Research Article

Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Evogliptin Tartrate in Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid and precise reversed – phase High performance liquid chromatographic (RP-HPLC) stability indicating method was developed and validated for the determination of Evogliptin tartrate in the bulk and tablets. The method involves the use of commonly available and inexpensive laboratory reagents. Chromatographic separation of evogliptin tartrate was achieved using a reverse phase Hypersil BDS C18 column (250mm × 4.6mm, 5µm) and Buffer: Methanol (45:55 v/v) as mobile phase at 1.0 ml/min flow rate. Detection was carried out at 265 nm. Rt was found to be 5.310 min for Evogliptin tartrate in stability indicating method different stress conditions were applied.

Keywords: Evogliptin tartrate, High Performance liquid Chromatography, Method Validation, Stability Indicating, Forced degradation study, ICH guideline.

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INTRODUCTION

Evogliptin tartrate is a novel, potent, and particular dipeptidyl peptidase IV inhibitor reduces blood sugar level brand name of drug –Valera ,which is used in the treatment of type 2 diabetes mellitus.

Evogliptin is administered as a monotherapeutic, oral antihyperglycemic drug, or administered in combination with other antidiabetic agents to treat type II diabetes mellitus, a chronic metabolic disease associated with insulin deficiency and insulin resistance.

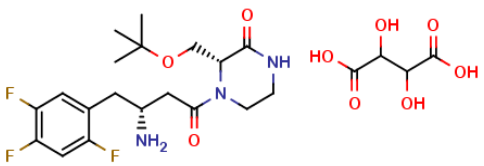

Figure 1

The International Conference on harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of active substances.

The stability indicating method (SIM) is an analytical method used to quantitate the decrease in the active pharmaceutical ingredients (API) in drug product due to degradation.

An ideal stability indicating method one that quantifies the standard drug alone and also resolves its degradation products and its process impurities. Consequently, the implementation of an analytical methodology to determine Evogliptin tartrate in bulk samples, the proposed method is simple, accurate, Linear specific, repeatable, stability indicating, reduces the duration of analysis and suitable for routine determination of Evogliptin tartrate in Pharmaceutical samples. The current method was validated in compliance with ICH guidelines and its updated international convention .

MATERIALS AND METHODS[6-8]

Instrumentation and Chromatographic Conditions

Shimadzu Prominence equipped with UV-Visible Detector Shimadzu SPD-10AVP used for the analysis. The column used was Hypersil BDS c18 column (250mm X 4.6mm, 5µm). Different mobile phases were tested in order to find the best conditions for the separation of Evogliptin tartrate and its degradation products. The optimum composition of mobile phase was determined to be Buffer(pH-4.5):Methanol(45:55%v/v). The flow rate was set to 1 mL min-1,UV detection was carried out at 265 nm at injection volume 20µL maintained. Retention time was 5.310 min.

Preparation of Standard Solution

1. Preparation of Evogliptin Tartrate Stock solution: Accurately weighed Evogliptin Tartrate 20 mg was transferred into 100 ml volumetric flask. Dissolved and dilute up to the mark with methanol to obtain final concentration 0f 200 µg/ml.

2. Working standard solution of Evogliptin Tartrate: 20µg/ml of Evogliptin Tartrate Working standard solution

was prepared by diluting 1ml of stock solution to 10 ml with methanol.

Selection Of Wavelength

The standard solution of Evogliptin Tartrate was diluted with methanol to get the concentration 20µg/ml and was scanned in the UV range 200-400 nm. The absorption maxima of drug were found to be 265 nm.

Method Development

The RP-HPLC method developed in this study was aimed at finding the chromatographic system capable of eluting and resolving Evogliptin tartrate. To develop the conditions various parameters such as mobile phase, pH, flow rate and solvent ratio were changed and suitable chromatographic condition has been developed for routine analysis of drug samples. Initial trails were carried out by using same column taking Methanol, Acetonitrile and Water in various proportion.

The chromatograms obtained after ions with flow rate of 1.0ml/min. Further trails were carried out varying the flow rate, changing the chromatographic column, pH conditions and mobile phase composition. The best resolution was reported during a trial when Mobile phase was taken as Buffer (pH-4.5): Methanol in the ratio (45:55) %v/v, flow rate of 1.0ml/min, and sharp peak was depicted at retention time of 5.310 min, peak was narrow, sharp and with high resolution compared to other peaks obtained in different trails. Thus, these chromatographic conditions was used for studying the different properties of drug such as degradedness and also used to validate various method parameters like linearity, precision, recovery, robustness, LOD and LOQ. Chromatographic condition was established such that it could be suitable for separation of drug and its degradation products separating impurities during elution from the chromatographic column. The proposed method is simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in the analysis of drugs.

Forced Degradation study

Preparation of Solutions

- *Standard Stock Solution I of Evogliptin tartrate (200µg/ml):*

20mg of Evogliptin tartrate was accurately weighed and transferred to 100ml volumetric flask and dissolved in Methanol and sonicated for about 10min. Volume was made up to the mark with Methanol to give a solution containing 200µg/ml Evogliptin tartrate solution.

- *Preparation of Sample Stock Solution:*

20 Tablets were weighed and powdered. Powder equivalent to 20mg Evogliptin tartrate was taken and transferred to 100ml volumetric and dissolved in 60mL diluent and sonicated for about 10min. Volume was made up to the mark with methanol to give a solution containing 200 µg/ml Evogliptin tartrate solution.

- *Preparation of 0.1M sodium hydroxide solution (0.1N NaOH):-*

  Sodium hydroxide (0.4gm) was transferred to a 100mL volumetric flask, dissolved in and diluted up to mark with water.

- *Preparation of 0.1M hydrochloric acid solution (0.1N HCl) :-*

  Hydrochloric acid (0.85ml) was transferred to a 100mL volumetric flask and diluted up to mark with water.

- *Preparation of Standard Solution for Stability:*

  1mL of Evogliptin tartrate Standard Stock Solution was transferred in 10ml volumetric flask. Volume was made up to the mark with Mobile Phase used for trials to give a solution containing 20µg/mL Evogliptin tartrate solution.

Acid Hydrolysis

- *Acid Degradation Blank*

2mL of 1M HCl was transferred to 10mL volumetric flask and then 2mL of 1M NaOH was added for neutralization and diluted up to the mark with Mobile Phase.

- *Acid Degradation Standard*

1 ml Evogliptin stock solution and 2mL of 1M HCl was transferred to 10mL volumetric flask kept for 4 hours and then 2mL of 1M NaOH was added for neutralization to stop the degradation further and diluted up to the mark with Mobile Phase.

- *Evogliptin tartrate and Formulation Acid Degradation*

1mL of Sample Stock Solution transferred in 10mL volumetric flask; to it 2mL of 0.1NHCl was added and kept for 4hrs and then 2mL of 1M NaOH was added for neutralization to stop the degradation further and diluted up to the mark with Mobile Phase.

Alkaline Hydrolysis

- *Alkaline Degradation Blank*

2mL of 0.1N NaOH was transferred to 10mL volumetric flask and then 2mL of 0.1N HCL was added for neutralization and diluted up to the mark with Mobile Phase.

- *Alkaline Degradation Standard*

1 ml Evogliptin stock solution and 2mL of 0.1N NaOH was transferred to 10mL volumetric flask kept for 3 hours and then 2mL of 0.1N HCL was added for neutralization to stop the degradation further and diluted up to the mark with Mobile Phase.

- *Evogliptin tartrate Formulation Alkaline Degradation*

1mL of Sample Stock Solution transferred in 10mL volumetric flask; to it 2mL of 0.1N NaOH was added and

kept for 3 hrs and then 2mL of 0.1N HCL was added for neutralization to stop the degradation further and diluted up to the mark with Mobile Phase.

3. Oxidation Degradation:

- *Oxidation Degradation Blank:*

2mL of 3% H_2O_2 was transferred to 10mL volumetric flask and diluted up to the mark with Mobile Phase.

- *Oxidation Degradation Standard:-*

1 mL Evogliptin Standard stock solution and 2mL of 3% H_2O_2 was transferred to 10mL volumetric flask kept for 10 hours and diluted up to the mark with Mobile Phase.

- *Evogliptin tartrate Formulation Oxidation Degradation:-*

1mL of Sample Stock Solution transferred in 10mL volumetric flask; to it 2mL of 3% H_2O_2 was added and kept for 10 hrs and diluted up to the mark with Mobile Phase.

Thermal Degradation

- *Thermal Degradation Blank:-*

Mobile Phase is taken as a blank solution.

- *Thermal Degradation Standard:-*

Kept standard powder at 105 degree in Oven for 16 hours. 1mL of Evogliptin tartrate Standard Stock Solution was transferred in 10ml volumetric flask. Volume was made up to the mark with Mobile Phase to give a solution containing 20µg/ml Evogliptin tartrate solution.

- *Evogliptin tartrate Formulation Thermal Degradation:-*

Kept sample powder at 105 degree in oven for 16 hours. 1mL of Evogliptin tartrate Sample Stock Solution was transferred in 10ml volumetric flask. Volume was made up to the mark with Mobile Phase to give a solution containing 20µg/ml Evogliptin tartrate solution.

Photo Degradation:

- *Photo Degradation Blank:-*

Mobile Phase is taken as a blank solution.

- *Photo Degradation Standard:-*

Kept standard powder at UV chamber for 1 hour. 1mL of Evogliptin tartrate Standard Stock Solution was transferred in 10ml volumetric flask. Volume was made up to the mark with Mobile Phase to give a solution containing 20µg/ml Evogliptin tartrate solution.

- *Evogliptin tartrate Formulation Photo Degradation*

Kept sample powder at UV chamber for 1 hour. 1mL of Evogliptin tartrate Sample Stock Solution was transferred in 10ml volumetric flask. Volume was made up to the mark with Mobile Phase to give a solution containing 20µg/ml Evogliptin tartrate solution.

Method Validation

Linearity

The calibration curve showed (Fig.1) good linearity in the range of 10-30µg/ml, for Evogliptin tartrate with correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of y=237.4x-48.67. Results are given in table 1.

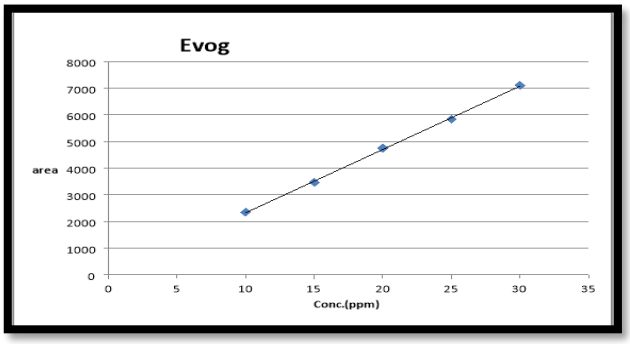

Figure 2: Linearity result of Evogliptin tartrate

Table 1: Table showing result of Linearity

Conc. (µg/ml)	Mean area ± S.D.	%RSD
10	2348.236±1.592	0.0678
15	3466.339±3.320	0.0958
20	4749.125±3.798	0.0799
25	5835.407±4.277	0.0732
30	7105.399±5.306	0.0746

Precision

Procedure

Result should be expressed as percentage relative standard deviation (%RSD) or co-efficient of variance.

Repeatability

Solutions of 20µg/ml (n=6) Evogliptin tartrate was prepared and peak area was measured with each solution and % RSD was calculated.(Table 2)

Table 2: Table showing result of Repeatability

	Evogliptin Conc. (20 µg/ml)
Sr no.	Area
1	4654.593
2	4730.161
3	4739.815
4	4749.193
5	4734.838
6	4655.58
Average	4710.68
SD	43.56
%RSD	0.92

Intraday Precision

Solutions of 10, 20, 30 µg/ml Evogliptin tartrate was prepared and peak area was measured containing analyzed three times on the same day and % RSD was calculated. (Table 3)

Table 3: Table showing result of Intraday Precision

EVOGLIPTIN TARTRATE		
CONC.(µg/ml)	**Area mean±S.D(n=3)**	**%RSD**
10	2329.757±30.78	1.33
20	4669.68±60.20	1.29
30	7000.41±43.24	0.62

Interday Precision

Solutions of 10, 20, 30 µg/ml Evogliptin tartrate was prepared and peak area was measured containing analyzed three times on different days and % RSD was calculated.(Table 4)

Table 4: Table showing result of Interday Precision

EVOGLIPTIN TARTRATE		
CONC.(µg/ml)	**Area mean±S.D.(n=3)**	**%RSD**
10	2325.24±20.13	0.87
20	4685±53.74	1.15
30	7009.67±67.56	0.96

Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80 %, 100 % and 120 %) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and results are calculated. (Table 5)

Table 5: Table Showing result of Accuracy

%Recovery	Target Conc. (µg /ml)	Spiked Conc. (µg/ml)	Final Conc. (µg/ml)	Conc. Obtained (µg/ml)	%Recovery	Avg	SD	%RSD
80 %	10	8	18	7.91	98.88	100.10	1.13	1.13
	10	8	18	8.09	101.10			
	10	8	18	8.03	100.32			
100 %	10	10	20	9.91	99.14	99.77	0.65	0.65
	10	10	20	10.04	100.44			
	10	10	20	9.97	99.74			
120%	10	12	22	12.03	100.23	99.75	0.49	0.49
	10	12	22	11.91	99.25			
	10	12	22	11.97	99.77			

Limit of Detection and Limit of Quantification

The LOD of was found to be 0.733 µg/ml and the LOQ 2.221µg/ml estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration.

Robustness

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio ((±2 %), change in pH (±0.2 units) and flow rate (± 0.2 ml/unit) were studied to determine the robustness of the method. The results were in favor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Evogliptin tartrate. The results are given in table 6.

Table 6: Table showing result of robustness

Sr. No	pH (+0.2units)	pH (-0.2 units)	Flowrate (+0.2 units)	Flow rate (-0.2units)	Mobile phase (+2%)	Mobile phase (-2%)
1	4501.222	4766.421	4553.459	4870.828	4592.875	4789.674
2	4535.492	4867.928	4630.479	4915.412	4540.084	4853.299
3	4563.791	4886.794	4649.547	4943.96	4654.215	4881.887
Avg	4533.50	4840.38	4611.16	4910.07	4595.72	4841.62
SD	31.33	64.74	50.87	36.86	57.12	47.20
%RSD	0.69	1.34	1.10	0.75	1.24	0.97

Assay of Evogliptin Tartrate Tablets

- Twenty tablets of Evogliptin tartrate (Valera) were weighed and powdered to 20 mg of Evogliptin tartrate and transferred to 100 ml volumetric flask, volume adjusted with methanol (200 µg/ml) peak area of the prepared solution was recorded and concentration of each drug was calculated using calibration curve equation.The results are given in table 7.

Table 7: Table showing result of assay of marketed formulation

Drug	Actual label claim of Drug(mg)	Conc. Of Drug found(mg)	Percentage of Drug found	Avg. of % Drug found	SD	%RSD
Evogliptin tartrate	5	4.79	95.78	96.00	0.24	0.25
	5	4.80	95.97			
	5	4.81	96.26			

RESULTS

Summary of forced Degradation study

Table 8: Table showing result of forced degradation study

Degradation type	Condition	Volume of stock solution (ml)	Time (hrs)	Final Dilution up to (ml)	%Degradation of standard	%Degradation of sample
Acidic	2ml 1 M HCL	1	4	10	19.48	18.92
Basic	2 ml 0.1 N NaOH	1	3	10	18.15	15.94
Oxidative	2 ml 3% H_2O_2	1	10	10	22.11	21.79
Thermal	105 ^{0}c in oven for 16 hours	1	16	10	14.27	14.98
Photo Degradation	UV chamber for 1 hour	1	1	10	11.33	13.40

CONCLUSION

- A simple, accurate and precise RP-HPLC method of Evogliptin tartrate in Pharmaceutical dosage form has been developed and validated.
- Separation of drug was carried out using mobile phase Buffer(pH-4.5):Methanol (45:55%v/v) at 5.310 min run time and 265 nm.
- Forced degradation study was carried out in various stress conditions like Acid, Base, Oxidation and Thermal .
- The maximum degradation of Evogliptin tartrate was observed in Oxidative degradation i.e. 22.11 % for standard and 21.79% for tablet .
- The peak of degraded component was in resolved from the peak of main component and do not interfere with the API peak.
- The forced degradation study gave future scope that the degraded product can be separated in sufficient quantity and characterized. Then it can be studied for its safety profile
- It is concluded that the developed method is specific. The test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the stability determination of Evogliptin tartrate in pharmaceutical formulation.

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