International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781

Development and Validation of RP- HPLC Method for estimation of Evogliptin in Pharmaceutical Dosage Form

Arpit Patel[1], Rakesh Patel[2] and Priyanka Yadav[2]
[1] *A-One Pharmacy College, Ahmedabad, M.Pharm, Pharmaceutical quality assurance (Sem IV),*
[2] *A-One Pharmacy College, Ahmedabad, Faculty of Pharmacy*

Date Of Submission: 01-05-2021 Date Of Acceptance: 10-05-2021

ABSTRACT: Evogliptin Tartrate, a member of dipeptidyl peptidase-4 inhibitors, is a recent drug marketed by Alkem Laboratory Limited, India for the treatment of Type 2 diabetes; it reduces degradation of endogenous glucagon-like peptide 1 (GLP-1) to× increase insulin secretion and satiety and decrease glucagon. Evogliptin can be used alone or in combination therapy.

A new sensitive and rapid RP-HPLC method was developed for the determination of Evogliptin in pharmaceutical dosage forms and it was validated according to ICH guidelines. The HPLC analysis was performed on the Model DG31000 system equipped with a ODS 5 µm (4.6 mm X 250 mm) C18 column, with a mixture of methanol:water:acetonitrile (70:20:10) and Octane 1 sulphonic acid sodium salt powder as mobile phase, at the flow rate of 1.0 mL/min. The detection was performed at the wavelength (λ) of 265, and the retention time of Evogliptin was around 3.6 min. The total run time was 10 min. The calibration plot gave linear relationship over the concentration range of 10–30 µg/ml. The LOD and LOQ were 0.99 and 3.00 µg, respectively. The accuracy of the proposed method was determined by recovery studies and was found to be in range of 99.87 to 100.47%. The repeatability testing for both standard and sample solutions showed that the method is precise within the acceptable limits. RSD% of the determination of precision was <2%. The results of robustness was within the acceptable limits as well. The proposed method showed excellent linearity, accuracy, precision, specificity, robustness, LOD, LOQ, and system suitability results within the acceptance criteria. In addition, the main features of the developed method are low run time and retention time around 3.6 min.

I. INTRODUCTION

Diabetes mellitus is evident from nearly 3000 years ago and despite the many research work that has been done, diabetes mellitus is still widely spreaded serious disease which has affected the life quality of millions of people worldwide. It is estimated that till 2035 year, the numbers of patients with diabetes mellitus will be increased to 592 millions. [1, 2].

The diabetes mellitus was distinguished to Type 1 and Type 2 until the year 1936 [2]. The main feature of Type 2 diabetes is the increased cell resistance to insulin and the dysfunction of the insulin-producing cell in the pancreas (β-cells) [1, 3].

Metformin is the first line of therapy for the treatment of Type 2 diabetes, but as the disease progresses, a drug in combination therapy is required to control [4].

After eating food, incretin hormones are secreted from the gastrointestinal tract in to the blood stream and stimulates the insulin secretion and help in controlling the glucose levels; thus, it helps the body against increase in blood glucose level. These hormones include glucagon-like peptide-1 and glucose-dependent insulin tropic polypeptide [5, 6]. Dipeptidyl peptidase-4 is an enzyme which is found in human body that helps to inactivate the incretin hormones and hence ending their hypoglycemic effect [2]. Evogliptin is a member of dipeptidyl peptidase-4 inhibitors is a recently developed drug, which is used for the treatment of Type 2 diabetes and it potentiates the effect of incretin hormones through inhibition of their degradation by the dipeptidyl peptidase-4 enzyme [2, 4]. Evogliptin can be used alone or in combination therapy, and it is approved in the India in 2018 [7]. Evogliptin is 4-[3-amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-[(2- methylpropan-2-yl)oxymethyl]piperazin-2-one;2,3-dihydroxybutanedioic acid ($C_{23}H_{32}F_3N_3O_9$), and it's structure is shown in Figure 1[8]

Analytical method validation provides assurity that the method will give reliable and repeatable result; which is crucial step in method development and validation with respect to specificity, linearity, range, precision, accuracy, detection, quantitation limits and robustness.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781

According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. [9–10].

Evogliptin is not official in any of the pharmacopoeia (i.e. USP, EP, and IP).

Literature survey reveals few methods reported for determination of Evogliptin but in biological samples using LC-MS/MS method [11-13].

In this research, a new sensitive and rapid RP-HPLC method was developed for the estimation of Evogliptin in tablet dosage forms, and this method was validated according to ICH guidelines.

II. MATERIALS AND METHODS

Instrumentation. Model DG31000 HPLC system was used for liquid chromatography method development and validation (Analyticals Technologies), equipped with a pump (model P3000), an auto sampler (ALS), and ODS 5 µm (4.6 mm X 250 mm) C18 column and the detector consisted of UV operated at 265 nm. Anal Chrom Software was used for data processing and evaluation.

Chemicals and Reagents. A pharmaceutical grade sample of Evogliptin Tartrate (purity 99.1%) was obtained from Vivan Laboratory. Valera tablet containing 5 mg Evogliptin was purchased from the local pharmacy store. Methanol, water, acetonitrile (HPLC grade) and Orthoposphoric acid, Sodium dihydrogen phosphate, Hydrochloric acid and Octane 1 sulphonic acid sodium salt powder (AR grade) were purchased from Merck.

Chromatographic Conditions. The mobile phase was prepared by mixing methanol:water:acetonitrile (70:20:10) and adding Octane 1 sulphonic acid sodium salt powder in 1000 mL of mobile phase. The pH of this mobile phase was adjusted to 3 using Orthoposphoric acid. Prior to use the mobile phase was filtered through Whatmann Filter and degassed by sonication for 10 min.

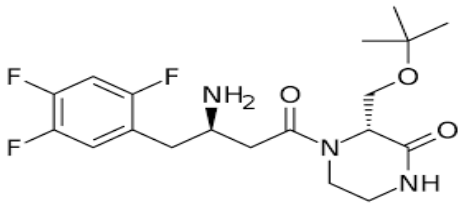


Figure 1: Chemical structure of Evogliptin

× The analysis was carried out on DG31000 series HPLC system. The analysis was carried out on an analytical column C18, 5 µm, 250 X 4.6 mm with a detection wavelength of 265 nm. The operating temperature of the column was set at 25°C. The injection volume was 20 µL, and the flow rate was maintained at 1.0 mL/min. The run time was 10 min.

Preparation of Standard Solution. A standard solution of Evogliptin tartrate was prepared by dissolving an accurately weighed amount of API powder of Evogliptin (10 mg) in 50 ml of the mobile phase and sonicated, and then 5 mL of the resulting solution was diluted to 50 mL by the same solvent to obtain a standard solution of Evogliptin (20 µg/ml).

Preparation of Sample Solution. Ten Evogliptin tablets were weighed, triturated in porcelain mortar, and mixed, and the average weight of tablet was calculated i.e. 149.7 mg. Accurately weighed amount (299.4 mg) of crushed tablet powder (equivalent to 10mg Evogliptin) was transferred to 50 mL volumetric flask, and 50 mL of the mobile phase was added and sonicated. 5 mL of the resulting solution was diluted to 50 mL by the same solvent to obtain a test solution of Evogliptin (20 µg/ml). The prepared solution was filtered through whatmann filters.

Method Validation. The method was validated as per ICH Q2(R1) guideline, and the validation parameters included specificity, linearity, range, accuracy, precision, LOQ, LOD and robustness [9- 17].

Specificity. Specificity is the ability of the analytical method to discriminate between the analyte and the other component(s) in the mixture. Specificity of the method was evaluated by injecting 20 µL solution of standard, sample and blank separately.

Linearity. To evaluate the linearity and range of the method, different standard solutions were prepared by diluting the standard stock solution with the mobile
phase in deferent concentrations of Evogliptin: 10, 16, 20, 24, and 30 µg/ml, which cover 50%, 80%, 100%, 120%, and 150% of the target concentration, respectively. Each of these concentrations was analysed under the same conditions. Linear regression analysis was used to estimate the linearity of the calibration curve by using the least square linear regression method.

Precision. The system precision and method precision (repeatability) of the proposed

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781

methods were determined by several measurements of standard solution and sample solution, respectively. Repeatability was estimated by six measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established by five assay determinations of the sample solution at the 100% concentration levels on the same day and on next day for intraday and interday precision. The RSD of obtained results was calculated to evaluate repeatability results.

Accuracy. The accuracy of the assay method was determined by recovery studies at three concentration levels (50%, 100%, and 150%), i.e., 10, 20, and 30 µg/ml, and three samples from each concentration were injected. The percentage recovery of added Evogliptin and RSD were calculated for each of the replicate samples.

Limit of detection (LOD) and Limit of Quantitation (LOQ). The LOD of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD= 3.3 σ/s & LOD= 10 σ/s

Where, σ = the standard deviation of the response.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte

Robustness. Robustness of the method was estimated by applying minor but deliberate changes in the experimental parameters, as below:

- Column temperature: ±1°C
- Flow rate: ±0.1 mL/min

Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD.

III. RESULTS AND DISCUSSION

Method Development and Optimization. Several physical and chemical properties of Evogliptin were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC-UV chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of trials were performed by varying the ratio of methanol, acetonitrile and water, acetate buffer, Octane 1 sulphonic acid sodium salt powder in 1000 mL as mobile phase and optimizing the chromatographic conditions. The results of method optimization are summarized in Table 1.

The mobile phase consisting of Methanol:water:acetonitrile (70:20:10) and octane 1 sulphonic acid sodium salt powder in 1000 mL of mobile phase with pH adjusted to 3 using orthoposphoric acid with a flow rate of 1 mL/min, injection volume 20 µl, run time 10 min, and column temperature 25°C at wavelength (λ) 265 was optimized as the best chromatographic conditions for the entire study where evogliptin was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 3.6 min (Figure 2).

Method Validation

Specificity. Specificity was evaluated by comparing the chromatograms of mobile phase blank solution, standard solution, and sample solution (Evogliptin 20 µg/ml). For this purpose, 20 µL from solutions mobile phase blank, standard solution, and sample solution were injected into the HPLC system separately, and the chromatogram results are shown in Figures 2–4. It can be observed that there is no coeluting peak at the retention time of evogliptin interference and it is observed that the chromatograms of test and standard solution were similar and no peak in blank solution. This result indicates that the peak of the analyte was pure and this confirmed the specificity of the method.

Linearity and Range. The linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. 5 concentrations (50% to 150% level) were injected and plotted. The results of linearity study (Figure 5) gave linear relationship over the concentration range of 10–30 µg/ml. From the regression analysis, a linear equation was obtained: y = 00775 X – 21004, and the goodness-of-fit (r2) was found to be 0.999, indicating a linear relationship between the concentration of analyte and area under the peak.

Limit of Detection and Limit of Quantification (LOD and LOQ). The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781

be quantitatively determined with suitable precision. The results showed an LOD and LOQ for Evogliptin of 0.99 and 3.00 µg, respectively.

Accuracy. The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of 99.87 – 100.47%, and % RDS values were in the range of 0.000373 - 0.218718% as shown in Table 2. The results of percentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% and not more than 2.0%, respectively, which indicates the accuracy of method. Precision. Precision of the method is defined as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is normally expressed as the relative standard deviation. The results of both system and method precision showed that the method is precise within the acceptable limits. The RSD, tailing factor, and number of theoretical plats were calculated for both solutions; all the results were within limits.

Acceptable precision was not more than 2.0% for the RSD and the tailing factor and not less than 1000 for number of plates, as shown in Tables 3 and 4.

Robustness. The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method, as mentioned in Section 2.0. The results of robustness testing showed that a minor change of method conditions, such as the temperature and flow rate, is robust within the acceptable limits. The results are summarized in Table 5.

Table 1: Results of method optimization

Column used	Mobile phase	Temperature and Flow rate	Wavelength	Observation	Result
ODS 5 µm (4.6 mm X 250 mm) C18 column	Methanol and NH2PO4 buffer (70:30)	Temperature 25°C Flow rate: 1 mL/min	265 nm	No peak observed	Method rejected
ODS 5 µm (4.6 mm X 250 mm) C18 column	Acetate buffer : methanol (60:40) 100 mL + octane sulphonic acid sodium salt (30 mg)	Temperature 25°C Flow rate: 1 mL/min	265 nm	Sharp peak observed at 4.5 min but extra peaks also observed	Method rejected
ODS 5 µm (4.6 mm X 250 mm) C18 column	Methanol:water:acetonitrile (70:20:10) + Octane 1 sulphonic acid sodium salt powder in 1000 mL as mobile phase, pH adjusted to 3 using orthoposphoric acid	Temperature 25°C Flow rate: 1 mL/min	265 nm	Sharp peak observed at 3.6 minute	Method

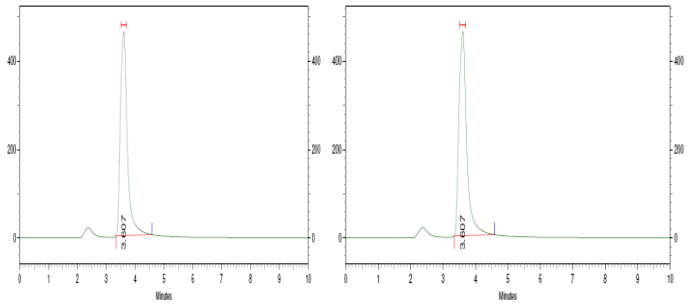
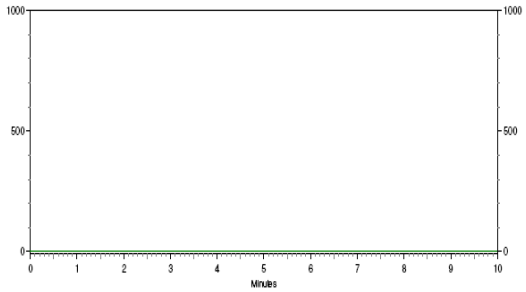


Figure 2: Chromatogram of Evogliptin standard solution

Figure 3: Chromatogram of Evogliptin sample solution

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781



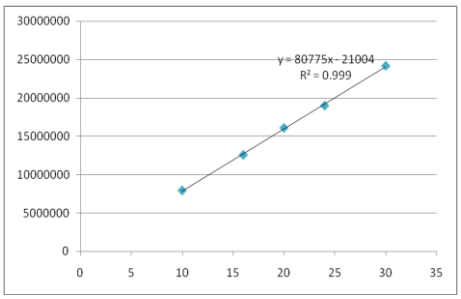


Figure 4: Chromatogram of blank solution **Figure 5:** Standard calibration curve of Evogliptin

Table 2: Recovery data of proposed HPLC method

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean	%RSD	SD
1	50 %	20	10	9.986	99.859	99.873	0.012131	0.012115
2		20	10	9.988	99.880			
3		20	10	9.988	99.880			
4	100 %	20	20	20.125	100.622	100.443	0.218718	0.219687
5		20	20	20.102	100.508			
6		20	20	20.0396	100.198			
7	150 %	20	30	30.143	100.477	100.477	0.000373	0.000374
8		20	30	30.143	100.477			
9		20	30	30.143	100.477			

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781

Table 3:Repeatibility data of proposed HPLC method

Sample	Area
1	15792486
2	15712887
3	15684882
4	15792476
5	15159545
6	15739042
AVG	15646886
SD	242565.2963
%RSD	1.55

Table 4: *Inter & Intraday precision data of proposed HPLC method*

Inter Day Precision		Intra Day Precision	
	Area		Area
1	16089281	1	15679323
2	16079282	2	15637212
3	16089701	3	15657129
4	16084607	4	15451683
5	16197922	5	15504022
AVG	16108159	AVG	15585874
SD	50356.3	SD	101430.4
%RSD	0.31	%RSD	0.65
Overall %RSD	1.80		

Table 5: Robustness data

Sr No.	Area at Flow rate (0.9 ml/min)	Area at Flow rate (1.1 ml/min)	Area at Column temperature 24^{0}C	Area at Column temperature 26^{0}C
1	16088651	16092282	16084652	16089701
2	16088235	16091976	16084695	16089219
3	16088489	16092278	16084658	16089629
%RSD	0.001303348	0.001090752	0.000144784	0.001615975

IV. CONCLUSION

The developed HPLC method is fast & simple and found specific, linear, accurate, precise, and robust. Hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Evogliptin. In addition, the main features of the developed method are short run time and retention time around 3.6 min. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under these chromatographic conditions.

REFERENCES

[1] A. B. Olokoba, O. A. Obateru, and L. B. Olokoba, "Type 2 diabetes mellitus: a review of current trends," Oman Medical Journal, vol. 27, no. 4, pp. 269–273, 2012.

[2] Introduction to Diabetes, https://www.healthline.com/health/diabetes

[3] C. Chen, C. M. Cohrs, J. Stertmann, R. Bozsak, and S. Speier, "Human beta cell mass and function in diabetes: recent ad-

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 775-781 www.ijprajournal.com ISSN: 2249-7781

vances in knowledge and technologies to understand disease pathogenesis," Molecular Metabolism, vol. 6, no. 9, pp. 943–957, 2017.

[4] M. Rendell, A. Drincic, and R. Andukuri, "Alogliptin benzoate for the treatment of type 2 diabetes," Expert Opinion on Pharmacotherapy, vol. 13, no. 4, pp. 553–563, 2012.

[5] W. Kim and J. M. Egan, "The role of incretins in glucosehomeostasis and diabetes treatment," Pharmacological Re- views, vol. 60, no. 4, pp. 470–512, 2008.

[6] National Library of Medicine, Jan 2021, https://pubchem.ncbi.nlm.nih.gov/compound /Evogliptin

[7] Approved drug list in India, https://cdscoonline.gov.in/CDSCO/Drugs

[8] Evogliptin details, Jun 2019, https://www.drugbank.ca/drugs/DB12625

[9] K. Zhang, P. Ma, W. Jing, and X. Zhang, "A developed HPLC method for the determination of alogliptin benzoate and its potential impurities in bulk drug and tablets," Asian Journal of Pharmaceutical Sciences, vol. 10, no. 2, pp. 152–158, 2015.

[10] K. Y. Kavitha, G. Geetha, R. Hariprasad, M. Kaviarasu, and R. Venkatnarayanan, "Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form," Journal of Chemical and Pharmaceutical Re- search, vol. 5, no. 1, pp. 230–235, 2013.

[11] Dae Y., Sookie L., Sun K., "Bioanalytical Validation For The Determination Of Evogliptin And Its Metabolites M7/M8 In Human Plasma By Liquid Chromatography-Tandem Mass Spectrometry", 2014

[12] Min K., Mi Y., Tae-eun K., "Multiple-dose pharmacokinetics and pharmacodynamics of evogliptin (Da-1229), a novel dipeptidyl peptidase iV inhibitor, in healthy volunteers, Drug Design, Development and Therapy", 2014, 709–172

[13] Mi-Jin K., Na-young K., Seunghyeong L., Yun-A J., "Evogliptin, a Dipeptidyl Peptidase-4 Inhibitor, Attenuates Renal Fibrosis Caused by Unilateral Ureteral Obstruction in Mice", Diabetes Metab J, 2019, 2020;44:186-192,

[14] ICH Guideline, "Validation of analytical procedures: text and methodology," in Proceedings of International Conference on Harmonization, Topic Q2 (R1), Geneva, Switzerland, No- vember 2005.

[15] S. Chandran and R. S. P. Singh, "Comparison of various international guidelines for analytical method validation," Pharmazie, vol. 62, no. 1, pp. 4–14, 2007.

[16] T. M. Kalyankar, P. D. Kulkarni, S. J. Wadher, and S. S. Pekamwar, "Applications of micellar liquid chroma- tography in bioanalysis: a review," Journal of Applied Phar- maceutical Science, vol. 4, no. 1, pp. 128–134, 2014.

[17] R. N. El-Shaheny, M. H. El-Maghrabey, and F. F. Belal, "Micellar liquid chromatography from green analysis per- spective," Open Chemistry, vol. 13, no. 1, pp. 877–892, 2015.

[18] M. M. Mabrouk, S. F. Hammad, F. R. Mansour, and M. M. Amer, "Development and validation of a reversed phase HPLC method for simultaneous determination of antidiabetic drugs alogliptin benzoate and pioglitazone HCl," Der Pharmacia Sinica, vol. 7, no. 2, pp. 32–40, 2016.

[19] K. S. Potdar, M. S. Kalshetti, and R. Y. Patil, "Development and validation of a novel RP-HPLC method for simultaneous estimation of alogliptin benzoate and pioglitazone HCL in pharmaceutical dosage form," International Journal of Chemical and Pharmaceutical Analysis, vol. 4, no. 3, 2017.

[20] Evogliptin, 'First Global Approval', 2015, 75(17), DOI: 10.1007/s40265-015-0496-5