

# DEVELOPMENT AND VALIDATION FOR EVOGLIPTIN IN BULK AND TABLET DOSAGE FORM USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-PDA METHOD

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## Abstract

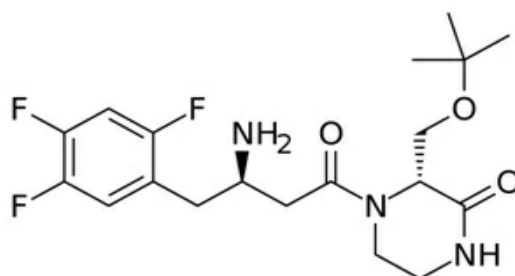
To develop a novel ultra-performance liquid chromatographic technique for the estimation of evogliptin in a API and tablet. The chromatographic separation was achieved using DIKMA Endoversil (2.1 x 50mm, 1.7µm) column, mobile phase was phosphate buffer, pH 4.2 and acetonitrile as a mobile phase (30:70) with a flow rate of 0.4 mL/min and eluent was monitored at 237 nm. The method was validated in accordance with International conference on harmonization guidelines. In this method evogliptin was eluted with retention time of 0.639 minutes. Calibration curve plots were found linear over the concentration ranges 2-50 µg/mL for evogliptin. Limit of detection was 0.2µg/ml and limit of quantification was found 0.5 µg/mL. The % assay of the marketed dosage form was found 97.8 %, even the present approach was found to be effective in the analysis of evogliptin in force degradation condition the experiential evidences of all the study results revealed the suitability of the estimation of evogliptin in API and tablet formulation.

**Keywords:** Evogliptin, UPLC, Method Development, Method Validation, ICH Guidelines.

## INTRODUCTION

Evogliptin is a new oral DPP-4 inhibitor developed for the treatment of patients with type 2 diabetes inadequately controlled by diet and exercise. DPP-4 inhibitors control glucose levels by preventing the breakdown of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which stimulate insulin secretion in response to the increased levels of glucose in the period following meals<sup>1</sup>. DPP-4 inhibitors enhance insulin secretion in a glucose-dependent manner, which potentially reduces hypoglycemia risks during monotherapy or combination therapy with other antidiabetic agents<sup>2</sup>. Evogliptin which is chemically 3-amino-4-(2,4,5-trifluorophenyl) butanoyl]-3-[(2-methylpropan-2-yl)oxymethyl] piperazin-2-one, with a molecular formula of C<sub>19</sub>H<sub>26</sub>F<sub>3</sub>N<sub>3</sub>O<sub>3</sub>, and molecular weight of 401.4 g/mole<sup>3</sup>. The chemical structure was shown in **Fig. 1**. Evogliptin is found effective, safe and widespread use for the treatment of type 2 diabetes<sup>4</sup>. Extensive literature review reveals that only pharmacokinetic study of evogliptin with in combination with metformin was reported.<sup>5</sup> Evogliptin was not included in any pharmacopoeia and no such chromatographic method was reported for the estimation of evogliptin using any of the instrumental methods till now. Only there is a recent availability of one UV spectrophotometric method for the estimation of evogliptin<sup>6</sup>. Therefore it is urgent necessary to develop an instrumental method for the estimation of evogliptin in bulk and marketed dosage form. By keeping the above things in mind, the authors developed an Ultra performance liquid chromatography method for the

evogliptin estimation and validate as per ICH guidelines<sup>7</sup>. And also performed the stability studies to investigate the degradation patterns of evogliptin in various stressed conditions as per ICH guidelines for stability studies<sup>8</sup>.



**Fig 1: Chemical structure of evogliptin**

## MATERIALS AND METHODS

### Chemicals and Reagents:

Pharmaceutical grade working standards Evogliptin (99.92%) was procured from the Alkem Pharmaceuticals Ltd, Sikkim, India. The tablets (Valera- 5 mg evogliptin, manufactured by Alkem pharmaceuticals, Sikkim, India) were purchased from the local market of Hyderabad, India. All required chemicals and reagents were purchased from Finer chemical Ltd, Fisher Scientific and Merck.

### Instrumentation Conditions:

The evogliptin was analysed using Ultra performance liquid chromatography (UPLC) Acquity Waters, PDA detector. Software: Empower 2, equipped with auto sampler and PDA detector. The analytical column DIKMA Endoversil (2.1 x 50mm, 1.7  $\mu$ m) UPLC with the flow rate 0.4 ml/min (isocratic) was utilised. The analytical balance 0.1mg sensitivity (Afcoset ER-200A), pH meter (Adwa – AD 1020).

### Preparation of 0.05 M phosphate buffer:

About 6.8043 grams of potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 4.2 with orthophosphoric acid.

### Preparation of mobile phase:

Accurately measured 450 ml (30%) of phosphate buffer and 550 ml of HPLC grade acetonitrile (70%) were mixed well and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45  $\mu$  filter under vacuum filtration.

### Standard Solution Preparation:

Accurately weigh 25mg of Evogliptin working standard and transferred into a 25ml clean dry volumetric flask, add 10ml of acetonitrile and sonicated to dissolve it completely and make volume up to the mark with the mobile phase to attain 1 mg/mL concentration. The working solution of Evogliptin was prepared by further pipette 1.5 ml of the above stock solutions into a 100 ml volumetric flask and dilute up to the mark with diluent to obtain 15  $\mu$ g /mL of Evogliptin .

### **Assay of marketed dosage form:**

Accurately weigh 20 valera (evogliptin 5 mg) tablets and triturated into a mortar and pestle and transfer the tablet powder equivalent to 100 mg Evogliptin into a 100 ml clean dry volumetric flask add about 10 ml of acetonitrile and sonicated it up to 10 minutes to dissolve completely and make volume up to the mark with the mobile phase. Then it is filtered through 0.44-micron injection filter which is considered as stock solution. Further pipette 0.15 ml of solution from the above stock solution using micropipette into a 10ml volumetric flask and dilute up to the mark with diluent. 10  $\mu$ L of the standard and sample solution was placed in the autosampler, and injected in triplicate into the chromatographic system. The areas for Evogliptin peak were measured and assay percentage was calculated.

### **Method Validation**

#### **Specificity:**

It was performed using placebo interference test of the evogliptin sample solution using 500 mg of placebo, which is equivalent to one tablet dissolved in 100ml of mobile phase and the placebo solution was treated like a standard solution. The solution was injected to the chromatographic system to assess the possible interfering peaks.

#### **System suitability:**

It was conducted<sup>9</sup> to rationalize whether analytical system is running properly. It was carried out by injecting the six replicates of standard solution of evogliptin. The %RSD of a range of optimised parameters like peak area, theoretical plates, retention time and asymmetric factor were calculated.

#### **Accuracy:**

To validate the accuracy of the present method recovery study was conducted at different levels (50%, 100%, and 150%) of pure evogliptin. The amounts of standard evogliptin was added to a fixed concentration to evogliptin tablet sample solution to attain the various levels. This study<sup>10</sup> were carried out three times and the percentage recovery as well as percentage mean recovery was calculated.

#### **Intra day & Inter day precision:**

The precision of the method was<sup>11</sup> evaluated by analysing the six sample solutions in triplicate (n=6) of 10  $\mu$ g/mL of evogliptin solution. The intra- and inter-day precision was determined by analysing for six times on the same day (intra-day study) and repeated on the second and also third day study (inter-day study). The chromatograms were recorded, peak area and retention time of Evogliptin was determined and relative standard deviation (RSD) was calculated.

#### **Detection and Quantitation limit:**

The limit of detection is defined as a concentration for which a signal-to - noise ratio of 3 was obtained and a signal-to - noise ratio of 10 was considered to be<sup>12</sup> for the limit of quantification. The standard solution of evogliptin was prepared by chronological dilution and injected into the chromatographic system in the range of 0.1-10  $\mu$ g / ml of Evogliptin in the decreasing order of concentration.

### **Linearity:**

Standard evogliptin solution was prepared as stated earlier in order to conduct the linearity analysis, different volumes of aliquot from the standard solution were diluted with mobile phase to achieve different concentrations in the 2-50 µg / ml range of evogliptin. In view of concentration versus peak area, the calibration curve was plotted for evogliptin and the data obtained were subjected to regression analysis.

### **Robustness:**

Robustness of the developed method was studied<sup>11</sup> by deliberately changing the chromatographic condition. Six sample solutions were prepared and analysed in triplicate utilising the optimised condition by purposely varying the analytical conditions like flow rate, mobile phase ratio, and detection wavelength at three different levels. All the optimised parameter was found within the limit. For the calculation of percentage RSD the tailing factor was considered.

### **Force degradation study of evogliptin:**

Force degradation study of the evogliptin solution<sup>12</sup> was carried out by using prescribed stress condition by ICH such as acidic, alkaline, oxidative, thermal and photolytic stress conditions. All the types of degradation studies have been performed in triplicate and mean peak area has been measured for the calculation.

### **Acid degradation:**

The acid degradation analysis was conducted at 60°C and 75% relative humidity using 1 M HCL using an environmental test chamber (Acamus Technologies, India). 0.5 ml of stock solution of evogliptin (1 mg / mL) was administered in a 10 ml volumetric flask, 0.5 ml of 1 M HCL was applied to the flask and held for 16 hours in the environmental test chamber. The solution was neutralised using 1 M NaOH after the suitable stress time and the mobile phase volume was composed, injected into the UPLC device.

### **Alkaline degradation:**

This study was performed at 60°C and 58% relative humidity using same environmental chamber. 0.5 ml of stock solution was taken in 10 ml volumetric flask mixed with 1 M 0.5 ml of 1M NaOH and kept for 16 hr. After the suitable stress period the solution was neutralized with 1 M HCL and make up the volume upto the mark with mobile phase and injected in to the UPLC system.

### **Oxidative degradation:**

At 40°C, 75% relative humidity, it was carried out in the same environmental chamber. To conduct the oxidation of the studied sample, 6 percent H<sub>2</sub>O<sub>2</sub> was used. For this reason, 0.5 ml of stock solution was taken in a 10 ml volumetric flask and 0.5 ml of 6 percent H<sub>2</sub>O<sub>2</sub> was applied to the flask and held for 16 hours at 60°C, eventually forming the volume up to the mobile phase mark and injected into the UPLC system.

### **Thermal degradation:**

It has been carried out in the environmental chamber at 40°C, 75% relative humidity and using the oven at 105°C. Accurately 0.5 ml of stock solution was taken in 10 ml volumetric flask and kept in chamber for 144 hr and for dry heat thermolysis 1 mg of dry drug in solid form was placed in oven at 110°C for 2 days.

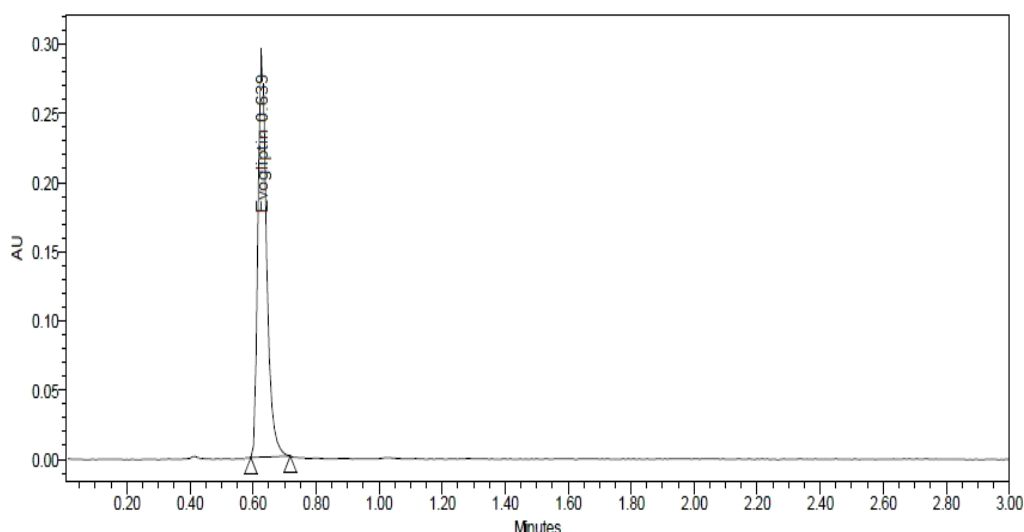
### Photolytic degradation:

This study was carried out during the daytime in sunlight (60000-70000 lux) for the duration of 48 hr. Accurately 0.5 ml of evogliptin stock solution was taken in a 10 ml volumetric flask and the volume was made up to the mark with mobile phase and analyzed.

## RESULTS

### Method Development

Various UPLC chromatographic conditions were tried to attain an optimised method for evogliptin estimation in API and tablet formulation. Several parameters such as mobile phase composition, column type, pH of mobile phase and diluents were varied during the initial trials. Various proportions of solvents, buffer, were tested in order to attain suitable composition of the mobile phase for the method optimization. Finally, evogliptin was eluted with good quality peak shape and low retention time with the mobile phase phosphate buffer, pH 4.2 and acetonitrile as a mobile phase (30:70) with a flow rate of 0.4 mL / min. The retention time 0.639 minute was observed for the evogliptin with PDA detection at 237 nm. As per ICH guidelines, the established technique was validated. The optimised chromatogram was shown in **Fig 2**.



**Fig 2: Optimised UPLC chromatogram of evogliptin**

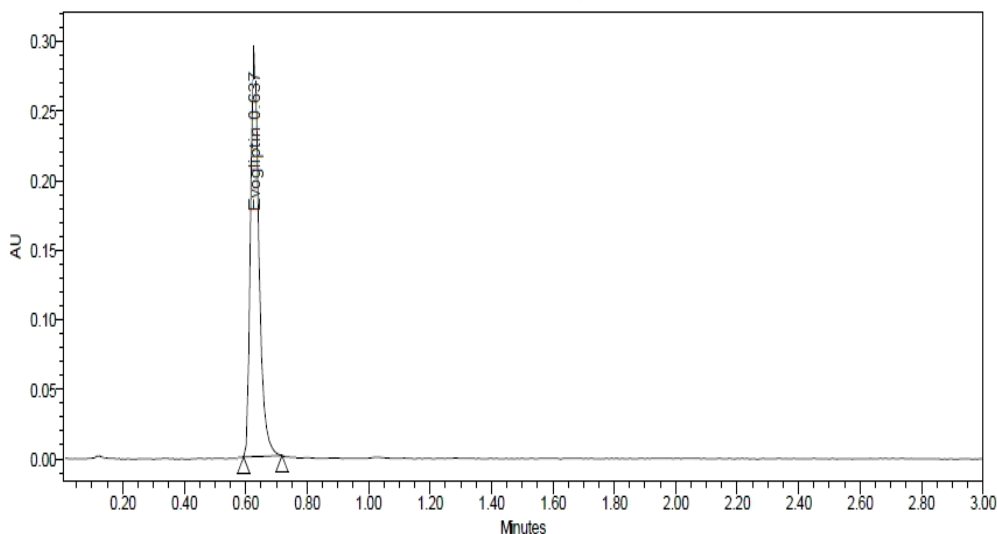
### Method validation

For the subsequent validation studies, the optimised method for bulk evogliptin was effectively applied. The result of the marketed tablet dosage type assay of evogliptin indicates that 97.80 percent was the average percentage purity. In **Fig 3**, the assay chromatogram was shown and the result was found in **Table 1**.

**Table 1: Assay of evogliptin marketed formulations**

Evogliptin marketed formulation	Labelled claimed	Amount obtained*	Percentage purity of evogliptin*
Evogliptin tablets (Valera) Contains evogliptin 5 mg, by Alkem Pharmaceuticals limited, Sikkim, India.	5 mg	4.89 mg	97.8%

\*average of three replicates



**Fig 3: Assay of marketed dosage form of evogliptin**

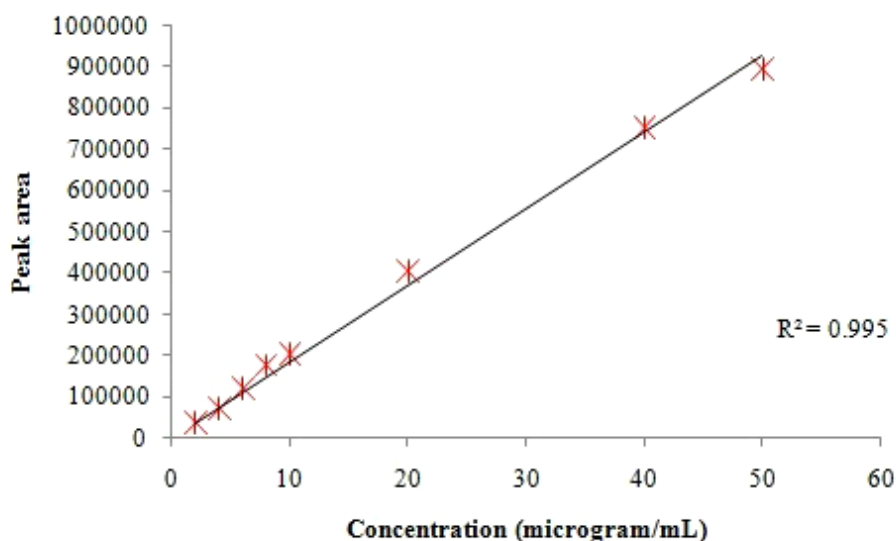
Both accuracy and precision were determined with standard quality control samples. The results of accuracy as a mean % recovery was found 98.72, and the % RSD was not more than 2 %, shown in Table 2. The % RSD of the intra and inter day precision study was found 0.63 and 0.47. The results of precision study were also shown in Table 2. The findings of the specificity analysis indicate no peaks in excipients at the retention time of the analyte. To confirm the adequate operation of the equipment used for analytical measurements, a system suitability study was conducted. Several parameters were taken into account. The 0.22 %, 0.97 %, 1.07 % and 1.43 % respectively were the relative standard deviation of peak area, theoretical plates, tailing factors and retention time. The linearity was determined for the range of concentrations 2-50 µg/ml. and the correlation coefficient was obtained 0.997 for the evogliptin mentioned in Table 2. The linearity graph was shown in Fig 4.

**Table 2: Summary of the results of validation parameters**

Parameters	Evogliptin
LOD µg/ml	0.2
LOQ µg/ml	0.5
Linearity range (µg/ml)	2-50 µg/ml
Regression co-efficient	0.998
% *Mean recovery (accuracy)	98.72
Intraday precision** (% RSD)	0.63
Inter-day precision** (% RSD)	0.47
% RSD of retention time* (robustness study)	0.79

\*Average of three replicates. \*\* Average of six replicates



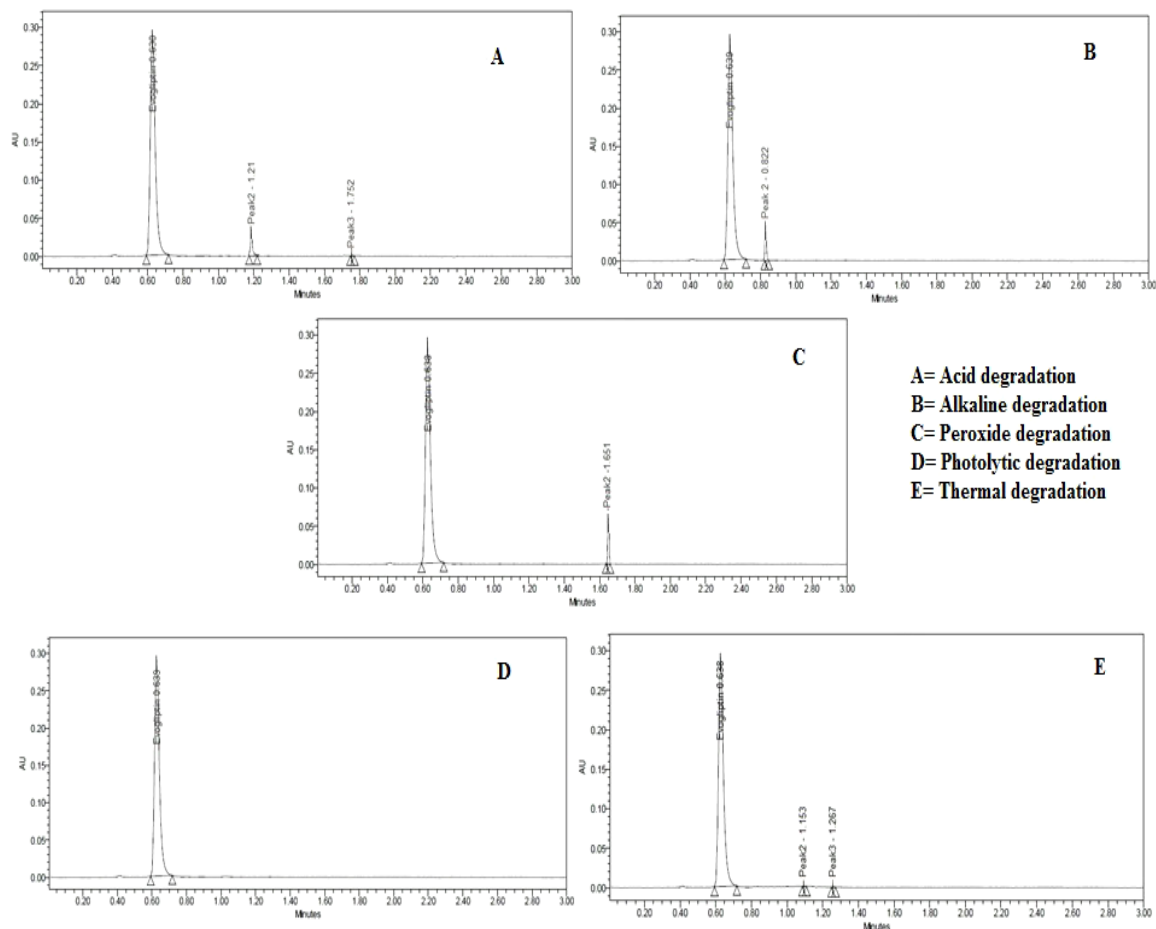


**Fig 4: Linearity graph of evogliptin**

The detection and quantitation limit of evogliptin were found to 0.2 µg/ml and 0.5 µg/ml respectively. Robustness study of the method was carried out by changing three parameters from the chromatographic conditions such as changes in mobile phase composition ( $\pm 2\%$ ), changes in flow rate ( $\pm 0.1$  ml/min), and detection wavelength ( $\pm 2$  nm) and the % RSD of the retention time which was considered as a tool parameter was established to be fewer than 0.79 as shown in **Table 2**. Degradation studies of evogliptin were performed under the influence of acid, alkali, oxidation, thermal, photolytic conditions. Degradation was found almost all stressed condition except photolytic influence. Acidic stressed condition shows degradation of 3.30%, alkaline stress condition shows 2.91 %, peroxide condition shows 0.76%. The thermal degradation shows 0.63 % degradation respectively, the results were revealed in **Table 3** and chromatograms shown in **Fig 5**.

**Table 3: Degradation results for evogliptin solution**

Stressed condition	Evogliptin				
	Mean Area*	% Degraded	Purity Angle	Purity Threshold	Peak purity
Acid	417292	3.30	1.83	2.55	Passes
Base	402632	2.91	0.89	1.74	Passes
Peroxide	381739	4.72	0.76	1.49	Passes
Photo	423867	0.22	0.09	1.36	Passes
Thermal	409539	2.08	0.63	2.99	Passes



**Fig 5: Force degradation chromatograms of evogliptin in various stressed conditions.**

## DISCUSSION

The optimised chromatographic conditions were developed after several trials in the current research paper. Evogliptin was eluted with a good peak form, very short retention time using a mixture of acetonitrile and phosphate buffer with a volume ratio of 70:30 and a flow rate of 0.4 mL / min by the use of the developed optimised condition.

The amount found was measured in the intraday and interday accuracy analysis and the calculated percent RSD was found acceptable and within the limits. The findings of the precision study showed that the methodology developed was found to be specific. The retention time of 0.639 minutes obtained for evogliptin is. In accordance with the guidelines, accuracy and accuracy were determined and the percent recovery was found as a result of accuracy within the acceptable limit, i.e. within 95-105% as shown in the result, confirming the accuracy of the method developed. The percentage assay of 97.8 % in the marketed dosage form, which indicates the suitability of the method developed for the study of evogliptin in the marketed dosage form. In the linearity study of the method the correlation coefficient was found near to 0.998 for evogliptin which indicates its specified linearity. The least squares method was used to establish the regression line and the curve was found linear. The limit of detection and quantitation values proved sensitivity of the developed method, and in 0.5 µg/ml



concentrations also found quantifiable for evogliptin. In the specificity result it was observed that no excipients peaks were found at the retention time of the evogliptin and justifies the specificity of the method. In the robustness study, the tailing factor was considered for the measurement tool and the % RSD of the tailing factor was found less than 2.0 which validates the robustness of the established approach, because no there was no significant changes were observed on the deliberate changes in the optimized parameters. The result of degradation studies of Evogliptin indicated that acidic and peroxide stressed condition leads to little more degradation in compare to other stressed condition, whereas in photolytic condition no degradation was establish. In every stressed condition, the chromatograms of evogliptin was found very specific.

## CONCLUSION

Based on the experiential evidences of the present developed method for the Evogliptin, authors are strongly declare about the novelty of the method over the available very few methods. The present UPLC method which is 'rapid' because it significantly reduced the total analysis time within 0.639 minutes, which can consider lowest analysis time required. The present method is "stability indicating" because not as much of degradation was observed in stressed conditions and excellent separation of evogliptin among the other degraded peaks also observed. The results of the validation parameters were noted and found under the acceptance criteria of ICH Q2B guidelines. Hence the present developed method can be employed as a novel, reliable, validated method can be apply for routine analytical and quality control assay of evogliptin in the bulk as well as tablet dosage form.

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## Conflict Of Interest

The authors confirm that this article content has no conflicts of interest.

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