Original Article

Derivative Spectrophotometric Method Development and Validation for the Estimation of Evogliptin Tartrate in Pharmaceutical Dosage Form

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ABSTRACT

Aim: A simple and economic method was developed as a derivative spectrophotometric study for estimation of evogliptin tartrate in the tablet dosage form. The developed derivative method was validated as per the ICH guideline. **Materials and Methods:** The maximum absorption of evogliptin tartare was found to be 267 nm and its first and second derivative wavelengths were measured at 275 nm and 277 nm respectively. Water was used as a solvent for all measurements. **Results:** The developed method was shown linear in the concentration range of 20-120 μg/ml for evogliptin tartrate and shows a good correlation coefficient. The precision of the developed method was less than the maximum allowable limit (% RSD < 2) specified by the ICH guidelines. Excellent % recovery (98% - 101%) with less than 2% RSD value indicates method was accurate. **Conclusion:** The developed UV – Visible method was simple eco-friendly, precise and accurate as per ICH guidelines. The proposed method will use in quality control for routine analysis of evogliptin tartrate in the pharmaceutical dosage form.

Keywords: Derivative method, Evogliptin tartrate (EVO), First derivative, Spectroscopy method, UV-visible method, Validation.

INTRODUCTION

Evogliptin tartrate is chemically (3R)-4-(3-amino-4-(2,4,5-trifluoropheny)butanoyl)-3-(tert-butoxymethyl)piperazin-2-one tartrate.[1] (Figure 1) Evogliptin belongs to class of DPP-4 inhibitors drug for safe and effective oral treatment of type -2 diabetes. DPP-4 inhibitors are reduced degradation of glucagon-like peptide 1 (GLP-1) simultaneously increase insulin secretion and decrease glucagon.[2] Evogliptin is effectively improve glycosylated haemoglobin (HbA1c) and suggests a lower risk for hypoglycemia.[3] Single oral dose of 1.25 mg to 60 mg have 50% bioavilibity and maximum concentration among healthy patient was 3 to 5.5 hr.[4-5] The pharmacokinetics of Evogliptin was affected by food. In clinical research, 5 mg of evogliptin was once daily administrated in diabetic patients for 12 weeks and showed significant glucose lowing effects.[6-8]

The literature survey indicates that few analytical methods involving liquid chromatography with tandem MS method was reported for determination of evogliptin tartrate in human plasma.[9] This technique is highly sensitive and for handling it qualified operator is needed. For routine analysis of drug that type methods are costly. Spectrophotometry is most widely useful method for the determination of drugs in the form of bulk and its dosage form. Only zero order UV spectroscopy method has been reported for determination of evogliptin tartrate in pharmaceutical dosage form.[10] However no derivative

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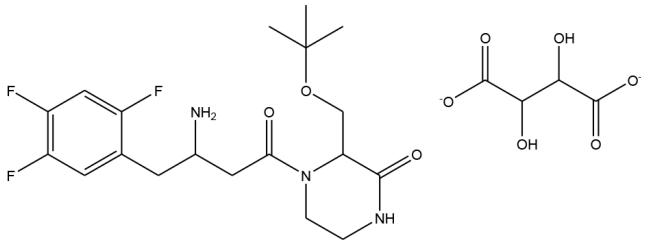

Figure 1: Structure of Evogliptin tartrate.

spectrophotometric method had been reported till date for determination of evogliptin tartrate. In present study, novel derivative spectroscopy method for estimation of evogliptin tartrate in pharmaceutical dosage form has been developed and validated as per ICH guidelines.[11]

MATERIALS AND METHODS

Reagents and Chemicals

Pure grade evogliptin tartrate (API) powder was procured as gift sample from Alkem Laboratory Limited, Sikkim, India. 5 mg of evogliptin tartrate containing marketed formulation name as Valera was procured from local market. Analytical grade chemicals and reagents were used for analysis.

Instrumentation

Shimadzu 2600 UV-visible double beam spectrophotometer with 1 cm quartz cuvettes were used for analysis and connected with computer loaded UV probe 2.35 software for spectral measurement. Spectrophotometer was fixed with spectral band width of 2 nm and all solutions were measured at medium speed with a sampling interval 0.5 nm. Acculab analytical balance (ALC-210.4, Huntingdon Valley, PA) was utilized for weighing purpose and also use sonicator (EN 30 US, Enertech Fast Clean, Mumbai, India) for sonication.

Preparation of Stock and Working Solution

Weigh accurately and transfer 50 mg of evogliptin tartrate into 50 ml of volumetric flask. Add sufficient amount of deionized water in volumetric flask for complete dissolving drug and made up to with deionized water. Required amount of stock standard solution were transferred in to the10 ml of volumetric flask and to get concentration range of 20-120 µg/ml evogliptin tartrate solution.

Preparation of Test Solution

Weigh 20 tablets of evogliptin tartrate and finely crushed. Weigh accurately equivalent amount of 50 mg tablets powder and transferred in to 50 ml of volumetric flask. Add 10 ml of methanol and 30 ml of deionized water in to flask and sonicate for 10 min with vigorous shaking. Allow stand for 15 min and made up to 50 ml with deionized water. Filter the solution with Whatman paper No 41 and used that filtered solution as sample stock solution (1 mg /ml). Further, sample working solutions were prepared by diluting with deionized water to get amount of evoglitpin tartrate in the range of calibration curve for assay analysis.

Selection of Analytical Wavelength

Solutions of evoglitpin tartrate were prepared by appropriate dilution in deionized water and spectrum was recorded in the range of 200-400 nm. All zero order (D^0) spectra were converted to first order (D^1) and second order (D^2) derivative spectrum using delta lambda ($\Delta\lambda$) 4 nm and scaling factor 1.0. The UV spectrum of evogliptin tartrate in deionized water has shown maximum absorbance at 267 nm, 275 nm and 277 nm for zero, first and second ordered spectrum respectively (Figure 2).

Method Validation

The proposed method has been validated as per ICH guideline parameters like Linearity, Precision, Accuracy, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

From appropriate volumes of stock solution, 20-120 µg/ml of evogliptin tartrate was prepared by using deionized water as a solvent. Absorption spectra of D^0, D^1 and D^2 were recorded in range of 200-400 nm of spectrophotometric condition. D^0, D^1 and D^2 absorbance at 267 nm, 275 nm and 277 nm respectively were recorded of evogliptin tartrate. The calibration curves were plotted against drug concentration by making five replicates (Figure 3).

Precision

The intra-day precision and inter-day precisions of the proposed method were determined by analysis of three different concentration of standard of evogliptin tartrate (40, 60 and 80 µg/ml) on the same day and on 3 different days and responses were recorded in triplicate.

Accuracy

The accuracy of the proposed method was checked by recovery studies of evogliptin tartrate by the addition of standard drug solution (32, 40 and 48 µg/ml) to pre-analysed sample solution (40 µg/ml) at three different concentration levels (80%, 100% and 120%) within the range of linearity for all drugs. Each solution was

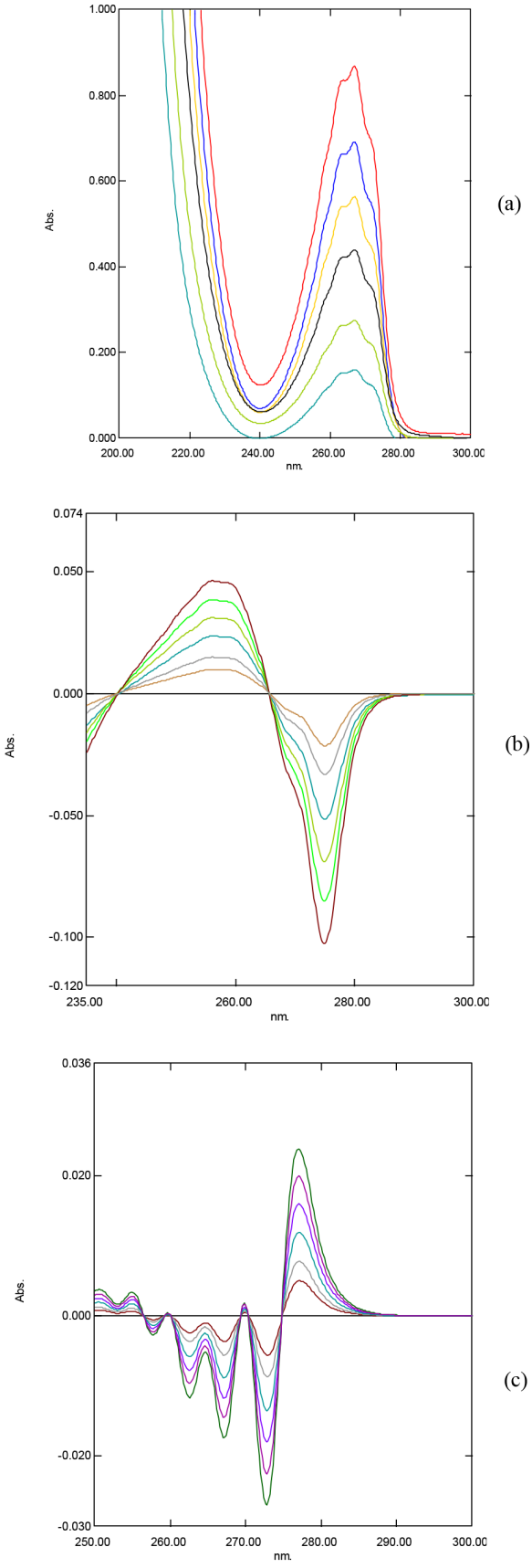

Figure 2: Overlay zero (a), first (b) and second (c) order derivatives UV spectra of standard evogliptin tartrate in deionized water (20-120 µg/ml).

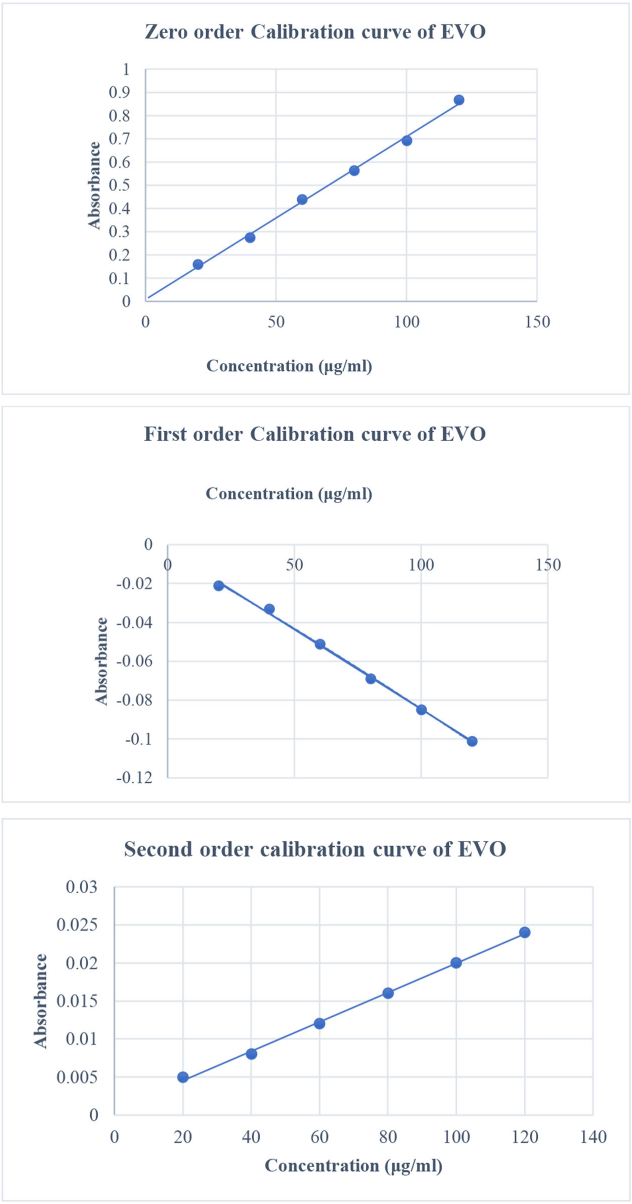

Figure 3: Calibration curves of evogliptin tartrate.

analysed in triplicates and recovery was calculated by measuring absorbance.

Limit od Detection (LOD) and Limit of Quantification (LOQ)

ICH guideline describes several approaches for determination of LOD and LOQ. The standard derivation of the response approach used for the calculation of LOD and LOQ.

LOD and LOQ of the drugs were calculated using equation as per ICH guideline.

$$LOD = 3.3\ \sigma / S \text{ and } LOQ = 10\ \sigma / S$$

Where, σ is standard deviation of the response and S is slope of calibration curve.

RESULTS AND DISCUSSION

A simple accurate and precise method was developed and validated as per ICH guideline for estimation of evogliptin tartrate in pharmaceutical dosage form. Evogliptin tartrate is freely water and methanol soluble.

Table 1: Validation parameters of zero, first and second order derivative spectrophotometry.

Parameter	Zero order	First order	Second order
Linearity range	20-120 µg/ml	20-120 µg/ml	20-120 µg/ml
Regression equation	y = 0.007x + 0.0085	y = -0.0008x - 0.0026	y = 0.0002x + 0.0007
Correlation coefficient	0.9968	0.9976	0.9982
Precision (% RSD)			
Intra - day (*n* = 3)	0.11 – 0.48	0.10 – 0.23	0.09 – 0.26
Inter – day (*n* =3)	0.60 – 1.43	0.30 – 0.76	0.30 – 0.93
% Recovery studies (*n* = 3)	99.93 – 101.46	99.83 – 101.56	98.85 – 101.04
LOD			
By calculation (µg/ml)	1.157	1.019	0.899
LOQ			
By calculation (µg/ml)	3.507	3.087	2.725

Table 3: Analysis of Marketed formulation.

Order	Amount of drug taken (Label Claim)	Amount of drug estimated* (mg/tab) ± SD	% Label claim* ± SD	% RSD
Zero	5 mg	4.98 ± 0.09	99.9 ± 0.92	0.38
First	5 mg	5.01 ± 0.07	100.2 ± 0.85	1.21
Second	5 mg	4.99 ± 0.081	99.87 ± 0.61	1.08

*Average of three determinants.

During method development, deionized water was used as solvent. Maximum wavelength at 275 nm for first order and 277 nm for second order were selected for the analysis of evogliptin tartrate.

The linearity in the concentration range of 20-120 µg/ml was evaluated with six samples in triplicate (n=3) for evogliptin tartrate. Absorbance verses concentration range of evogliptin tartrate was plotted in the form of calibration curves along with regression equation and correlation coefficient. The correlation coefficient is 0.9968, 0.9976 and 0.9982, indicates proposed analytical method showed good linearity.

Inter-day precision and intra-day Precision were determined at three replicates having three different concentration of evogliptin tartrate (40, 60 and 80 µg/ml). Percentage relative standard deviation (% RSD) was

Table 2: Accuracy studies for zero, first and second order at three concentration level.

Zero order						
Drug	**% Level**	**Conc. of sample taken (µg/ml)**	**Conc. of Std. added (µg/ml)**	**Amount Recovered (µg/ml) Mean* ± SD**	**% Recovery**	**% RSD**
EVO	80	40	32	32.4 ± 0.84	101.46	0.84
	100	40	40	40.4 ± 0.92	100.08	
	120	40	48	47.9 ± 0.50	99.93	
First order						
Drug	**% Level**	**Conc. of sample taken (µg/ml)**	**Conc. of Std. added (µg/ml)**	**Amount Recovered (µg/ml) Mean* ± SD**	**% Recovery**	**% RSD**
EVO	80	40	32	32.7 ± 0.75	101.56	0.87
	100	40	40	39.9 ± 1.40	99.83	
	120	40	48	48.4 ± 0.84	100.09	
Second order						
Drug	**% Level**	**Conc. of sample taken (µg/ml)**	**Conc. of Std. added (µg/ml)**	**Amount Recovered (µg/ml) Mean* ± SD**	**% Recovery**	**% RSD**
EVO	80	40	32	31.6 ± 1.12	98.85	1.12
	100	40	40	39.8 ± 1.63	99.58	
	120	40	48	48.5 ± 0.79	101.04	

*Average of three determinants.

found to be less than 2 indicates method was precises. All validation parameters were tabulated in Table 1.

The accuracy of evogliptin tartrate was carried out at three different levels (80%, 100% and 120%). The % recovered concentration of evogliptin tartrate was found to be 98-100%. The % RSD was less than 2 shows good recovery of evogliptin tartrate from formulation (Table 2). The proposed method was applied to tablet formulation of evogliptin tartrate. % RSD less than 2 indicates, the developed method was implied for routine quantification of evogliptin tartrate in pharmaceutical dosage form (Table 3).

CONCLUSION

The proposed derivative spectrophotometric developed simple, specific, precise, accurate and robust method for estimation of evogliptin tartrate in pharmaceutical dosage form. The good recovery of method showed that no interference of excipient. Water has been used as a solvent, making this developed method economic and eco-friendly. The method was validated as per ICH guidelines and can be used for routine analysis and quality control assay of evogliptin tartrate in dosage form.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPP: Dipeptidyl peptidase; **API:** Active pharmaceutical ingredients; **RSD:** Relative standard deviation; **SD:** Standard deviation; **std:** Standard; **Conc.:** Concentration; **ml:** Millilitre; **µg:** Microgram; **%:** Percentage; **v/v:** Volume by volume; **mg:** Milligram; **Hr:** Hour; **nm:** Nanometre.

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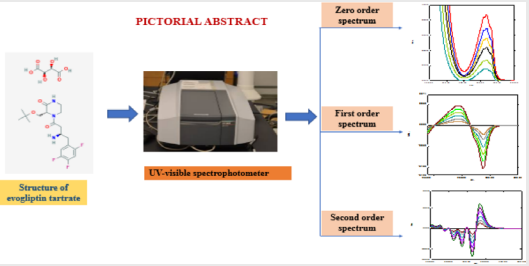
PICTORIAL ABSTRACT



SUMMARY

Developed derivative UV-spectrophotometric method for the analysis of evogliptin tartrate and validate the developed method as per ICH Q2 (R1) guidelines.

About Authors

Mrs. Khushbu Patel, M. Pharm (Pharmaceutical chemistry) currently is working as Assistant Professor, Shri Sarvajanik Pharmacy college, Mahesana.

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