (first line agents) for patients with insufficient control of hyperglycemia.[2,3] Evogliptin tartarate (DA-1229) (Figure 1) is chemically *(3R)-4-[(3R)-3 amino-4-(2,4,5-trifluorophenyl) butanoyl]-3-[(tert-butoxy) methyl] piperazin-2-one piperazin tartarate*, a novel oral DPP-4 inhibitor, was recently developed by Dong-A ST for treatment of type 2 diabetes improve glycemic control mainly via stimulation of glucose-mediated incretin secretion, resulting in increased insulin secretion and decreased glucagon release.[4] These glucose-dependent mechanisms of DPP-4 inhibitor suggests a lower risk for hypoglycemia and positively affect metabolic abnormalities such as obesity, hypertension and dyslipidemia, which are associated with type 2 diabetes.[5]

In patients with type 2 diabetes mellitus, once-daily administration of Evogliptin 5 mg for 12 weeks showed significant glucose-lowering effects while in healthy volunteers, a single administration of Evogliptin showed a long half-life (≥30 hrs) and the pharmacokinetics of Evogliptin was not affected by food.[6] Multiple-dosing studies signify that, 5–20 mg dose of Evogliptin exhibited linear pharmacokinetics and the

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Correspondence:
Dr. Yogesh P Agrawal
Assistant Professor, Government College of Pharmacy, Near Thiba Palace, Ratnagiri-415612, Maharashtra, INDIA.
Phone no: +91 9423720983
Email id: yogeshpagrawal@yahoo.co.in

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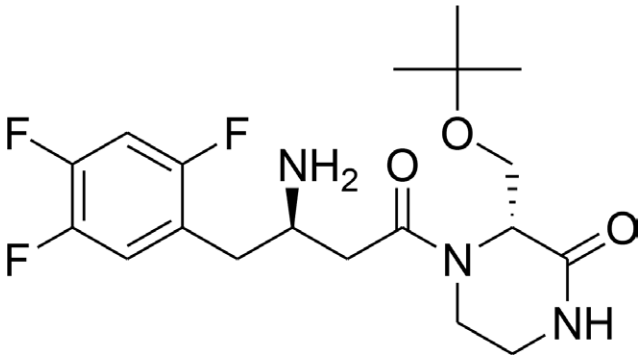

Figure 1: Molecular Structure of Evogliptin.

inhibitory effect on DPP-4 activity was sustained over 24 hrs.[7-9]

Literature survey reveals that only Liquid Chromatography with tandem MS[10] or Orbitrap MS methods were reported for the determination of Evogliptin in plasma, urine[11] and liver of humans.[12] Although these techniques have significant scientific merits such as good sensitivity and low detection limits, but they require long treatment procedures by the qualified operator with high cost implications. Alternatively, no assay procedure has been reported for the determination of this drug in bulk and its Pharmaceutical formulations. Spectrophotometry continues to be very popular amongst the various methods available for the determination of drugs, because of their simplicity, specificity and low cost. In recent advancement, electrochemical procedures offers imperative advantages, such as simplicity with cost effectiveness, rapid response, selectivity and high sensitivity[13,14] in the detection of analyte in various sample matrices and can be utilized for determination of Evogliptin in future. In present study, for the first time, a simple new spectrophotometric method for quantification of Evogliptin tartarate has been developed and validated according to the International Conference on Harmonization (ICH) guidelines[15] and successfully applied for assay of Pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation

A Shimadzu UV–visible spectrophotometer (Model UV-1800, Shimadzu Corporation, Spectrophotometric Division, Kyoto, Japan) with 10 mm quartz cuvettes were used to record the UV absorption spectra. Absorption of solutions was measured at medium speed with a sampling interval of 0.1 nm. The instrument has a fixed slit width of 1 nm. Shimadzu high precision analytical balance (Model AUX-220, Shimadzu Corporation, Kyoto, Japan) was utilized for weighting. Deionised water prepared by Extrapure-10 water purification system (Lablink Corporation Pvt. Ltd., Mumbai, India) was used throughout the experiments.

Chemicals and Reagents

Pharmaceutical grade Evogliptin (API) in the form of Evogliptin tartarate powder was procured as gift sample from Alkem Laboratory Limited, Mumbai, India. Market Formulation Valera (containing 5 mg Evogliptin Tartarate) used for method verification which was purchased from Indian local market. All chemicals and reagents were of analytical grade.

Preparation of Standard

Evogliptin Standard solution stock (1mg/mL)

Weighed and transferred 50 mg of Evogliptin Tartarate into a 50 mL flask and made up to volume with deionised water. Further, the working standard solutions were prepared by diluting stock solutions with deionised water.

Tablet test sample Preparation

Weighed 20 tablets and crushed to fine powder. Accurately weighed Powder equivalent of 50 mg of Evogliptin Tartarate to a 50 ml volumetric flask to that added 10 ml of methanol vortex for 5 min to dissolve, further added 30 mL of water and sonicate for 10 min with vigorous shaking. Allow to equilibrate at room temperature and diluted upto 50 mL with water. Centrifuged at 5000 RPM for 5 min. and filtered the supernatant solution through Whatman's filter paper No. 41 and first few ml was rejected. Use filtered solution as sample stock solution of Evogliptin Tartarate (1mg/mL) for assay analysis.

Procedure for construction of calibration curve

Required amount of working standard solutions of Evogliptin Tartarate were transferred into the 10 mL volumetric flasks to get ten solutions in the concentration range of 10-100 µg/mL. All these solutions were scanned in the range of 200-400 nm against deionised water as blank in spectrum mode and observed the maximum absorbance at wavelength 267 nm (λ_{max}) for Evogliptin Tartarate (Figure 2). Further, the calibration curves were created by plotting a graph between peak amplitude against corresponding concentrations. In addition, regression equations were figured.

Procedure for assay of sample solutions

The sample solution was prepared by accurately transferring 2 mL sample stock solution into 10 mL

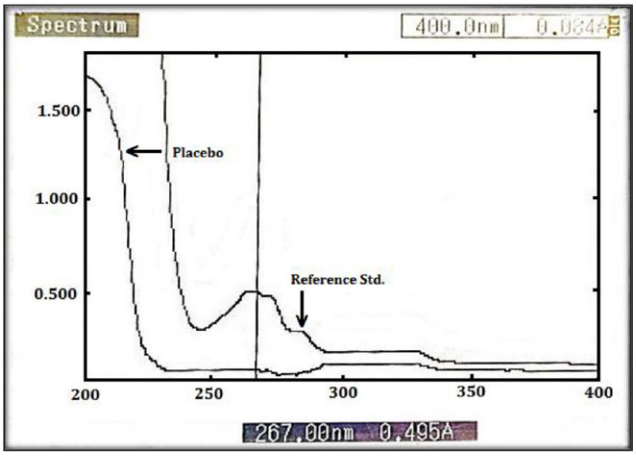

Figure 2: UV-spectra of Reference sample and Placebo in deionized water.

volumetric flask and the final volume was made with deionised water. Further, this solution was diluted with water to get the amount of Evogliptin in the range of calibration curve. The sample solution was scanned in the range of 200 nm-400 nm and concentration of Evogliptin was determined from the calibration curves of developed method.

Stability study of solution

Samples prepared for repeatability study were preserved for 24 hr at room temperature and analyzed on the subsequent day to test for short-term stability. The % RSD was determined and found to be less than 2.0 % which shows that stability of the solution at bench top.

Specificity in the presence of excipient

The established method was evaluated for the specificity using placebo. The over laid UV absorption spectra of placebo, blank and drug was compared in order to verify that none of the component interfered with the quantification of the drug (Figure 2).

Intra-day (repeatability) and inter-day (intermediate) precision study

Repeatability and intermediate precision of newly developed method was analyzed using the sample stock solution (1mg/ml). Three different aliquots were diluted to 10 ml to obtain the concentrations of 40, 60 and 80 µg/ml. This procedure was repeated in the subsequent days.

RESULTS AND DISCUSSION

An objective of this study was to develop an improved simple, selective, accurate, reproducible and sensitive spectrophotometric assay method for Evogliptin tartarate in bulk and pharmaceutical formulation. Evogliptin tartarate is very soluble in water and dilute methanol while freely soluble in organic solvents like ethanol, chloroform and ethyl acetate. During the development phase, deionized water was used as the diluent resulted in preferable outcome in UV analysis showing 267 nm as wavelength of maximum absorption (λ_{max}) and none of the component interfered with its quantification.

Validation of developed Method

The proposed developed method was validated for linearity, sensitivity, selectivity, stability, accuracy and precision as per ICH guidelines,[13] to prove that the proposed method can be used for the intended purpose.

Linearity

The linearity of the suggested method was established by evaluating the six samples in triplicate (n=3) in the concentration range of 10-100 µg/mL for Evogliptin tartarate and the amplitude determined was plotted against corresponding concentration to generate the calibration curves (Figure 3) along with regression

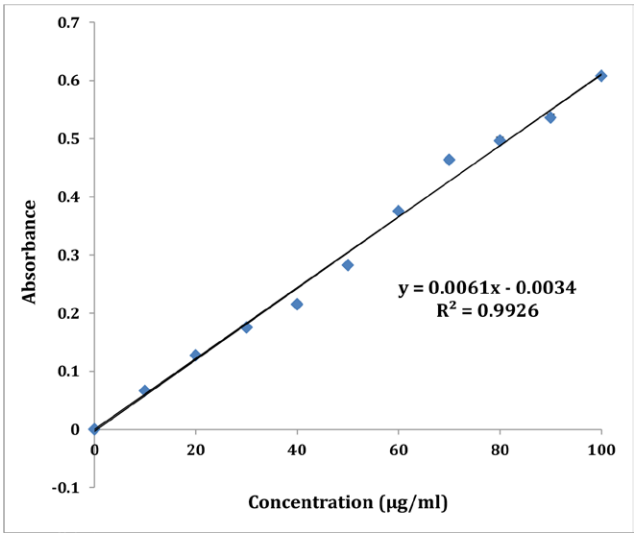

Figure 3: Calibration Curve of Evogliptin Tartarate in deionized water.

Table 1: Regression and validation parameters of Evogliptin tartarate

Linearity Range in µg/mL	10 to 100 µg/mL
Regression Equation	Y= MX + C
Slope (M)	0.0061
SD of slop	0.002
Intercept (C)	-0.0034
% RSD	1.23
Correlation coefficient (R^2)	0.9926
LOD in µg/mL	1.08 µg/mL
LOQ in µg/mL	3.27 µg/mL

Each value is average of Three determinations (n=3)

Table 2: Precision and accuracy data for three different concentrations of Evogliptin.

Conc. (µg/ml)	Intra-day Precision (*n*= 3)				Inter-day Precision (*n*= 9)			
	Absorbance Measured (Mean + SD)	RSD (%)	Conc. Found (%)	% RE	Absorbance Measured (Mean + SD)	RSD (%)	Conc. Found (%)	% RE
40	0.241 + 0.002	.829	98.77	-1.23	0.236 + 0.001	.423	96.72	-3.28
60	0.365 + 0.001	.274	99.72	-0.28	0.359 + 0.002	.557	98.08	-1.92
80	0.486 + 0.003	.617	99.59	-0.41	0.479 + 0.004	.835	98.15	-1.85

Each value is average of determinations

Table 3: Short term stability for three different concentrations of Evogliptin (n=3)

Conc. declared (µg/ml)	Conc. found (µg/ml) (Mean + SD)	RSD (%)	Average potency (%)
40	39.84 + 0.051	0.128	99.60
60	58.95 + 0.014	0.023	98.25
80	79.08 + 0.076	0.096	98.85

Each value is average of three determinations (n=3)

Table 5: Determination of Evogliptin Tartarate in Pharmaceutical preparation using proposed spectrophotometric methods.

Batch	Label claim (mg)	Amount found (mg) (Mean+ SD)	% Recovery	RSD (%)
Batch A	5 mg/tablet	4.903 + 0.008	98.0	0.163
Batch B	5 mg/tablet	4.927 + 0.005	98.4	0.101
Batch C	5 mg/tablet	4.951 + 0.006	99.0	0.121

Each value is average of three determinations (n=3)

Table 4: Recovery studies for three different concentration of Evogliptin (n=3).

Drug	Label claim	Conc. (µg/mL)	Recovery level (%)	% Recovery Estimated (Mean ± SD)	% RSD
Std. Evogliptin Tartarate	5 mg/tablet	90	80	99.2 ± 0.345	0.347
		100	100	99.6 ± 0.546	0.548
		110	120	99.5 ± 0.648	0.650

Each value is average of three determinations (n=3)

equations. Table 1, showed results of regression equations with a correlation of coefficient (R^2) greater than 0.992, proposed that, the analytical methods showed good linearity. Further, the standard deviation of a slope, intercept and residuals were found to be low, confirming the good linearity of the methods.

Sensitivity

Limit of detection and limit of quantification were calculated to confirm the sensitivity of the method. LOD was determined by multiplying 3.3 with the ratio of the standard deviation of intercept to the slope of the calibration curve. LOQ was determined by multiplying 10 with the ratio of the standard deviation of intercept to the slope of the calibration curve. The LOD and LOQ for Evogliptin Tartarate were observed to be 1.08µg/mL and 3.27µg/mL respectively indicated the good sensitivity of the proposed methods.

Precision and Accuracy

Intra-day and inter-day accuracy and precision were determined by evaluating Evogliptin Tartarate at three different concentration (40, 60 and 80) covering the entire calibration range. For intra-day, all these solutions were analyzed by proposed methods on the same day in triplicate (*n*=3). For inter-day, same solutions were analyzed on three successive days in triplicate (*n*=9). The precision was expressed as %RSD (Table 2) and was found to less than 2%, indicating good precision of the procedures. Accuracy was expressed as the percentage relative error (%RE). The low %RE indicated the accuracy of the suggested procedures.

Stability of solutions

Standard solution were prepared and analyzed spectrophotometrically at initial and after 24 hr (short-term) to check the stability. The % RSD for peak areas was determined (Table 3) and found to be less than 2.0 % which shows that stability of the solution.

Recovery studies

Recovery studies were executed at three diverse levels (80%, 100% and 120%) to confirm the specificity and accuracy of the anticipated approaches by the standard addition method. To the previously analyzed formulation consisting of 50 µg/mL of Evogliptin Tartarate and 40, 50 and 60 µg/mL of Evogliptin were added separately. All these solutions were analyzed by proposed methods and peak amplitudes were determined. The concentration of the added amount of Evogliptin was calculated

using the regression equation and subtraction of initial formulation concentration. The good recovery of Evogliptin from the formulation with low % RSD (Table 4) confirmed the specificity and accuracy of the suggested approaches.

Application of proposed methods

Application to a marketed pharmaceutical preparation

Proposed spectroscopic procedures were utilized for quantification of Evogliptin Tartarate from three different batches of market formulation "Valera" (label claims 5 mg Evogliptin Tartarate). The results were tabulated in Table 5. The recovery results were inconsistent with the labeled amount with % RSD <2%, indicating the suitability of the suggested procedures for regular quality control of Evogliptin Tartarate in pharmaceutical preparations.

CONCLUSION

Developed selective, accurate, precise, sensitive and robust UV spectroscopic method for the first time, for concurrent quantification of Evogliptin Tartarate in pure form and pharmaceutical tablets formulations with low cost. A good recovery of method showed that there is no interference of excipients used in the formulations at analyte. Water has been used as a solvent, making this method economic and eco-friendly. The outcome of the validation study showed that the developed UV spectrophotometric method for Evogliptin Tartarate will serve as a standard protocol for routine analysis of Evogliptin tartarate in bulks, Pharmaceutical formulations and would be of great help to Pharmaceutical industries in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

API: Active Pharmaceutical Ingredients; **UV:** Ultra Violet Spectroscopy; **DPP:** dipeptidyl peptidase; **conc.:** Concentration; **std.:** Standard; **RSD:** Relative Standard Deviation; **SD:** Standard Deviation; **RPM:** Rotation per minute; **ml/mL:** Mili Liter; **lit.:** Liter; **µl:** Micro Liter; **µg:** Micro Gram; **mg:** Mili Gram; **Gm:** Gram; **Kg:** Kilo Gram; **Min:** Minute; **Hr.:** Hour; **°C:** Degree centigrade; **%:** Percentage; **v/v:** Volume by volume; $\lambda_{max:}$ Wavelength maxima; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation.

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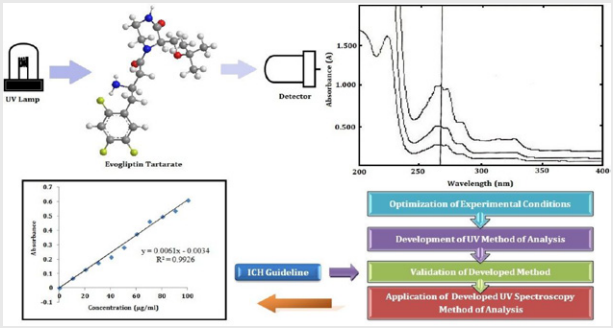
PICTORIAL ABSTRACT



SUMMARY

Developed UV-spectrophotometric method for the analysis of Evogliptin tartarate and validated as per ICH Q2 (R1) guidelines

About Authors

Dr. Yogesh P. Agrawal, M.Pharm, Ph.D (Pharmaceutical Chemistry) currently is working as Assistant Professor, Government College of Pharmacy, Ratnagiri. Competent professional with over 10 years of experience in Teaching, Research and Training on handling of Analytical Instruments

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