JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

NOVEL METHOD DEVELOPMENT, VALIDATION, AND STABILITY INDICATING ASSAY METHOD FOR EVOGLIPTIN TARTRATE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT:

To develop a novel, simple, precise, accurate, reproducible, and validate stability indicating assay method by RP-HPLC for determination of Evogliptin tartrate in the pharmaceutical dosage form. The adequate separation was achieved on Hypersil BDS C18 (250 mm x 4.6 mm 5 µ) column in isocratic mode with a mobile phase consisting of methanol: water: TFA mixture (70: 30: 0.1% v/v) with flow rate 1 ml/min. The detection was carried out at 264 nm. The retention time of Evogliptin tartrate API and tablet was found to be 4.03 min and 4.02 min respectively. The method was validated as per ICH guidelines. Linearity was established for Evogliptin tartrate in the range of 75.25-225.75 µg/ml with an R^2 value of 0.999. The mean recovery of Evogliptin tartrate was found to be in the range of 100.58 ± 0.4. The stress degradation studies of Evogliptin tartrate were carried out under acidic, basic, peroxide, thermal and photolytic conditions. Degradation was observed in acid and base stressed sample, but not in peroxide, thermal, and photolytic stressed sample. The proposed method was found to be specific, accurate, precise, and robust can be used for estimation of Evogliptin tartrate in API and pharmaceutical dosage form.

1. Introduction

Evogliptin is a new oral hyperglycemic agent of the DPP-4 (dipeptidyl peptidase-4) inhibitor class developed by Dong-A. Evogliptin (brand name: Sugarnon) selectively and potently reduces blood glucose levels by inhibiting dipeptidyl peptidase IV, which is primarily responsible for the rapid degradation of incretin hormones, including glucagon-1-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide. (1, 2) Evogliptin is a derivative of piperazine in clinical development as a new DPP-IV inhibitor for the treatment of

type 2 DM. Its IUPAC name is 4- [3-amino-4- (2, 4, 5-trifluropenyl) butanoyl] -3 - [(2-methyl propane-2-yl) oxymethyl] piperazine-2-one; 2,3-dihydroxybutanedioic acid. Its empirical formula is $C_{23}H_{32}F_3N_3O_9$. It is a white powder consisting of a molecular weight of 551.5 g / mol. and soluble in a polar solvent such as water, methanol, etc. EVT is not official in any pharmacopeia. A literature survey of EVT revealed that no analytical method has been reported for its determination in bulk drugs and its pharmaceutical dosage form. Also, there is no stability-indicating assay method reported for the estimation of Evogliptin tartrate. Since the literature has not mentioned any method for the determination of this drug from the bulk drug, as well as its formulation. The aim of this work was to develop and validate the RP-HPLC stability-indicating assay method for the estimation and determination of Evogliptin tartrate in the bulk and pharmaceutical dosage form.

2. Material and Methods

The development and validation of the method were performed using the Acme 9000 separation model with the UV/Vis detector model. Data were obtained using Atochro -3000 and the column used was Hypersil BDS C18 (250 mm, 4.6 mm 5 μ).

3. Instrumentation

Younglin separation model, Acme 9000 with UV/Vis detector and Atochro-3000 is used for current research. Chromatographic separation is performed on Hypersil BDS C18 (250 mm x 4.6 mm 5 μ) with the mobile phase of methanol, water, and TFA (70:30:0.1%v/v). The substances of the mobile phase were filtered before use through a membrane filter (0.45μm). The advanced chromatographic condition is shown below

4. Chromatographic condition

- Column: Hypersil BDS C_{18} (250 mm x 4.6 mm 5 μ)
- Flow rate: 1.0 ml/min
- Run time: 15 min
- Wavelength: 264nm
- Injection Volume: 20 μl
- Detector: UV/Vis Detector
- Elution: Isocratic
- Mobile phase: Methanol, Water, TFA was used in the ratio of (70:30:0.1) (v/v)
- Column temperature: Ambient

5. Chemical products and reagents

A pure Evogliptin tartrate sample was obtained from Ozone international (Mumbai). HPLC grade water, HPLC grade methanol, TFA AR grade, $NH_4H_2PO_4$ AR grade, KH_2PO_4 AR grade, a digital ultrasonic cleaner has been used to improve drug dissolution. A digital pH meter was used for pH adjustment. The formulation VALERA (5mg) was purchased from the local market.

6. Preparation of Standard solution: select the UV spectrum

An accurately weighed quantity of about 10 mg of EVT was transferred to the 10 ml volumetric flask, dissolved in methanol, and volume was made up to the mark with the same solvent. (Concentration: 1mg/ml).

Aliquots of standard stock solutions were suitably diluted with methanol diluents to obtain a concentration of 100 µg/ml of each drug. The solutions were scanned in the 400-200 nm range in a 1 cm cell against the blank. The wavelength of absorption (lambda max) was found to be 264 nm. So the wavelength selected for the determination of EVT is 264 nm.

7. Selection of mobile phase

The pure drug EVT was injected into the HPLC system and run in different solvent systems and their ratio. Different mobile phases like methanol and water, methanol and buffer, methanol and buffer, methanol, water, TFA and methanol, water, and TFA tried. It was found that Methanol, water, and TFA give a satisfactory result as compared to other mobile phases. Finally, the optimal composition of the mobile phase determined to be Methanol: water: TFA (70:30:0.1 v/v).

8. Preparation of the mobile phase

A. Preparation of Ammonium Dihydrogen Orthophosphate Buffer

Firstly 0.01M of Ammonium Dihydrogen orthophosphate buffer ($NH_4H_2PO_4$) was prepared by dissolving 11.5 gm KH_2PO_4 in 1000 ml of HPLC grade water. Mobile phase prepared by mixing 80 ml of Methanol with 20 ml of phosphate buffer and lastly mobile phase mixture adjusted to pH 6.5 with ortho phosphoric acid. The prepared mobile phase was then filtered through a 0.45 µ membrane filter, sonicated for 10 min and degassed to remove trapped air. They were used as mobile phases

B. Preparation of Potassium Dihydrogen Phosphate Buffer

Firstly 0.1M of Potassium Dihydrogen phosphate buffer (KH_2PO_4) was prepared by dissolving 1.36gm of KH_2PO_4 in 1000 ml of HPLC grade water. Mobile phase prepared by mixing 80 ml of methanol with 20 ml of phosphate buffer and lastly mobile phase mixture adjusted to PH 4.5 with ortho phosphoric acid. The prepared mobile phase was then filtered through a 0.45 µ membrane filter, sonicated for 10 min and degassed to remove trapped air. They were used as the mobile phase.

C. Preparation of standard solutions for chromatography:

I. EVT Standard Stock Solution:

A weighed quantity of 150.1 mg of EVT was dissolved in diluents (mobile phase) and the volume was filled with a 100 ml mark to obtain a stock solution of 1501 µg/ml. The resulting solution was filtered through a 0.45 µ membrane filter and sonicated for 10 min with intermittent shaking.

II. EVT working standard solution:

Pipette out 10 ml of a standard stock solution of EVT and further dilute it with 100 ml of diluent to obtain 150.1 µg/ml of EVT. This solution was filtered 0.45 µ membrane filter paper and sonicated for 10 min with intermittent shaking.

9. Calibration curve of EVT

A. Preparation of the mobile phase:

Accurately measure 1400 ml of HPLC grade methanol, 600 ml of HPLC grade water, and 2 ml of AR grade TFA were mixed, shaken well, and then filtered through a 0.45 µm membrane filter and degassed with digital ultrasound for 10 min.

B. Diluent preparation

The mobile phase itself was selected as diluents for preparation of standard and sample solution

C. Preparation of calibration curve

A large quantity of 150.50 mg of EVT was dissolved in diluent and obtained up to 100 ml of volume with the same solution obtained of 1505 µg/ml. From 1505 µg/ml Appropriate aliquots such as 0.5 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.5 ml taken in a 10 ml volumetric flask, and prepared for labeling with diluents, whereby the resulting solution becomes 75.25, 120.4, 150.50, 180.6, 225.75 µg/ml.

D. Analysis of the marketed preparation

Each tablet was weighed and powdered with mortar and pestle. Accurate weight quantities of pre-analyzed tablet powder equivalents to 15 mg of EVT were taken and dissolved in diluent and a volume of up to 10 ml of the volumetric flask was prepared. The resulting solution was filtered through a syringe filter and sonicated for 10 min with intermittent shaking. Pipette 1 ml of the above solution into a 10 ml volumetric flask and dilute to the mark with diluents. The resulting solution was filtered through a 0.45 µ membrane filter and sonicated for 30 min with intermittent shaking

Equal volumes (20 µl) of standard and sample solutions were injected separately after stationary phase equilibration. The content of EVT was calculated by comparing a sampling peak with that of the standard.

Acceptance criteria: The % assay as between 95-105%.

10. Validation parameter as per ICH guidelines

A. Preparation of diluents

The mobile phase itself was selected as diluents for the preparation of standard.

B. System suitability

The system suitability parameter established for the present developed RP-HPLC method includes the number of theoretical plates, tailing factor. For this, the HPLC system was equilibrated using the initial mobile phase composition, followed by 3 injections of the standard solution of concentration containing 150.1 µg/ml of EVT.

Acceptance criteria: The relative standard deviation of the area of analyte peak in standard chromatogram should not be more than 2%, TP: NLT 2000, TF: NMT 2.0.

C. Linearity and range

Appropriate aliquots of standard EVT stock solution(150.1 µg/ml each) were taken in different 10ml volumetric flask and the resultant solution was diluted up to the mark with diluent (mobile phase) to obtain different concentration range of 50-150%.of the standard concentration. Calibration curves were constructed by plotting the concentration of EVT versus the corresponding peak area.

Acceptance criteria: The correlation coefficient should be not less than 0.995.

D. Specificity

A study conducted to establish the specificity of the proposed method involving injecting blank using the chromatographic condition defined for the proposed method. Equal volumes (20 µl) of blank (diluent), standard, and sample solutions were injected separately into the chromatographic system in above defined

chromatographic condition and injected into the HPLC system to check interference if any at the retention time of EVT.

Acceptance criteria: No peak shall be eluted at the retention time EVT in the blank.

E. Precision

The precision study was performed using an intraday and intraday precision method. The proposed method was determined by analyzing the EVT solution at different time intervals and on different days. Injected standard preparation and sample preparations into the HPLC system record the chromatograms and measure peak responses for the EVT peak. The precision was expressed in terms of standard deviation and % RSD.

Acceptance criteria: The RSD of the % assay values should not be more than 2.00%

F. Accuracy

The accuracy of the method was evaluated by the recovery studies by the known quantity of EVT was added to the predetermined sample solution at three different concentrations at 80%, 100% and 120% by spiked level. For each level, their solution was prepared and injected in HPLC. The accuracy was calculated as the percentage of the drug recovered from the formulation and added concentration. % Recovery = (amount of drug recovered) / (amount of drug added) x 100.

Acceptance criteria: Recovery should be between 98 and 102%, RSD of recovery should not be more than 2.00 %.

G. Robustness

The robustness method was determined by varying the method parameter, such as a change in flow and a change in wavelength. Under analytical conditions, inject into the HPLC system at -0.05 (0.95 ml/min) and +0.05 (1.05 ml/min). The HPLC system also works with a variation in wavelength at -2 (262 nm) and +2 (266 nm). Keeping other parameters constant, the chromatographic response was measured. The robustness was calculated as the % assay and RSD.

Acceptance criteria: Overall RSD should not be more than 2.0%

11.Stress degradation studies

Standard preparation:

A. Preparation of diluents

The mobile phase itself was selected as diluents for the preparation of standard.

B. C. Preparation of Stock EVT solution

Accurately weighed a quantity of 150.2 mg EVT was dissolved in diluents (mobile phase) and volume was made up to 100 ml mark with diluent and filter with a 0.45 µm membrane filter then sonicate it for 10 min. to obtain a 1502 µg/ml stock solution.

C. Acid degradation:

10 ml of stock solution transfer in 100 ml of the volumetric flask, add 10 ml of 0.1 N HCL solutions, and refluxed on a water bath at 60°C for about 30 minutes. The sample after the stress was neutralized with 0.1 N NaOH and volume made up to 100 ml to mark with diluent and filter through a 0.4 µm membrane filter before analysis and injected into the HPLC system. From the peak area found in the chromatograph, the % degradation

was calculated.

D. Base degradation:

10 ml of stock solution transfer in 100 ml of the volumetric flask, add 10 ml of 0.1 N NaOH solution, and refluxed on a water bath at 60°C for about 30 minutes. The sample after the stress was neutralized with 0.1 N HCL and volume made up to 100ml to mark with diluent and filter through a 0.4 μm membrane filter before analysis and injected into the HPLC system. From the peak area found in the chromatograph, the % degradation was calculated.

E. Oxidative/ Peroxide degradation

10 ml of stock solution transfer in 100 ml of the volumetric flask, add 10 ml of 0.3 % H_2O_2 solution, and refluxed on the heating mantle at 60^0C for about 12 h. After cooling, volume made up op to 100 ml to the mark with diluents and filtered it with a 0.45 μm membrane filter before analysis and injected the solution to the system. From the peak area found in the chromatograph, the % degradation was calculated.

F. Thermal degradation

The pure drug was heated in the oven for 1h which is maintained the temperature at 60°C and cool at room temperature. Accurately weighed quantity of the above drug to 150.1 mg EVT was dissolved in diluent (mobile phase) and volume was made up to 100 ml mark (stock solution). Pipette out 10 ml from a standard stock solution of EVT was further diluted it with 100ml diluent and filter it with a 0.45 μm membrane filter then sonicate it for 10 min. to obtain 150.1μg/ml of EVT and injected the solution to the system. From the peak area found in the chromatograph, the % degradation was calculated.

G. Photolytic degradation

Pure drug of EVT was taken in the Petri plate and spread as a thin layer and this is exposed to UV light at 254 nm in a UV chamber for 1 hr. Laure accurately weighed quantity of 150.3 mg EVT was dissolved in diluent (mobile phase) and volume was made up to 100 ml mark (stock solution)

Pipette out 10 ml from a standard stock solution of EVT was further diluted it with 100ml diluent and filter it with a 0.45 μm membrane filter then sonicate it for 15min. to obtain 150.3 μg/ml of EVT and injected the solution into the system. From the peak area found in the chromatograph, the % degradation was calculated.

12.Result and Discussion

A. Selection of analytical wavelength

The standard disposition of EVT is examined in the range of 200-400 nm for the methanol as blank. From the spectrum, the detecting wavelength selected for the estimation of the drug was 264 nm as shown in Figure 1.

I. Method Development trail and optimization of the methods

In the present investigation, a new analytical RP-HPLC method was developed for the determination of EVT tablet dosage form. The mobile phase was selected as it showed a sharp peak with symmetry and significant reproducible retention time. During the method development process, several trials were performed using different aqueous, organic, acidic phases, a good peak shape was observed when using the Hypersil BDS C18 column (250 mm x 4.6 mm, 5 μm) and Methanol: Water: TFA (70: 30: 0.1) as a mobile phase at a flow rate

of 1.0 ml/min. For quantitative analytical purposes, the wavelength was selected as 264 nm.

The total run time of the analysis was 15 min and the retention time of EVT was 4.03. The final optimized chromatogram is shown in figure 3. The detail of the method trial was presented in Table 1.

II. Analytical Method validation

The development of the method has been validated according to the ICH guidelines.

B. System suitability

The result is all within acceptable limits summarized in Table 3. The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

C. Specificity

Blank interference: No interference was observed from Blank at the retention time of EVT standard and sample peaks. It means that my method was specific to the HPLC system. (Figure 2). The result was given in Table 4.

D. Linearity and range

The standard calibration curve showed good linearity in the range of 75.25-225.75 µg/ml for Evogliptin tartrate API with a correlation coefficient of 0.999. The response of the drug was found to be linear in the investigation concentration range (Table 4). A typical calibration curve has the regression equation of $y= 4.066x-26.59$ for EVT.

E. Assay of marketed formulation

Experimental results of the amount of EVT in tablet expressed as a percentage of label claims were in good agreement with the label claims. The Result for analysis of marketed formulation was given in table 5.

F. Precision

The result was calculated in terms of % RSD for both interlay and intraday precision study which was found to be 1.91% and 1.05% respectively this confirms that the method was precise. The result was given in table 6-6.1.

G. Accuracy

The mean % recovery was found in the range of 100.58 ± 0.4 and that of the assay was 99.34. The result of % recovery was given in Table 7.1 and that assay was given in table 7 indicate the method is accurate.

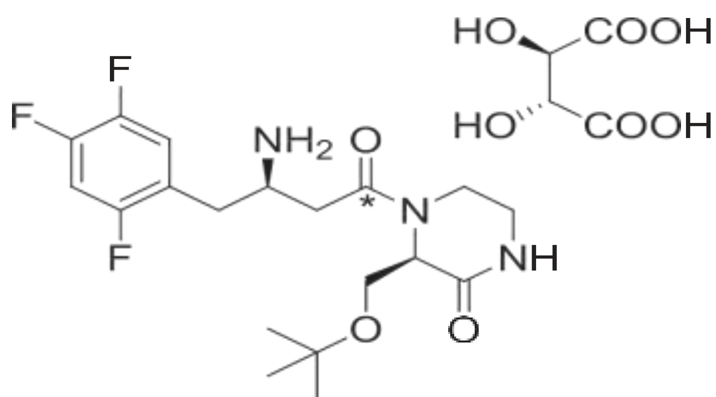
H. Robustness

The result of the assay of two test preparation was not affected by varying the condition. Fully agree with the result obtained under the original condition. The result was given in table 8-8.3. The % RSD for (Retention time, peak area, and % Assay) was not more than 2% for EVT which was in agreement with system suitability. The above results indicate that the test method is robust for all variable conditions outlined in the above tables.

I. Stability Studies

Stability indicating RP-HPLC method was performed in different stress conditions using the Methanol: Water: TFA (70:30:0.1 v/v) as the mobile phase suggested the following degradation behavior.

The chromatograms obtained on stress degradation were shown in Figures 7, 8, 9, 10, and 11. The result of all degradation studies is given in Table 9.



Structure of Evogliptin tartrate

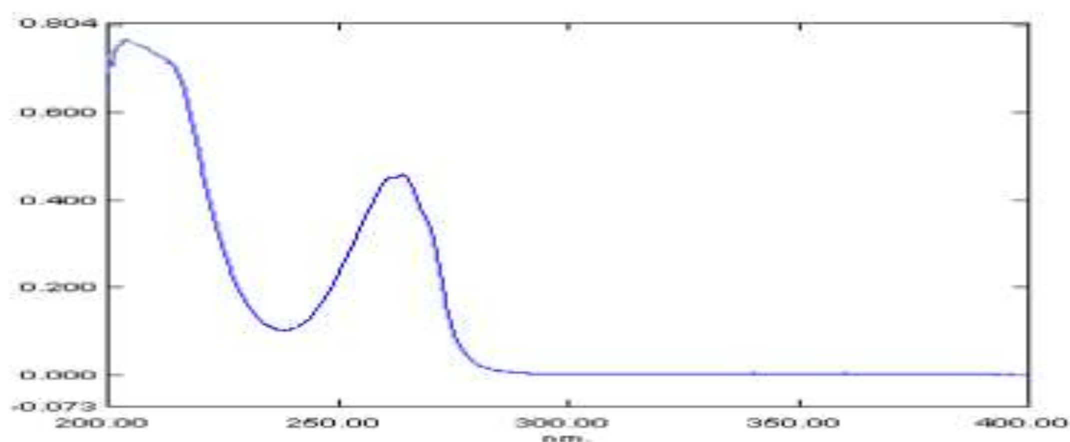


Figure 1: UV absorption spectrum of EVT

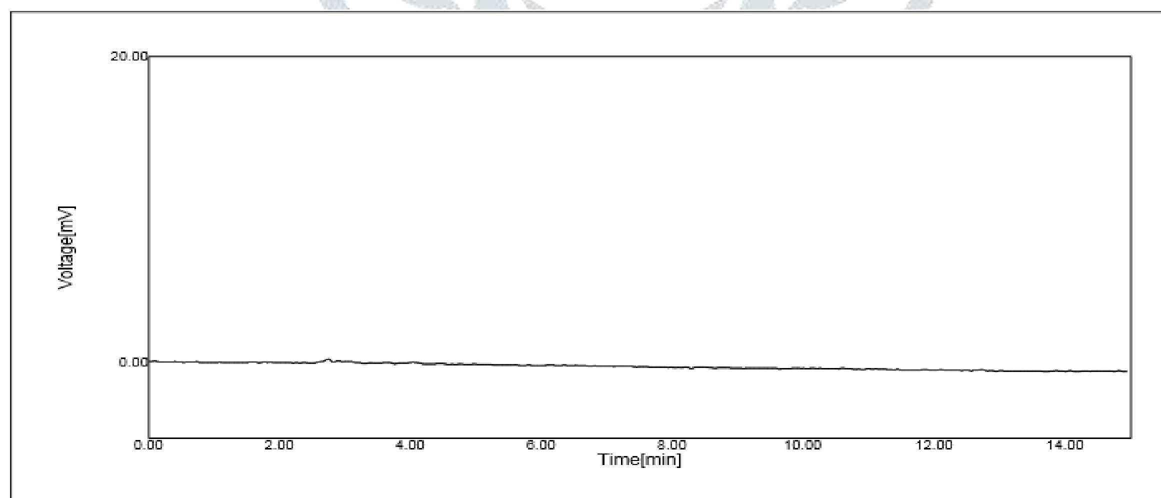


Figure 2: Blank Chromatogram: Diluent (Mobile phase)

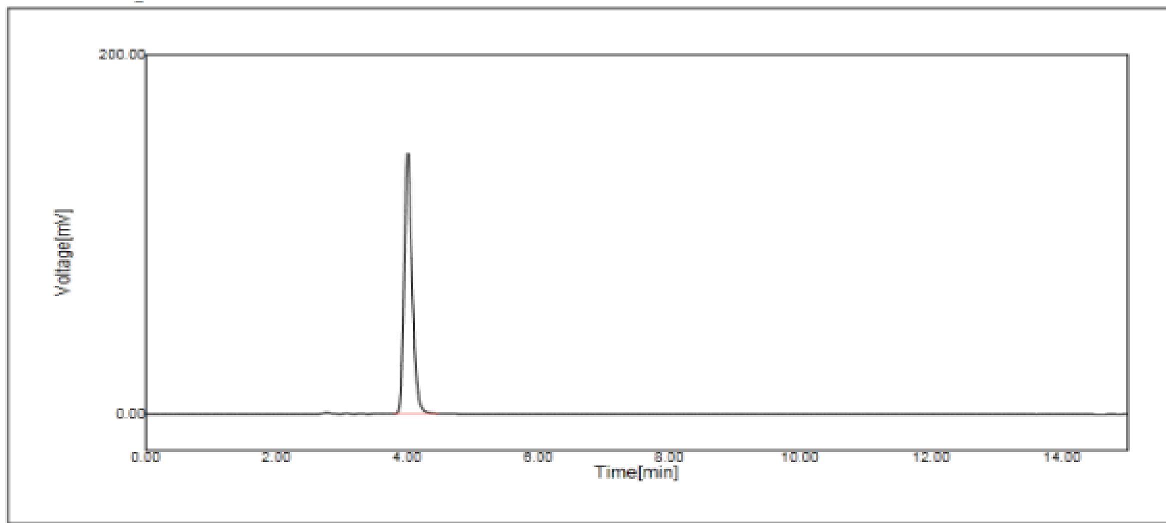


Figure 3: Final optimized chromatogram

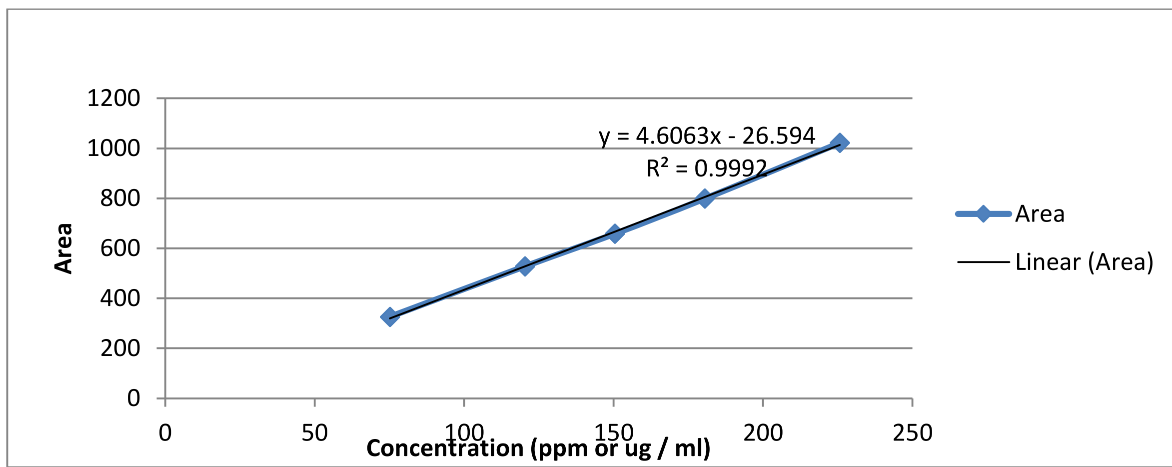


Figure 4: Calibration graph of EVT

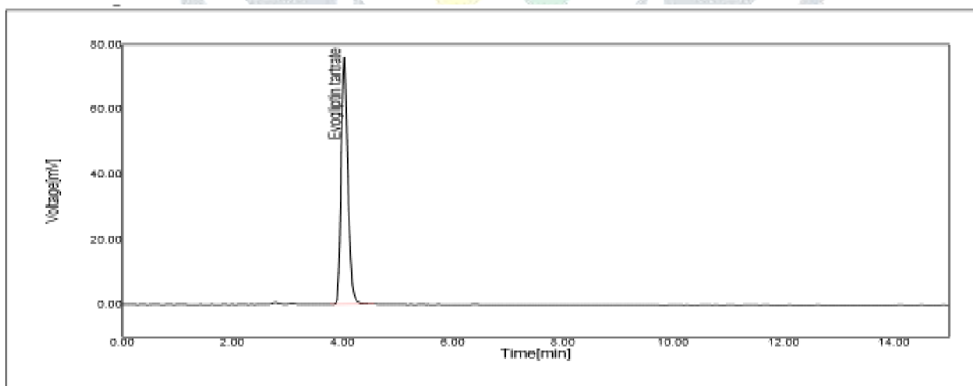


Figure 5: Chromatogram of standard Evogliptin tartrate for Assay

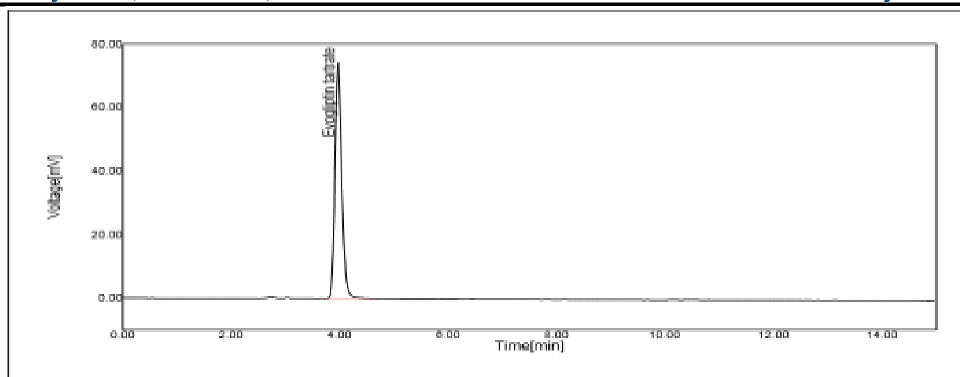
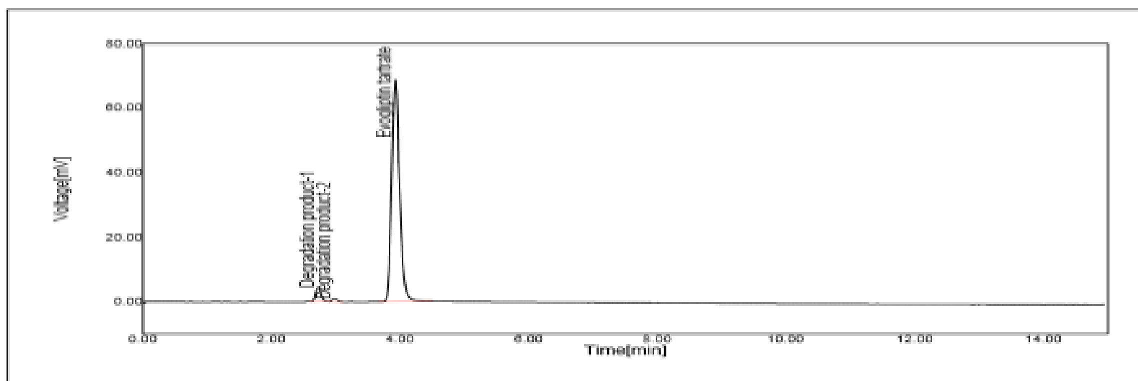
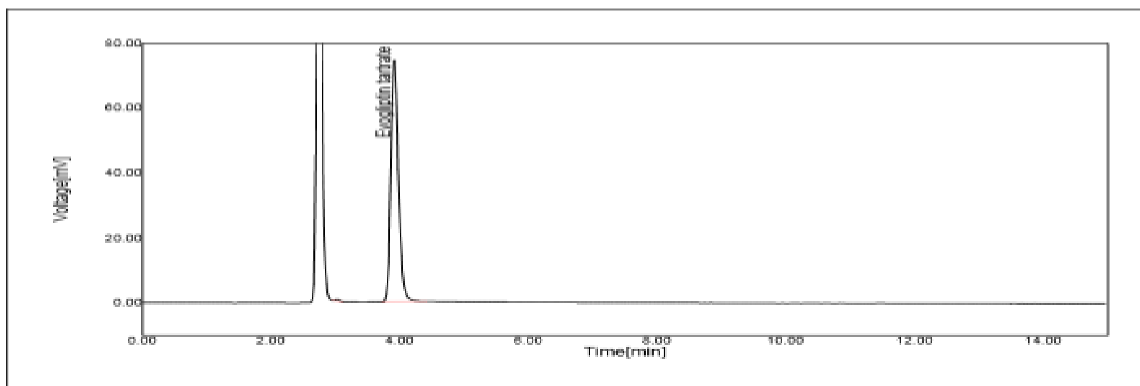


Figure 6: Chromatogram of test Evogliptin tartrate for Assay



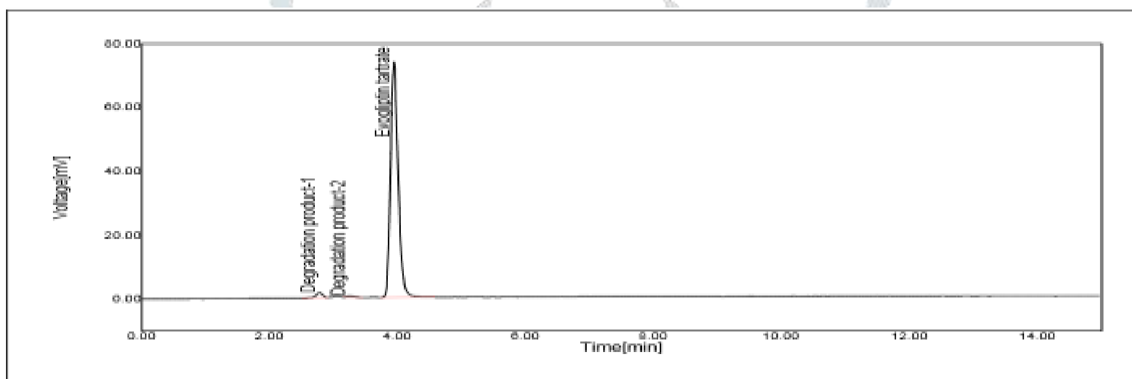
No.	Name	RT[min]	Area[mV*s]	Area%	Resolution
1	Degradation product-1	2.73	26.0220	4.23	0.00
2	Degradation product-2	2.98	4.8923	0.79	2.17
3	Evogliptin tartrate	3.93	584.6782	94.98	5.29
Sum			615.5925		

Figure 7: Chromatogram for Acidic Degradation Study



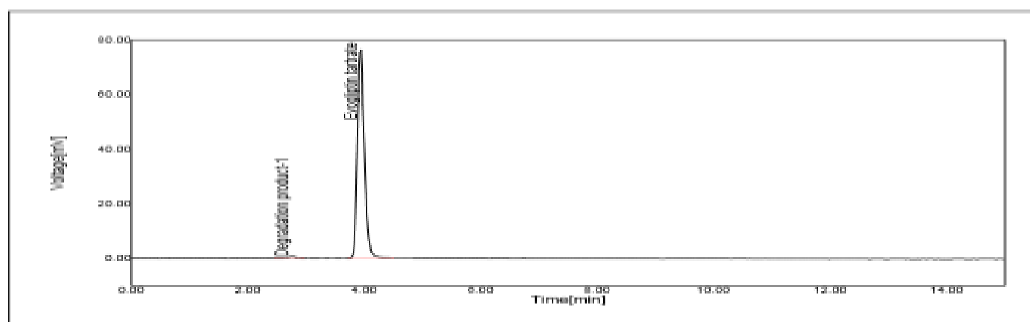
No.	Name	RT[min]	Area[mV*s]	Area%	Resolution
1		3.05	2.7461	0.44	0.00
2	Evogliptin tartrate	3.93	615.1326	99.56	6.14
Sum			617.8786		

Figure 8: Chromatogram for Basic Degradation Study



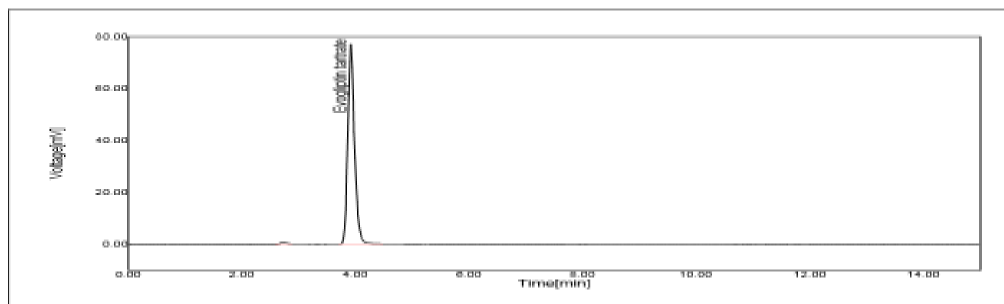
No.	Name	RT[min]	Area[mV*s]	Area%	Resolution
1	Degradation product-1	2.78	11.7272	1.85	0.00
2	Degradation product-2	3.25	3.3629	0.53	2.98
3	Evogliptin tartrate	3.95	620.0323	97.62	3.97
Sum			635.1224		

Figure 9: Chromatogram for Peroxide Degradation Study



No.	Name	RT[min]	Area[mV*s]	Area%	Resolution
1	Degradation product-1	2.77	5.4580	0.85	0.00
2	Evogliptin tartrate	3.95	634.1935	99.15	5.60
Sum			639.6515		

Figure 10: Chromatogram for Thermal Degradation Study



No.	Name	RT[min]	Area[mV*s]	Area%	Resolution
1		2.73	4.1038	0.63	0.00
2	Evogliptin tartrate	3.92	642.2974	99.37	6.43
Sum			646.4011		

Figure 11: Chromatogram for Photolytic Degradation

Table 1: Method development trials of Evogliptin tartrate

Sr. No.	Column	Mobile Phase	RT	TF	TP	Peak Characteristics
1	C18	Methanol: Water (80:20) 1ml/ min at 264nm	3.28	2.69	8241	TF was not observed within acceptance criteria
2	C18	Methanol and 0.01M $NH_4H_2PO_4$(80:20) 1ml/ min at 264nm	3.74	1.38	1058	TP was not observed within acceptance criteria
3	C18	Methanol and 0.1M KH_2PO_4 (80:20) 1ml/ min at 264nm	3.16	2.18	3512	TF was not observed within acceptance criteria
4	C18	Methanol : water: TFA (80:20:0.1) 1ml/ min at 264nm	2.83	1.02	8592	Retention is much reduced.
5	C 18	Methanol : water: TFA (70:30:0.1) 1ml/ min at 264nm	4.03	1.25	7589	All parameter is in range as per ICH

Table 2: Observation of system suitability parameters

Sr. No.	Peak Area	Retention time (min)	Tailing factor	Theoretical plate
Injection-1	664.2321	4.00	1.16	4252
Injection-2	660.2625	3.96	1.17	5021
Injection-3	656.6426	4.02	1.18	5921
Mean	**660.3791**	**3.99**	**--------**	**----------**
SD	**3.7961**	**0.030**	**-------**	**-------**
%RSD	**0.57**	**0.77**	**-------**	**-------**
Limit	**NMT2**	**------**	**NMT 2**	**NLT 2000**

Table 3: Result of specificity

Sr. No	Area	RT	TF	TP
Blank Injection	-	-	-	-
Standard Injection	663.6243	4.00	1.15	4172
Test Injection	652.2014	4.02	1.12	4172

Table 4: Result for linearity

Con.(µg/ml)	Area	RT (min)	Statistical analysis	
75.25	326.2234	4.03	Regression Equation	4.066x-26.59
120.4	527.7645	4.01		
150.50	658.3597	4.02	Slop	4.066
180.6	799.0125	4.02	Intercept	26.59
225.75	1021.8860	4.01	Correlation Coefficient	0.999

Table 5: Analysis in Tablet formulation

Weight of Standard	Standard Peak area	Weight of test	Test Peak area	Amount observed in mg	Amount claim in mg	% Assay
149.8	642.0656	329.9	649.7189	6.8934	6.8690	100.35
	637.1528	327.1	640.7254	6.8561	6.8690	99.81
	646.6958	**Mean**				**100.08**
Mean	**641.6958**					
SD	**4.7722**					
%RSD	**0.74**					

Table 6: Observation and Result of Inter day study

Weight of Standard	Standard Peak area	Weight of Test	Sample peak area	EVT observed in mg	% Assay	Initial assay
149.8	661.7375	323.9	643.9967	6.7466	98.22	100.35
	665.5179	325.8	656.9840	6.8425	99.61	99.81
	659.3562	**Overall mean**			**99.50**	
Mean	**662.2039**	**Overall SD**			**0.91**	
SD	**3.1072**	**Overall %RSD**			**0.91**	
% RSD	**0.47**					

Table 6.1: Observation and Result of Intraday study

Weight of Standard	Standard Peak area	Weight of Test	Sample peak area	EVT observed in mg	% Assay	Initial assay
152.1	668.6260	326.4	631.7961	6.7348	98.05	100.35
	643.4725	327.5	632.7961	6.8608	99.88	99.81
	654.8210	**Overall mean**			**99.52**	
Mean	**655.6398**	**Overall SD**			**1.0105**	
SD	**12.5967**	**Overall %RSD**			**1.05**	
% RSD	**1.92**					

Table 7: Result for accuracy (Assay)

Weight of Test	Peak area of Standard	Peak area of Test	Amount observed in mg	% Assay
326.1	663.6243	652.2014	6.8037	99.05
325.9	661.2558	655.6536	6.8439	99.63
	660.6711	**Mean**	**6.8238**	**99.34**
Mean	**661.8504**			

Table 7.1: Observation and Result for recovery study

Level of % recovery	Weight of test	Test peak area	Amount of standard added in mg w.r.t test	Amount found in mg w.r.t. test	Amount recovered in mg	% Recovery	Mean	SD	% RSD

80%	327.5	1193.6033	5.4998	12.3983	5.5745	101.36	101.09	0.2311	0.23
	327.6	1192.2582	5.4931	12.3692	5.5454	100.95			
	327.8	1192.7425	5.4898	12.3667	5.5429	100.97			
100%	327.4	1313.0557	6.8769	13.6433	6.8195	98.17	99.11	0.8297	0.84
	327.6	1315.2545	6.8727	13.6577	6.8339	99.44			
	327.5	1316.9825	6.8748	13.6798	6.8561	99.73			
120%	327.7	1462.8906	8.2447	15.1862	8.3624	101.43	101.56	0.1350	0.13
	327.8	1465.2514 0	8.2422	15.2060	8.3822	101.70			
	327.4	1465.5475 0	8.2372	15.1894	8.3647	101.56			
Mean							**100.58**	**0.3999**	**0.4**

Table 8: Observation and Results of robustness study

Change Flow (0.95ml/min)						**Original flow**
Weight of Standard	Standard Peak area	Weight of Test	Test peak area	EVT observed in mg	% Assay	% Assay
150.4	656.7729	324.6	651.3958	6.8420	99.61	100.35
	665.6483	325.2	645.8571	6.7717	98.58	99.81
	662.5245	**Cumulative mean**			**99.59**	
Mean	**661.6486**	**Cumulative SD**			**0.741**	
S.D	**4.5021**	**Cumulative %RSD**			**0.74**	
%RSD	**0.68**					

Table 8.1: Observation and Result of robustness study

Change flow (1.05ml/min)						**Original flow**
Weight of Standard	Standard peak area	Weight of Test	Test peak area	EVT observed in mg	% Assay	% Assay
	611.1598	327.9	618.1824	6.8318	99.46	100.35

149.1	621.1325	326.1	610.228	6.7802	98.71	99.81
	619.2341	**Cumulative mean**			**99.59**	
Mean	**661.6486**	**Cumulative SD**			**0.6879**	
S.D	**5.29549**	**Cumulative %RSD**			**0.69**	
%RSD	**0.86**					

Table 8.2: Observation and Result of robustness study

Change wavelength (262nm)						**Original flow**
Weight of Standard	Standard Peak area	Weight of Test	Test peak area	EVT observed in mg	% Assay	% Assay
150.6	599.5911	326.2	582.4085	6.7369	98.08	100.35
	601.1128	325.9	584.6279	6.7688	98.54	99.81
	595.3576	**Cumulative mean**			**99.20**	
Mean	**598.68727**	**Cumulative SD**			**1.0622**	
SD	**2.98223**	**Cumulative %RSD**			**1.07**	
%RSD	**0.50**					

Table 8.3: Observation and Result of robustness study

Change wavelength (266nm)						**Original flow**
Weight of Standard	Standard Peak area	Weight of Test	Test peak area	EVT observed in mg	% Assay	% Assay
150.6	653.993	326.6	637.9136	6.8076	99.11	100.35
	650.5078	325.4	641.8624	6.8750	100.09	99.81
	639.9245	**Cumulative mean**			**99.69**	
Mean	**648.1418**	**Cumulative SD**			**0.5343**	
SD	**7.3266**	**Cumulative %RSD**			**0.54**	
%RSD	**1.13**					

Table 9: Observation and Result of forced degradation study

Stress condition	**Time**	**% area EVT observed after degradation**	**% of degradation**	**Observation**

Acidic	30min	94.98	5.02	Significant Degradation observed
Basic	30min	97.62	2.38	Significant Degradation observed
Peroxide	12h	99.56	0.44	No Significant degradation
Thermal	1h	99.15	0.85	No Significant degradation
Photolytic	1h	99.37	0.63	No Significant degradation

Conclusion.

A novel, simple, precise, accurate, RP-HPLC method has been developed for the estimation of Evogliptin tartrate in the pure and marketed formulation. The developed method is specific, Linear accurate, precise, and robust. Stress studies are performed as acid degradation, base degradation, oxidation degradation, thermal degradation, and photo degradation. Degradation study indicates the stability of the drug. All the peak of the degradation products formed during stress degradation studies were well separated from the analyte peak. Hence, this method regarded as more specific, stability-indicating, and can be successfully used for routine analysis for the determination of Evogliptin tartrate in the tablet dosage form.

Acknowledgment.

The authors are thankful to the principle and management of Vidya Bharti College of pharmacy, Sant Gadge Baba Amravati University. India for providing equipment and necessary facilities to carry out research work and thankful to Peaks analytical research & training center for providing facilities to complete research work.

Abbreviation.

HPLC: High performance liquid chromatography, **RP-HPLC:** Reverse-phase high-performance liquid chromatography, **UV:** Ultraviolet, **ICH:** International Conference on Harmonization, **RT:** Retention time, **RSD:** Relative standard deviation, **NLT:** Not less than 2, **NMT:** Not more than 2, **EVT:** Evogliptin tartrate, **HCl:** Hydrochloric acid, **NaOH:** Sodium hydroxide, **H_2O_2:** Hydrogen peroxide, **T.F.A.:** Trifluoroacetic acid. **R^2:** Correlation Coefficient, **w.r.t.:** With respect to, **$NH_4H_2PO_4$:** Ammonium Dihydrogen Orthophosphate, **KH_2PO_4:** Potassium Dihydrogen Phosphate, **AR:** Analytical reagent.

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