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DEVELOPMENT AND VALIDATION OF EVOGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM AND ITS STRESS DEGRADATION STUDIES

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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 in the bulk and tablets. The method involves the use of commonly available and inexpensive laboratory reagents. Chromatographic separation of evogliptin was achieved using a reverse phase Zodiac C18 column (150mm × 4.6mm, 5µm) and Solvent A, 20mM KH_2PO_4 water-methanol (70:30%, v/v); Solvent B, acetonitrile-methanol (90:10, v/v) as mobile phase at 1.0 ml/min flow rate. Detection was carried out at 260 nm. Rt was found to be 3.463 min for Evogliptin tartrate in stability indicating method different stress conditions were applied.

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

INTRODUCTION

Evogliptin 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 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

Instrumentation and Chromatographic Conditions

Shimadzu Prominence equipped with UV-Visible Detector Shimadzu SCL-10AVP used for the analysis. The column used was Zodiac-100 c18 column (150 × 4.6 mm ID, 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 Solvent A, 20mM KH_2PO_4 water-methanol (70:30%, v/v); Solvent B, acetonitrile-methanol (90:10, v/v). The flow rate was set to 1 mL min-1,UV detection was carried out at 260 nm at injection volume 20µL maintained. Retention time was 3.463 min.

Preparation of Standard Solution

1. Preparation of Evogliptin Stock solution: Accurately weighed Evogliptin 10 mg was transferred into 10 ml volumetric flask. Dissolved and dilute up to the mark with methanol to obtain final concentration 0f 1000 µg/ml.

2. Working standard solution of Evogliptin : 10µg/ml of Evogliptin Working standard solution was prepared by diluting 1ml of stock solution to 10 ml with methanol.

Selection Of Wavelength The standard solution of Evogliptin was diluted with methanol to get the concentration 10µg/ml and was scanned in the UV range 200-400 nm. The absorption maxima of drug were found to be 260 nm.

Method Development

The RP-HPLC method developed in this study was aimed at finding the chromatographic system capable of eluting and resolving Evogliptin. 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 Solvent A, 20mM KH_2PO_4 water-methanol (70:30%, v/v); Solvent B, acetonitrile-methanol (90:10, v/v) flow rate of 1.0ml/min, and sharp peak was depicted at retention time of 3.463 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 (1000µg/ml):** 10mg of Evogliptin was accurately weighed and transferred to 10ml 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 1000µg/ml Evogliptin solution.

• **Preparation of Sample Stock Solution:** 10 Tablets were weighed and powdered. Powder equivalent to 10mg Evogliptin was taken and transferred to 100ml volumetric and dissolved in 40mL diluent and sonicated for about 10min. Volume was made up to the mark with methanol to give a solution containing 1000 µg/ml Evogliptin 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 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 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 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 andkept 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% H2O2 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% H2O2 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% H2O2 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 solution.

• Evogliptin 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 with correlation coefficient (r2) of 0.999. A typical calibration curve has the regression equation of y=237.4x-48.67. Results are given in table 1.

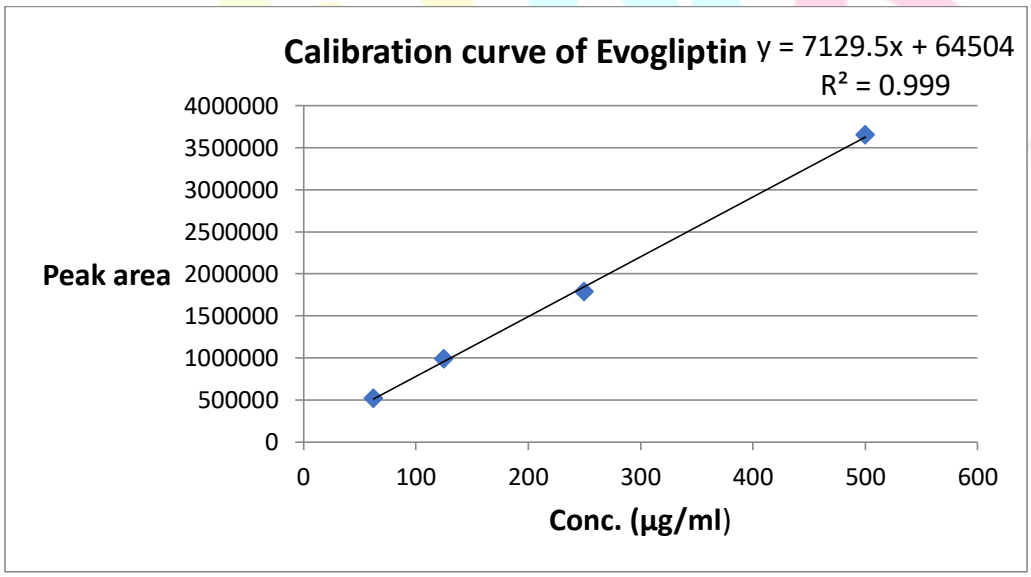

Figure 2: Linearity result of Evogliptin

Table 1: Table showing result of Linearity

Sr no.	Concentration (µg/ml)	Area
1	500	3651239
2	250	1784270
3	125	989825
4	62.5	516631

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

Sr no.	Drug Name: Evogliptin
	Peak Area (Conc- 500 ppm)
1	4178076
2	4239084
3	4143869
4	4090808
5	4144048
6	4241243
Mean	**4172854.667**
STD. DEV.	**59147.611**
RSD (%)	**1.41**

Intraday Precision

Solutions of 10, 20, 30 µg/ml Evogliptin 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

Drug Name: Evogliptin				
S. No.	**Concentration (ppm)**	**Area**	**SD**	**%RSD**
1	500 ppm	4227471	76922.01956	1.78
	500 ppm	4362341		
	500 ppm	4359004		
2	500 ppm	4174199	74476.09867	1.75
	500 ppm	4306662		
	500 ppm	4299424		
3	500 ppm	4390750	51781.6388	1.17
	500 ppm	4494056		
	500 ppm	4436085		

Interday Precision

Solutions of 10, 20, 30 µg/ml Evogliptin 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

Drug Name: Evogliptin				
Sr. no.	**Concentration (ppm)**	**Area**	**SD**	**%RSD**
DAY 1	500 ppm	4161909	65390.94	1.55 %
	500 ppm	4279242		
	500 ppm	4170549		
DAY 2	500 ppm	4080979	37745.30	0.92 %
	500 ppm	4035614		
	500 ppm	4110552		
DAY 3	500 ppm	4118728	63871.85	1.52 %
	500 ppm	4240675		
	500 ppm	4212649		

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)

Conc. (%)	**Ref. Std (mg)**	**Marketed drug (mg)**	**Recovery (mg)**	**% Recovery**	**Peak area**	**Mean ± SD**	**% RSD**
80%	50	40	88.91	98.78	641273	98.51	0.38
	50	40	88.43	98.25	642092		
100%	50	50	101.32	101.32	812781	101.59	0.38
	50	50	101.87	101.87	819735		
120%	50	60	110.96	100.72	959836	101.03	0.44
	50	60	111.49	101.35	960385		

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.

RESULTS

Summary of forced Degradation study

Conditions: saxagliptin	No. of degradants	% degradation
Acid (0.1N NaOHl) + 45˚C + 12 Hrs.	1 degradant	0.19%
Base (0.1N/M HCl) + 60˚C + 12 Hrs.	3 degradants	4.40%
Thermal (45˚C) + 12 Hrs.	0 degradants	0%
Oxidation (6% H_2O_2) + Room Temp.	0 degradation	0%

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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