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Development and Validation of RP- HPLC Method for Estimation of Evogliptin in Formulation

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ABSTRACT

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 ACCLAIMED mix mode HILIC-1 (5µ, 150 X 4.6 mm. ID). Ammonium acetate-acetonitrile (30:70 v/v) as mobile phase, at the flow rate of 1.0 mL/min. The detection was performed at the wavelength (λ) of 205 nm, and the retention time of Evogliptin was around 4.8 min. The total run time was 20 min. The calibration plot gave linear relationship over the concentration range of 5–65 µg/ml. The LOD and LOQ were 0.75 and 2.5 µg/ml, respectively. UV detection was monitored at205nm for Evogliptin as the compound exhibit optimum absorption at this selected wavelength. 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.

INTRODUCTION

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. In this research, a new sensitive and rapid RPHPLC method was developed for the estimation of Evogliptin in tablet dosage forms, and this method was validated according to ICH guidelines.[1,2]

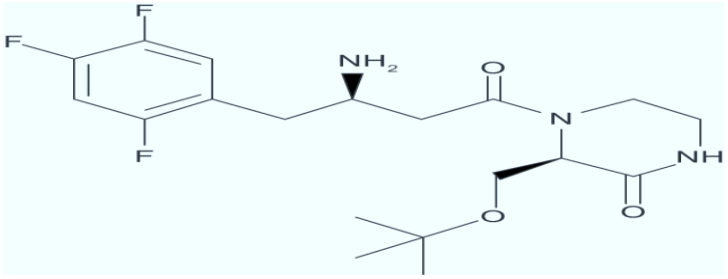

Figure 1 Evogliptin

MATERIALS AND METHODS

Research work was carried out in Acclaimed mix-mode HILIC-1 column (5µ, 150 X 4.6 mm. ID) with isocratic elution technique, consisting 25 mM ammonium acetate-acetonitrile (30:70 v/v).

Column:	ACCLAIMED mix mode HILIC-1; 5µ, 150 X 4.6 mm. ID.
Mobile Phase:	15 ml Ammonium acetate-acetonitrile (30:70 v/v)
Flow rate:	1 ml/min
Elution mode:	Isocratic elution mode
Wavelength selected:	205 nm
Temperature:	Room temperature
Run time:	7 minutes

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. [3,4]

System suitability

The purpose of the system suitability test is to ensure that the complete testing system, including instruments, reagents, columns, analysts etc., is adequate for the intended analysis. The following parameters are usually determined: theoretical plate count, tailing factors, resolution, and reproducibility.[4]

Peak	Ret. Time	Area	Area%	T. Plate	Resolution	Tailing F.	Separation
Evogliptin	4.726	9855047	35.7892	1492.072	3.722	1.15	2.769

Specificity[5]

Specificity is the ability of the analytical method to discriminate between the analyte and the other component(s) in the mixture.

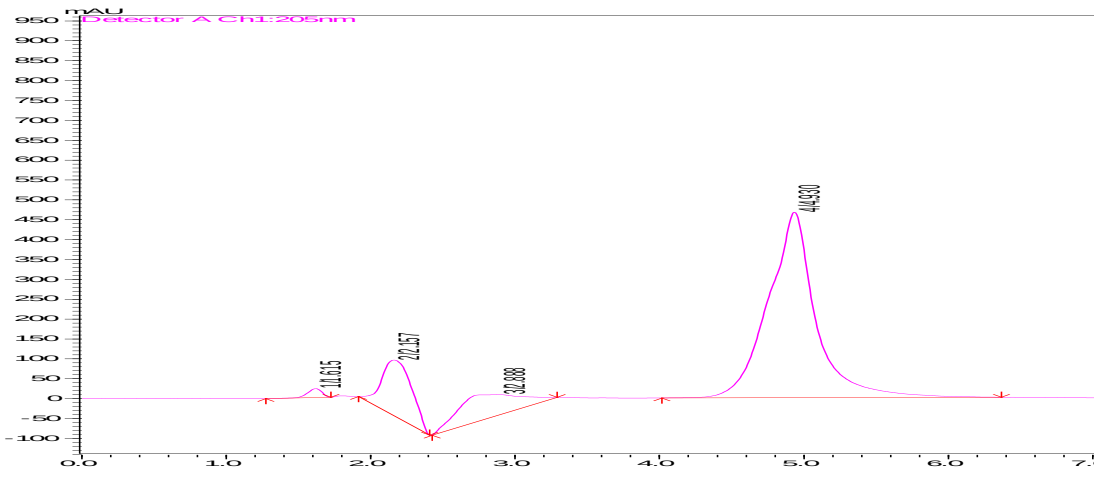

Fig.2 Chromatogram of Evogliptin standard solution

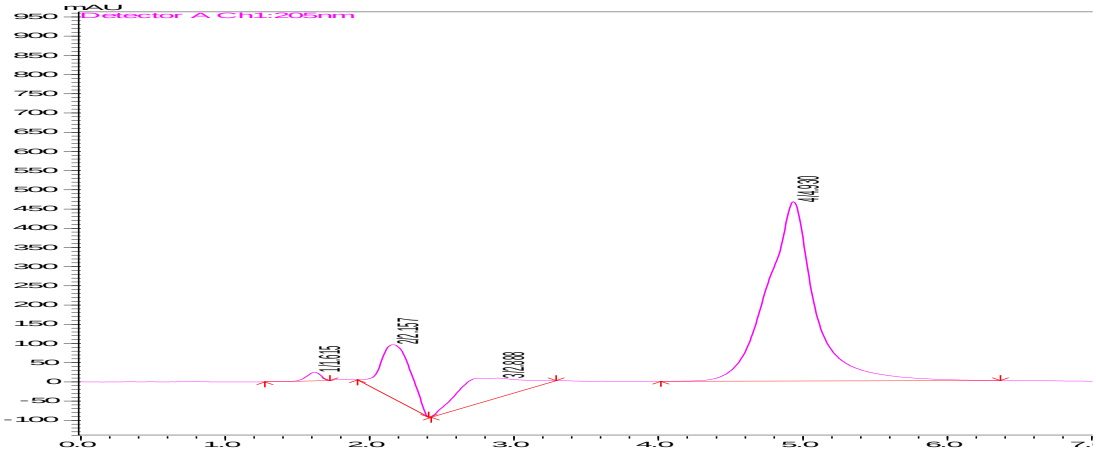

Figure 3: Chromatogram of Evogliptin sample solution

Repeatability [6]

Implementing the procedure, the homologous mixture Evogliptin (300 ug/ml) were tested for 6 injections within the same day. The% RSD was calculated and found it is less than 2%.

Table 1: Repeatability data of Evogliptin

Sr. No.	**Evogliptin**
1	27711844
2	27889184
3	28614552
4	27429374
5	27935553
6	27280776
Mean	**27810213**
STD. DEV.	**479877.87**
RSD (%)	**1.50**

Precision studies [7]

The precision of RP-HPLC method reflects its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions.

Table 2: Precision data of Evogliptin

Sr. No.	Concentration (ppm)	Area
1	300 PPM	27711844
2	300 PPM	27889184
3	300 PPM	28614552
1	300 PPM	27429374
2	300 PPM	27935553
3	300 PPM	27280776
1	300 PPM	28717193
2	300 PPM	27975395
3	300 PPM	27990835
	Mean % RSD	**1.50**

Linearity and range [8]

The linearity of any RP-HPLC Method represents its ability to explicit the results that should proportional to the concentration of studied analytes within a selected range. Therefore, over the tested range of 5-65 µg/ml for Evogliptin.

Table 3: Linearity data of Evogliptin

Sr. No.	Concentration (µg.mL^{-1})	Area	Average (Mean)
1	500	8037947	8037947
2	250	4015541	4015541
3	125	2022212	2022212
4	62.50	1024711	1024711
5	31.25	522355	522355

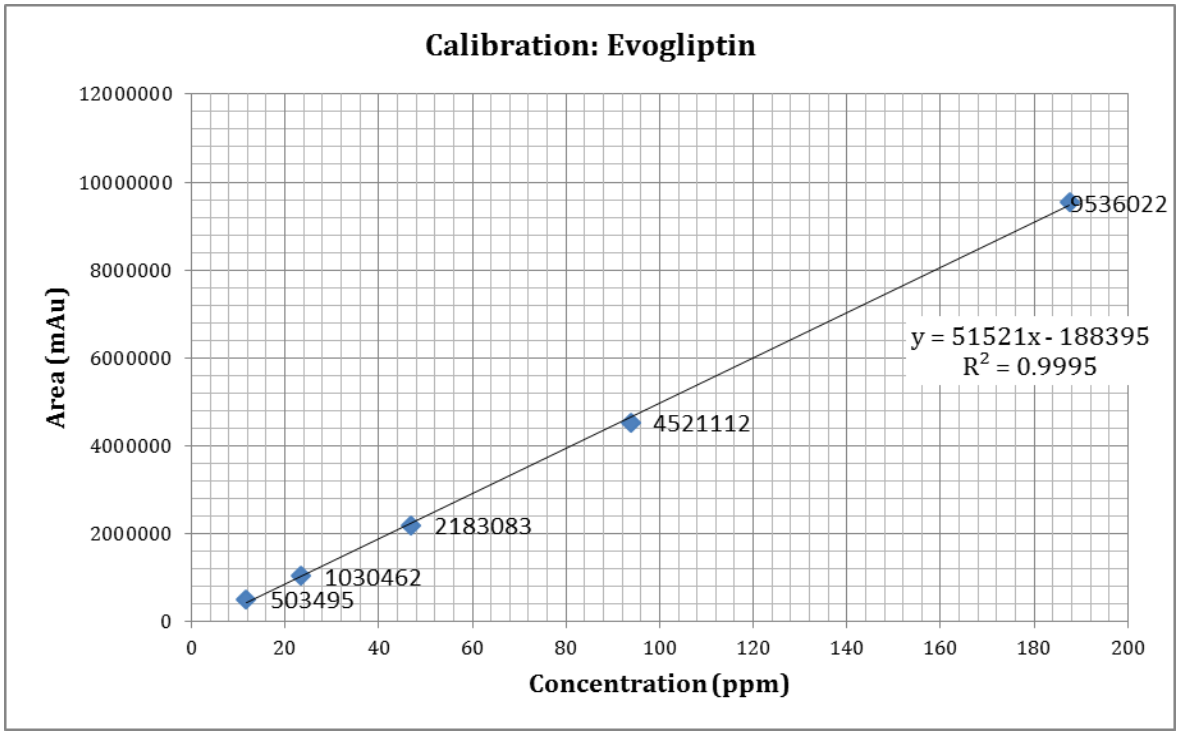

Fig. 4 Calibration curve of Evogliptin

Robustness for the chromatographic method[9,10]

Robustness of RP-HPLC Method represents its ability to remain unaffected by small but deliberate variations in separation parameters to ascertain its reliability during routine analysis. In this method, robustness was established by making deliberate changes in flow rate (1.0 ± 0.2 ml/minutes), and temperature (28°C ± 2°C). Capacity factor (k'), resolution (Rs) and peak tailing (*Tf*) of selected Evogliptin were almost unchanged which clearly signified that the proposed RP-HPLC Method obliged all minimum requirements led by the ICH guidelines.

Table No 4: Robustness data of Evogliptin

Variables	tR(min)	k'	Tf	Rs	N
Flowrate (+0.2 mL.min^{-1})	7.01	4.09	0.86	5.51	2924
Flowrate (-0.2 mL.min^{-1})	10.42	4.23	0.94	5.12	1650
Temperature (+2˚C)	8.39	4.18	0.89	6.13	3069
Temperature (-2˚C)	8.39	4.13	0.89	5.95	2893
Mean±S.D.	8.59	4.45	0.95	5.86	

Limit of quantification (LOQ) and Limit of detection (LOD)[11]

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the regression equation. As observed, the LOD and LOQ of Evogliptin were 0.75 and 2.5 µg/ml.

Accuracy [12]

Percentage recoveries of three different concentrations; 80%, 100% and 120% (injected thrice) to determine the Evogliptin was calculated to determine the drug recovery (%) and variation in RSD% and results obtained were reported.

Table No 5: Accuracy data of Evogliptin

Conc. (%)	S. N.	Drug. added	Amt. rec.	% recovery	Peak Area (500 ppm)	Mean Rec %	% RSD
80%	1	400	400.1	44.46	31338357	100.09	0.10
	2	400	400.12	44.46	31339924		
	3	400	400.81	44.53	31393969		
100%	1	500	500.25	50.03	39182737	100.13	0.07
	2	500	500.88	50.09	39232083		
	3	500	500.77	50.09	39223467		
120%	1	600	600.17	54.56	47009102	100.07	0.02
	2	600	600.24	54.57	47014585		
	3	600	600.43	54.58	47029467		

CONCLUSION

The developed HPLC method is fast and 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 4.8 min. The method was validated in accordance with ICH guidelines.

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