**World Journal of Pharmaceutical Sciences**
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
**Research Article**

# STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF EVOGLIPTIN IN ITS PHARMACEUTICAL DOSAGE FORM

**Aqsa Iffath Fatima[1], Dr. V. Murali Balaram[2], Dr. Anupama Koneru[3]**

[1]M. Pharmacy, Department of Pharmaceutical Quality Assurance, sultan ul uloom college of pharmacy, Banjara hills, Mount hills, Hyderabad, 500034.
[2]Professor and HOD, Department of Pharmaceutical Analysis, sultan ul uloom college of pharmacy, Banjara hills, Mount hills, Hyderabad, 500034.
[3]Professor and Principal, Department of Pharmacology, Sultan Ul Uloom College of pharmacy, Banjara hills, Mount hills, Hyderabad, 500034.

***Received: 08-10-2025 / Revised Accepted: 11-10-2025 / Published: 14-10-2025***

## ABSTRACT
A basic, exact, accurate sensitive and specific RP-HPLC technique for pharmaceutical dose form Evogliptin measurement. Run through Sunfire C18 (4.6mm x 150mm, 5µm). Chromatogram Acetonitrile taken in the ratio 35:65 was pumped through column at a flow rate of 1.0ml/min in a mobile phase with 0.5gm of sodium hydrogen phosphate. Temperature maintained at thirty-degree Celsius. Selected a wavelength optimised at 240.0 nm. Evogliptin's retention time came up as 3.328 min. The Evogliptin's % RSD was 0.4%; the Method Precision of Evogliptin was determined to be 0.5%.For Evogliptin, recovery was 100.05%. The y = 42144x + 9007.4 regression equation of Evogliptin yields LOD, LOQ values of 0.12, 0.36. Retention durations were lowered and run time was lowered; so, the created approach was easy and cost-effective that one could apply in regular Quality control tests in different sectors.

**Key Words:** Evogliptin, Method development, Validation, RP-HPLC

## INTRODUCTION[1-10]
Modern dietary habits and sedentary lifestyles are significant contributors to the rising rates of diabetes. Diets high in processed foods, sugars, and saturated fats, coupled with decreased physical activity, increase the risk of developing Type 2 diabetes at younger ages. This shift in lifestyle behaviors underscores the importance of nutrition education and promotion of active living among young people. Diabetes, once considered primarily an affliction of older adults, has become a pressing concern for younger generations globally. This article explores the multifaceted impact of lifestyle choices, technological advancements, and prevailing health trends on the prevalence and management of diabetes among today's youth and young adults.[1]
Diabetes is a condition that happens when your blood sugar (glucose) is too high. It develops when your pancreas doesn't make enough insulin or any at all, or when your body isn't responding to the effects of insulin properly. Diabetes affects people of all ages. Most forms of diabetes are chronic (lifelong), and all forms are manageable with medications and/or lifestyle changes.[2]
Antidiabetic drugs are medicines developed to stabilise and control blood glucose levels amongst people with diabetes. Antidiabetic drugs are commonly used to manage diabetes.[3] Diabetes is caused by the body's inability to produce or respond to the pancreatic hormone insulin. One of the important physiological actions of insulin is to control blood glucose levels. Glucose is an important nutrient for cellular metabolism, and cells must receive neither too little nor too much. A deficiency in the pancreatic secretion of insulin, or lack of tissue sensitivity to the hormone, leads to diabetes, the primary feature of which is elevated blood glucose levels (hyperglycemia).[4]
Antidiabetic agents comprise a chemically and pharmacologically heterogeneous group of drugs. The objective in treating diabetes mellitus is to prevent undue rises in blood glucose throughout each successive 24-hour

**Address for Correspondence**: Aqsa Iffath Fatima, M. Pharmacy Department of Quality Assurance, sultan ul uloom college of pharmacy, banjara hills, Mount hills, Hyderabad, 500034, Medchal Dist., Email: aqsaiffathfatima44@gmail.com.

**How to Cite this Article:** Aqsa Iffath Fatima, STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF EVOGLIPTIN IN ITS PHARMACEUTICAL DOSAGE FORM, World J Pharm Sci 2025; 13(03): 217-224; https://doi.org/10.54037/WJPS.2022.100905

period, without producing clinical hypoglycemiaHollander (1998). It is now widely accepted that good control of blood glucose prevents the development of microvascular (retinopathy, nephropathy) and neuropathic long-term complications of the disease in both type 1The Diabetes Control and Complications Trial Research Group, 1993 and, the much more common, type 2 diabetes.[5] A significant fraction of the patients diagnosed with ORG fulfill the definition of diabesity: diabetes and obesity or prediabetes. Thus, antidiabetic therapy should be implemented in accord with recent treatment guidelines for diabetic kidney disease (DKD).[6]

**ANALYTICAL BACKGROUND**

Evogliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor used primarily for the treatment of Type 2 diabetes mellitus. As a DPP-4 inhibitor, evogliptin works by blocking the enzyme responsible for breaking down incretin hormones like GLP-1 (glucagon-like peptide-1), which in turn stimulates insulin secretion and inhibits glucagon release, thus helping to regulate blood glucose levels. Studies have shown that evogliptin, like other DPP-4 inhibitors, effectively lowers HbA1c levels and improves glycemic control in patients with Type 2 diabetes, either as monotherapy or in combination with other antidiabetic medications. Clinical studies have shown that evogliptin effectively reduces HbA1c levels, a marker of long-term blood glucose control, when used either as monotherapy or in combination with other antidiabetic medications.[7] Evogliptin's role in diabetes management is particularly beneficial due to its oral administration and convenient dosing regimen, which enhances patient adherence to treatment. It is often prescribed when lifestyle modifications alone are insufficient to achieve glycemic targets, providing an additional therapeutic option for patients with T2DM. In conclusion, evogliptin represents a valuable treatment option in the management of Type 2 diabetes, offering effective glycemic control through its inhibition of DPP-4 enzyme activity. Its efficacy, safety profile, and convenience make it a preferred choice for many healthcare providers managing patients with T2DM.[8] Evogliptin is chemically known as (3R)-4-[(3R)-3-amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-[(tert-butoxy)methyl]piperazin-2-one [9]

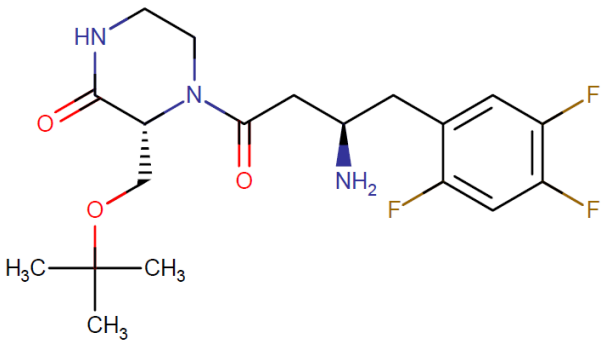


**Figure 1 structure of Evogliptin**

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Evogliptin, In the review of literature, more economical methods were observed[10-12], hence a simple, cost-effective stability-indicating simultaneous estimation of Evogliptin by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

**MATERIALS:**

Evogliptin pure drug (API), Evogliptin formulation (Valera), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

**INSTRUMENTATION**

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

**Table 1: Chromatographic Conditions:**

| | |
|---|---|
| **Mobile phase** | Acetonitrile: $NaHPO_4$(65:35 v/v) |
| **Flow rate** | 1 ml/min |
| **Column** | Sunfire C18 (4.6 x 150mm, 5µm) |
| **wave length** | 240 nm |
| **Column temperature** | 26°C |
| **Injection volume** | 10µL |
| **Run time** | 6.0 min |

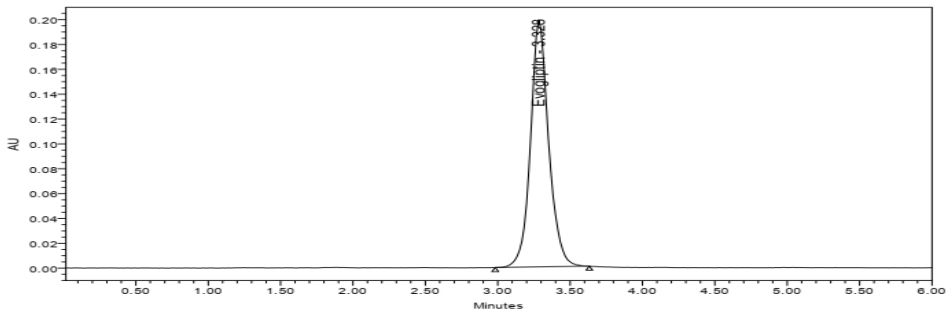


**Figure 2: Optimized Chromatogram**

**Methods:**

**Preparation of Standard stock solutions:** Accurately weighed 5mg of Evogliptin is transferred to 25ml volumetric flask. 3/4 ThE of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Evogliptin).

**Preparation of Standard working solutions (100% solution):** 1ml from stock solution was pipetted out, taken into a 10ml volumetric flask, and made up with diluent. (20µg/ml of Evogliptin).

**Preparation of Sample stock solutions:** Taken 10 tablets and weighed each tablet average weight and Equivalent to average weight of One Tablet (Equivalent to 5mg) was taken directly transferred into a 50ml volumetric flask, 10ml of diluents was added and sonicated for 25 min and further centrifuged for 30 min at 3000rpm and further the volume was made up with diluent and filtered by HPLC filters (100µg/ml of Evogliptin)

**Preparation of Sample working solutions (100% solution):** 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Evogliptin)

**Validation:**

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solution of Evogliptin (50ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

**Specificity:** Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

System suitability:

The system suitability parameters were determined by preparing standard solution of Evogliptin (20ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

**Specificity (Selectivity):** Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 4 and experimental data is given in Table 3

**Table: 2 System suitability parameters for Evogliptin**

| S no | Evogliptin | | |
|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing |
| 1 | 3.284 | 3551 | 1.06 |
| 2 | 3.285 | 3536 | 1.06 |
| 3 | 3.288 | 3521 | 1.07 |
| 4 | 3.290 | 3542 | 1.06 |
| 5 | 3.290 | 3552 | 1.06 |
| 6 | 3.301 | 3564 | 1.06 |

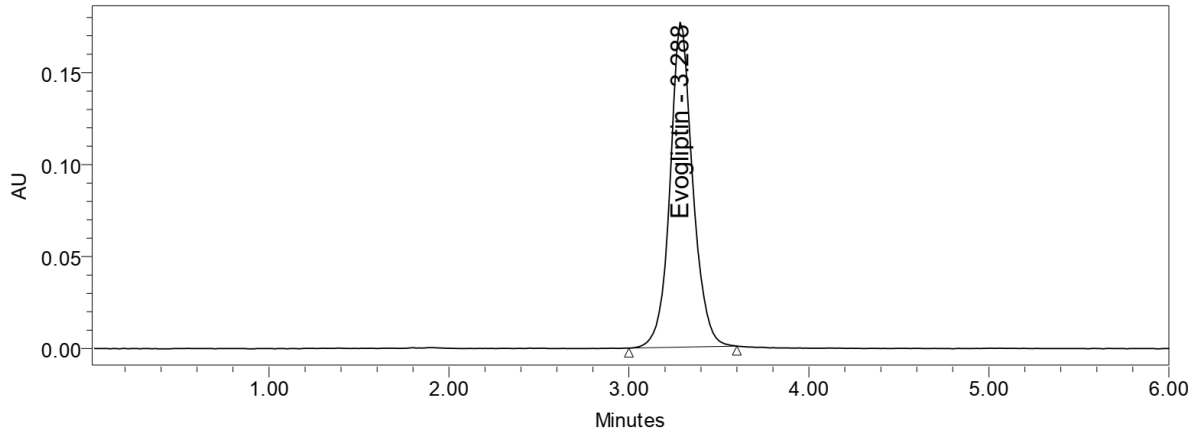


**Figure 3: System Suitability Chromotogram of Evogliptin**

**Table 3: Specificity Data**

| Peak name | Rt | Area | USP plate count | Tailing |
|---|---|---|---|---|
| Evogliptin | 3.328 | 850474 | 3527 | 1.06 |

**Specificity:**

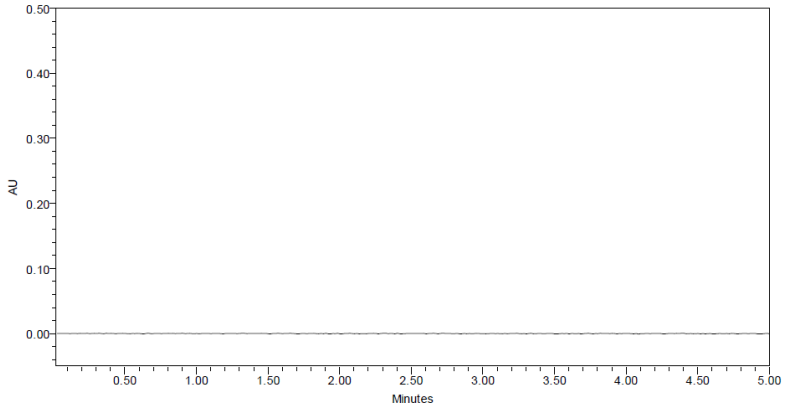


**Figure 4 Chromatogram of blank.**

The forced degradation conditions are mentioned in Table 3 and the results are mentioned in Table 4

**Table 4: Forced degradation conditions for Evogliptin**

| Stress condition | Solvent | Temp($^0$C) | Exposed time |
|---|---|---|---|
| **Acid** | 2N HCL | 60$^0$c | 30 mins |
| **Base** | 2N NAOH | 60$^0$c | 30 mins |
| **Oxidation** | 20% $H_2O_2$ | 60$^0$c | 30 mins |
| **Thermal** | Diluent | 105$^0$c | 6 hours |
| **Photolytic** | Diluent | - | - |
| **Hydrolytic** | Water | 60$^0$c | |

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

**Table 5: Degradation profile results**

| Degradation Condition | % Drug Un Degraded | % Drug Degraded |
|---|---|---|
| Acid | 93.96 | 6.04 |
| Base | 98.96 | 1.04 |
| Oxidation | 93.68 | 6.32 |
| Thermal | 98.76 | 1.24 |
| Photolytic | 99.19 | 0.81 |
| Hydrolytic | 98.13 | 1.87 |

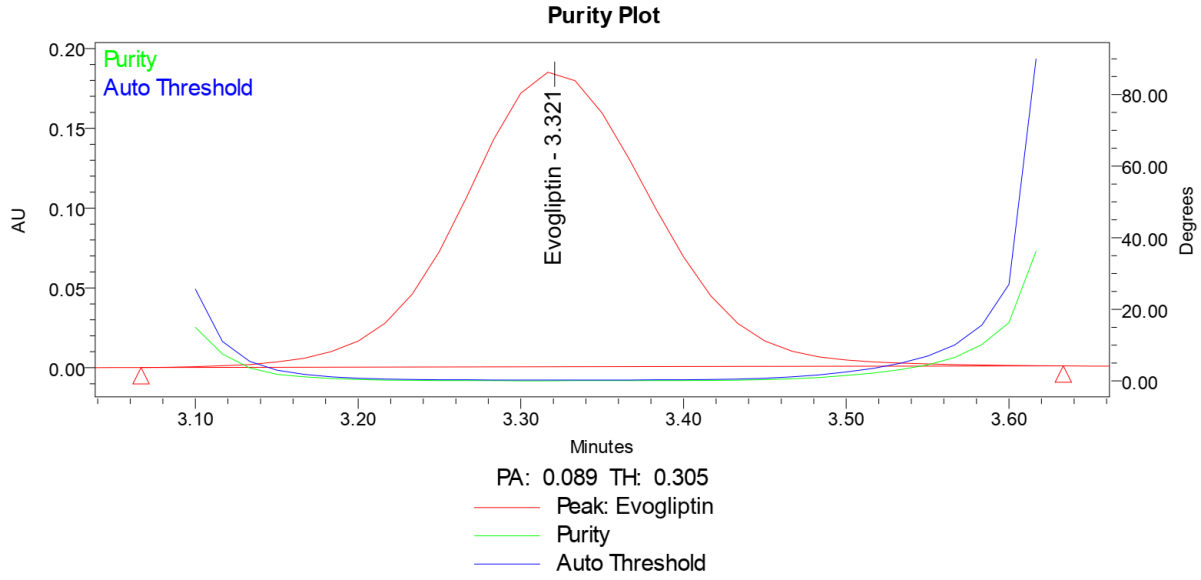


**Figure 5: Purity Plots of Evogliptin**

**Limit of detection (LOD)** The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

**Limit of quantitation (LOQ):** The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Evogliptin are listed in Table 5.

**Table 6: Summary of limit of detection**

| Sample | Conc (µg/ml) |
|---|---|
| LOD | 0.12 |
| LOQ | 0.36 |

**Linearity:** The linearity of the method was demonstrated for Evogliptin by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Evogliptin was 0.999. This indicates good linearity (Figures 8).

**Linearity:**

Calibration data is given in table 4 and regression data in table 4 and calibration curve in figure 4, 5

**Table 7: Calibration data of Evogliptin**

| Evogliptin | |
|---|---|
| Conc (µg/mL) | **Peak area** |
| 0 | 0 |
| 5 | 214605 |
| 10 | 428076 |
| 15 | 647223 |
| 20 | 855666 |
| 25 | 1071970 |
| 30 | 1261617 |

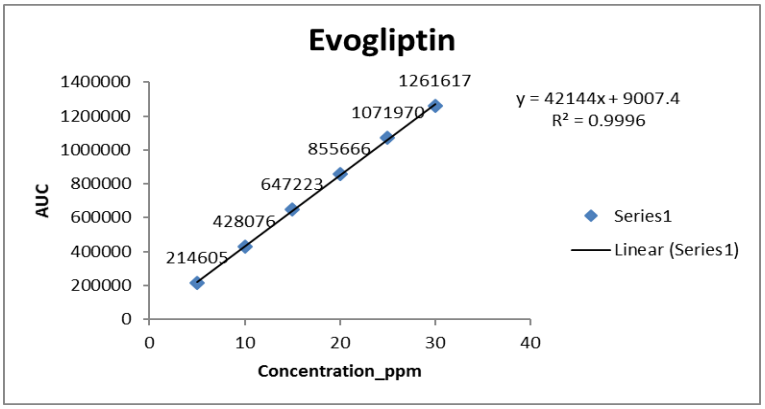

**Figure 6: Calibration curve of Evogliptin**

**Table 8: regression data**

| Parameter | Evogliptin |
|---|---|
| Conc range (µg/mL) | 5-30µg/ml |
| Regression Equation | y = 42144x + 9007.4 |
| Co-relation | 0.999 |

**Accuracy:** The accuracy of the method was determined by using solutions containing spiked samples of Evogliptin at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

**Table 9 Accuracy table of Evogliptin**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % recovery |
|---|---|---|---|
| 50% | 10 | 10.05 | 100.50 |
| | 10 | 10.03 | 100.29 |
| | 10 | 10.03 | 100.32 |
| 100% | 20 | 20.00 | 99.99 |
| | 20 | 19.97 | 99.87 |
| | 20 | 19.99 | 99.96 |
| 150% | 30 | 29.75 | 99.16 |
| | 30 | 30.05 | 100.18 |
| | 30 | 30.07 | 100.22 |
| Mean % recovery | | | 100.05 |

**System Precision:** The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Evogliptin. Results of peak area are summarized in Table 10

**Table 10 System precision table of Evogliptin**

| S. No | Area of Evogliptin |
|---|---|
| 1. | 859577 |
| 2. | 853677 |
| 3. | 850757 |
| 4. | 852769 |
| 5. | 852757 |
| 6. | 853758 |
| **Mean** | **853883** |
| **S.D** | **2992.0** |
| **%RSD** | **0.4** |

**Method Precision:** The precision of the method was determined by analyzing a sample of Evogliptin). Data obtained is summarized in Table 11

**Table 11 Repeatability table of Evogliptin**

| S. No | Area of Evogliptin |
|---|---|
| 1. | 850647 |
| 2. | 853758 |
| 3. | 850657 |
| 4. | 859757 |
| 5. | 850474 |
| 6. | 857433 |
| **Mean** | **853788** |
| **S.D** | **3989.2** |
| **%RSD** | **0.5** |

**Intermediate precision:** It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

**Table 12 Intermediate precision table of Evogliptin**

| S. No | Area of Evogliptin |
|---|---|
| 1. | 859664 |
| 2. | 850475 |
| 3. | 855646 |
| 4. | 850574 |
| 5. | 852374 |
| 6. | 850474 |
| **Mean** | **853201** |
| **S.D** | **3747.0** |
| **%RSD** | **0.4** |

**Robustness:** The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Evogliptin

| Condition | %RSD of Evogliptin |
|---|---|
| Flow rate (-) 0.9ml/min | 0.2 |
| Flow rate (+) 1.1ml/min | 0.9 |
| Mobile phase (-) 30B:70A | 0.2 |
| Mobile phase (+) 40B:60A | 0.5 |
| Temperature (-) 27°C | 0.4 |
| Temperature (+) 33°C | 0.5 |

**Assay data: -**

Valera Tablet bearing the label claims Evogliptin 40 mg. Assay was performed with the above formulation. Average % Assay for Evogliptin obtained was 99.69%. Assay data shown in table no 8.


**Figure 7: Evogliptin marketed drug**

**Formula to calculate assay:**

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{}{L.C} \times 100$$

| | |
|---|---|
| AT | Avergage peak area of sample in test solution |
| AS | Mean peak area of sample in standard solution |
| WS | Weight of sa,ple working standard taken in mg |
| P | Assay of sample working standard in % in dried basis |
| L.C | Label claim |
| FV | filled volume (1ml of a vail) |

**Table 14: Assay Data of Evogliptin**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 859577 | 850647 | 99.32 |
| 2 | 853677 | 853758 | 99.69 |
| 3 | 850757 | 850657 | 99.32 |
| 4 | 852769 | 859757 | 100.39 |
| 5 | 852757 | 850474 | 99.30 |
| 6 | 853758 | 857433 | 100.11 |
| **Avg** | **853883** | **853788** | **99.69** |
| **Stdev** | **2992.0** | **3989.2** | **0.466** |
| **%RSD** | **0.4** | **0.5** | **0.5** |

## CONCLUSION

Finally, the results of the Evogliptin HPLC analysis show that the drug's concentration and purity can be precisely measured using this method. This approach is well-suited for regular quality control and pharmacokinetic research because to its constant repeatability, crisp peak resolutions, and dependable retention durations. When it comes to evaluating the analytical properties of Evogliptin, HPLC is an indispensable instrument that guarantees its effectiveness and safety for use in clinical settings.

## ACKNOWLEDGEMENT:

The authors are thankful to, Department of Pharmaceutical Analysis, Sultan-ul-uloom, Affiliated to JNTUH India and Spectrum Pharma Research Solutions, Hyderabad, Telangana, India for providing with the gift sample of Evogliptin Pure API.

## REFERENCES

1. Danaei G, et al. Global burden of diabetes in the year 2000-2045: projections of the global burden of disease study. Diabetes Care. 2021;44(8):1916-25.
2. https://my.clevelandclinic.org/health/diseases/7104-diabetes
3. https://www.diabetes.co.uk/diabetes-medication/antidiabetic-drugs.html
4. John A. Thomas, Janet L. Stringer, Combination Therapy for Patients with Type 2 Diabetes: Repaglinide in Combination with Metformin, The Editors of Encyclopaedia Britannica. 2024.
5. Brian L. Furman, in xPharm: The Comprehensive Pharmacology Reference, 2007.
6. Michal Herman-Edelstein, ... Avry Chagnac, in Seminars in Nephrology, 2021.
7. Kim YG, et al. Efficacy and safety of evogliptin treatment in subjects with type 2 diabetes. Diabetes Obes Metab. 2017;19(8):1147-1154. doi:10.1111/dom.12942.
8. Gallwitz B. Safety and efficacy of dipeptidyl peptidase-4 inhibitors for treating type 2 diabetes. Expert Opin Drug Saf. 2016;15(4):505-515. doi:10.1517/14740338.2016.1155877
9. https://go.drugbank.com/drugs/DB12625
10. Surya Prakash Gupta, Sachin Singh, RP-HPLC Based Stability Indicating Assay of Evogliptin and Metformin: Development and Validation of the Analytical Method, International journal of pharmaceutical science and nonotechnology, 16 (2) (2023)
11. Baburao. N. Chandakavathe, Akash. D. Rajmane, Omkar. J. Shiddanagoudar, Shubham. P. Bet, Development and Validation of RP- HPLC Method for Estimation of Evogliptin in Formulation, Ijppr. Human, 2023; Vol. 26 (3): 360-368
12. Arpit Patel, Rakesh Patel and Priyanka Yadav, International Journal of Pharmaceutical Research and Applications, Volume 6, Issue 2 Mar-Apr 2021, pp: 775-78.