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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LINAGLIPTIN IN TABLET DOSAGE FORM

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ABSTRACT

A specific, accurate, precise, linear and cost effective HPLC method was developed for estimation of Linagliptin. Separation of the Linagliptin was achieved on a Column C18 (250 mm x 4.6 mm, 5 µm) Xterra @ RP 18 is suitable, using a mobile phase consisting of a mixture of 50 volumes of buffer solution and 50 volumes of methanol (v/v). The flow rate was 1mL/min and detection wavelength was 293 nm. The linearity was found in the concentration of 0.0075, 0.0125, 0.0200, 0.0250, 0.0375, mg/mL with a correlation coefficient (R2) of 0.999. The retention time of Linagliptin was 5.58 minutes. The predicted method was validated as per the International Conference for Harmonization guidelines for the parameters: Linearity, Accuracy, Robustness, Precision, Specificity etc. This method can be used for routine analysis of quality control of Linagliptin in tablet dosage form.

KEYWORDS: Linagliptin Tablet, HPLC, Method Validation, potency, Column.

INTRODUCTION

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor used to manage hyperglycemia in patients with type 2 diabetes mellitus.[2] Linagliptin is a selective, orally administered, xanthine-based DPP-4 inhibitor. GLP-1 lowers blood glucose levels by augmenting the glucose-stimulated insulin release.[1,3] Moreover, GLP-1 inhibits glucagon secretion, slows gastric emptying, and induces satiety. The plasma half-life of GLP-1 is limited to a few minutes because of rapid proteolytic degradation by the enzyme DPP-4. Inhibition of DPP-4 prolongs the half-life of active GLP-1 and thereby increases plasma insulin levels and lowers plasma glucose levels. Since GLP-1 activity ceases when the glucose concentration falls below 55 mg/dL, prolongation of the half-life of GLP-1 by DPP-4 inhibitors bears little risk of hypoglycaemia.[1] Linagliptin is a selective, competitive, reversible inhibitor of human DPP-4 with a 50% Inhibitor Concentration (IC50) of 1 nM. The therapeutic dose of linagliptin will be 5 mg. Linagliptin is predominantly excreted unchanged via the faeces.[5] Renal excretion is a minor pathway of elimination of linagliptin at therapeutic doses. Thus, linagliptin is especially suited for the treatment of patients with renal impairment without the need for dose adjustment. The claim indication for linagliptin is the treatment of type 2 diabetes mellitus to improve glycaemic control in adults.[1] Linagliptin, sold under the brand name Tradjenta among others, is a medication used to treat type 2 diabetes mellitus.[7] It is generally less preferred than metformin and sulfonylureas as an initial treatment.[7] It is used together with exercise and diet.[10] It is not recommended in type 1 diabetes.[10] It is taken by mouth.[10] Common side effects include inflammation of the nose and throat. Serious side effects may include angioedema, pancreatitis, joint pain.[8,10]

Use in pregnancy and breastfeeding is not recommended.[8] Linagliptin is a dipeptidyl peptidase-4 inhibitor. It works by increasing the production of insulin and decreasing the production of glucagon by the pancreas.[10] Linagliptin was approved for medical use in the United States in 2011.[10] In 2018, it was the 177th most commonly prescribed medication in the United States, with more than 3 million prescriptions.[9] As of August 2021, linagliptin is available as a generic medicine in the US.

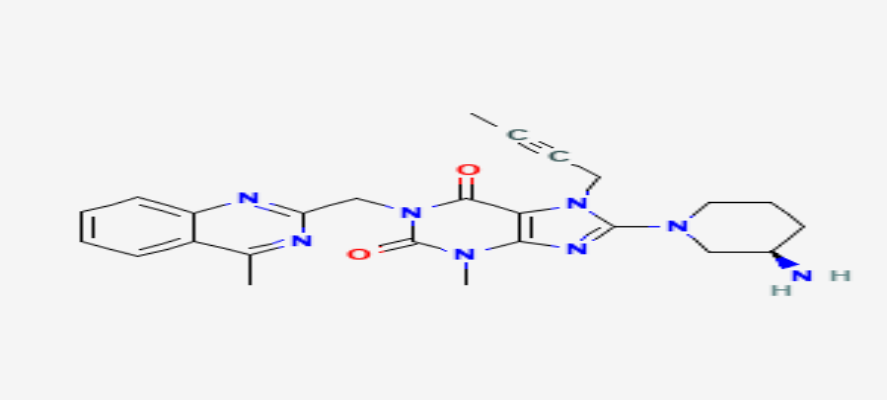

Fig. 1: Chemical structure of Linagliptin.

MATERIALS AND METHODS

Chemicals and Solvents: Linagliptin Tablet was obtained from Modern Pharmacy, Linagliptin was obtained from Sonali Scientific Store; Methanol (HPLC grade) was Sigma Aldrich Germany; Phosphoric acid (AR grade) and Potassium Dihydrogen Phosphate were procured from Merck Germany; HPLC grade water was taken from Nipa pharma Ltd., Bangladesh.

Preparation of Buffer: Dissolve 6.8 g of Potassium Dihydrogen Phosphate to 1000 ml of water.

Preparation of mobile phase and diluent: Mixed 50 volumes of buffer solution with 50 volumes of methanol. The mixture was degassed in a sonicator for about 15 min and it was then filtered through 0.45µm membrane filter under vacuum. The filtrate was stored at room temperature to use as mobile phase as well as diluent.

Preparation of Standard solution: Weigh accurately about 25.0 mg of Linagliptin working standard into a 50 ml volumetric flask. Add about 20 ml of diluent and sonicate to dissolve. Make up the volume with diluent. Pipette 5 ml into a 100 ml volumetric flask and volume with diluent. Before injection filter through 0.22 µ membrane filter and collect the sample in a clean and dry vial. Concentration of Linagliptin: 0.025 mg/ml.

Preparation of Sample Solution: Weigh and finely powder not fewer than 25 Tablets. Weigh powdered sample equivalent to 2.5 mg of Linagliptin into a 100 ml volumetric flask. Add about 20 ml of diluent and sonicate for 20 minutes. Make up the volume with diluent. Filter the solution through Whatman paper #1. Before injection filter through 0.22 µ membrane filter and collect the sample in a clean and dry vial. Concentration of Linagliptin: 0.025 mg/ml.

System Suitability: Chromatograph the standard solution onto the HPLC system.

Acceptance criteria: The tailing factor of the Linagliptin peak is not more than 2.0 and the percent relative standard deviation for six replicate injections is not more than 2.0% for Linagliptin peak. Separately inject equal volumes of the working standard solution and the sample solution onto the HPLC system and record the chromatograph.

Chromatographic Analysis: The analysis of Linagliptin was performed out by HPLC which contained PDA Detector equipped with temperature controlled auto sampler, a quaternary low pressure gradient pump and column oven. Chromatographic analysis was performed using Column C18 (4.6 mm x 250 mm internal diameter and 5µm particle size). Isocratic elution with flow rate 1 mL per minute was selected. The detector was PDA and wavelength was set at 293 nm, the injection volume was 20µL with a run time of 10 minute, column oven temperature 30°C. The column was stabled for 60 minute with the mobile phase flowing through the system. The mobile phase was prepared and degassed then sonicated for 15 minute before use. The column and HPLC system was kept at 30°C temperature.

Chromatogram with system suitability solution and working standard: System suitability solution and working standard was prepared according to above method. By this same way accurately weighed 25.00 mg and transferred into 100 mL volumetric flask for linearity stock solution and from this stock solution transferred 3mL, 5mL, 8mL, 10mL, 15mL into 100mL volumetric flask and volume upto the mark with diluent for the concentration of 30%, 50%, 80%, 100%, 150% respectively. Each of the solution (20 µL) was injected by auto injector into the column at a flow rate 1mL per minute of mobile phase and the corresponding

chromatogram was (Fig. 2) recorded. It is explicit from the **Fig. 2** that the chromatogram was quite good and it could be used for qualitative and quantitative analysis of Linagliptin. Retention time of the chromatogram was ascertained from the replicates and it was found as 5.58 min.

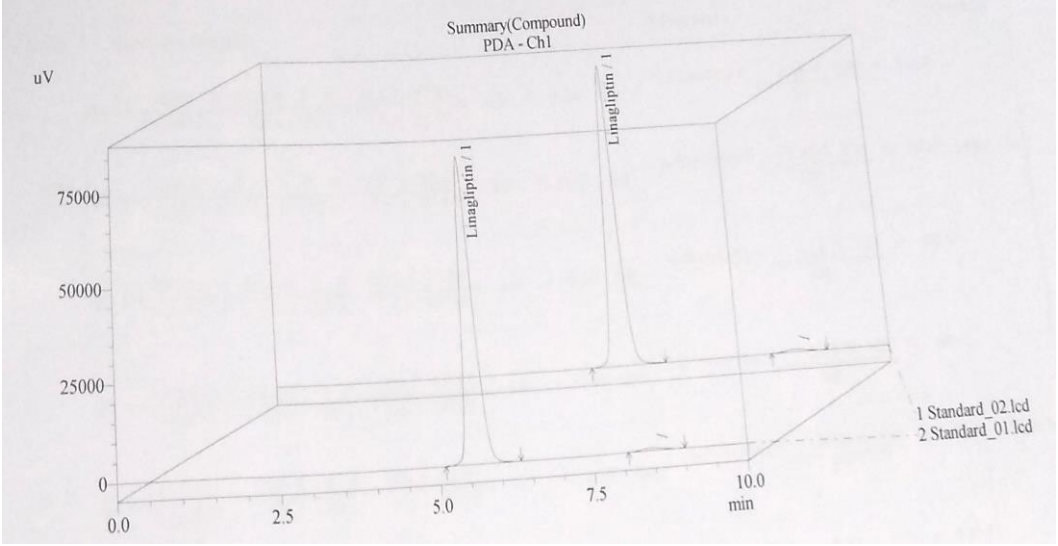

Fig. 2: A Representative chromatogram of Linagliptin (RT= 5.58 min) from standard solution under optimized conditions.

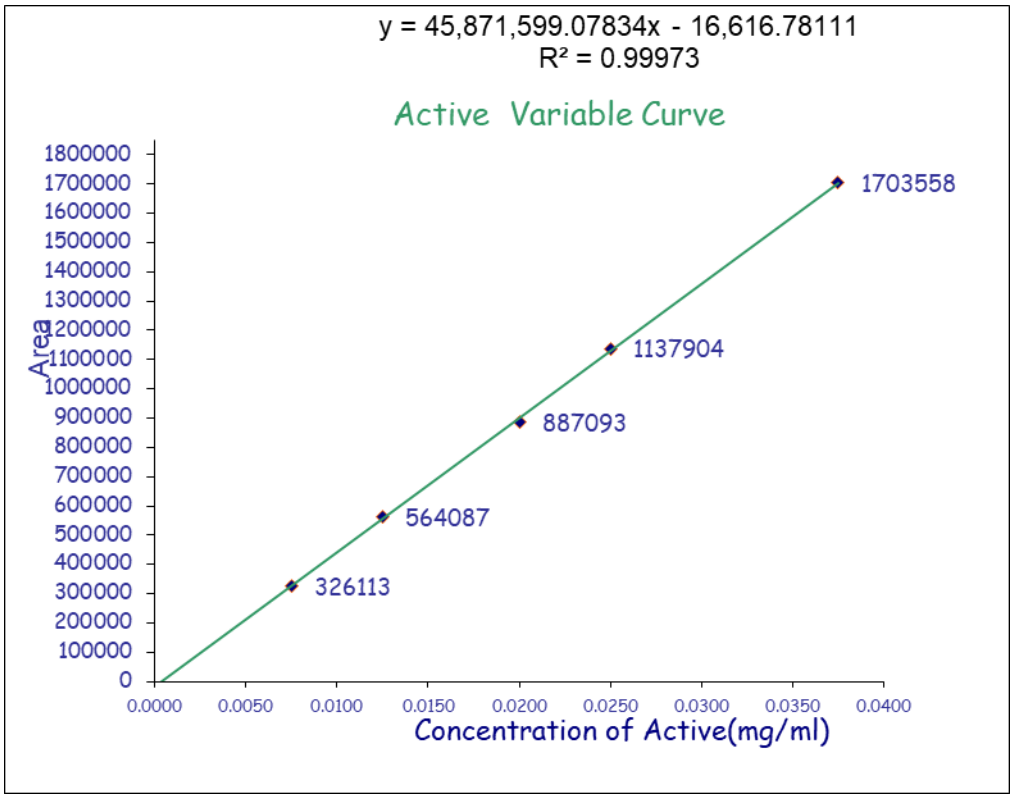

Fig. 3: Calibration curve for Linagliptin only (Working standard).

Calibration Plot: The calibration graph was constructed by plotting concentrations of the drug against area (µV) of the chromatogram (**Fig. 3**) at RT=5.58 min for Linagliptin and it was found linear. The regression equation for the curve was found as y=45,871,599.07834x – 16616.78111 with correlation coefficient (R2) 0.99973. It was used to estimate the amount of Linagliptin.

Validation of the proposed method: The System Suitability, Specificity, Linearity, Accuracy, Precision, Robustness and Range parameters of method validation were conducted systematically to validate the raised HPLC method as per ICH guidelines.

a) ***System suitability:*** In validating the method, it was important to check system suitability which was done by

the relative standard deviation (RSD) calculation of the peak area of six replicates of standard. Results and relevant discussion are presented in the results and discussion section.

b) ***Linearity:*** Linearity of the analytical method was performed by three studies regression analysis of Linagliptin without excipients, regression analysis of Linagliptin with different concentration of excipients, regression analysis of Linagliptin with fixed concentration of excipients and.

c) ***Limit of Quantification (LOQ*****):** LOQ was destined based on STDEV of response and slope method. Linearity was conducted in the specified range of the reference sample solution concentration. Linearity graph of concentration in mg/mL (X-axis) versus peak response (Y-axis) was plotted. LOQ was calculated using correlation coefficient, slope of regression line and standard deviation of regression line.

d) ***Limit of Detection (LOD):*** LOD was destined based on STDEV of response and slope method. Linearity was conducted in a specified range of reference sample solution concentration. Linearity graph of concentration in mg/mL (X-axis) versus peak response (Y-axis) was plotted. LOD was calculated by correlation coefficient, slope of regression line and standard deviation of regression line.

e) ***Range:*** The specified range is normally explained from linearity studies. Range has been calculated from the lower and upper concentration of analyte in the sample for which it has been demonstrated that the analytical procedure provides an acceptable degree of linearity, precision and accuracy.

f) ***Specificity:*** Specificity of the procedure was performed from assessing unequivocally the analyte in the presence of component i.e. excipients that are expected to be present in a dosage form. Regression equation (**Fig. 3**) was used to assess the content of analyte in the test sample.

g) ***Placebo effect:*** Placebo effect was studied by running the blank, placebo and active solution in HPLC.

h) ***Accuracy:*** In case of assay of the drug in the formulated product, accuracy of the method was determined first. To do so a blank matrix (Placebo); the excipients (all ingredients as per formulation of Linagliptin tablet) simulated Linagliptin Sample (excipients + Active Pharmaceutical ingredient) (50%, 100% and 150%) were run separately in three replicates in the HPLC.

i) ***Precision:*** Precision of a method for validation purpose was performed from repeatability, intermediate precision and reproducibility. Repeatability precision was carried out by Six (6) determinations at the fixed level of test sample concentration in homogeneous solution. Intermediate precision was determined from the HPLC measurements in different days using different equipment within the different laboratory by different analysts. Reproducibility of the stated HPLC method was verified involving analysts, other than those involved in repeatability and intermediate precision experiments, where six (6) determinations were executed immediately one after the other in a different laboratory.

j) ***Robustness:*** Robustness of the method was performed from the stability study of Linagliptin sample solution at 30ºC temperature (25ºC - 35ºC) at different time (0hr, 24hr) with time interval.

RESULT AND DISCUSSIONS

Linagliptin is a diabetes drug that works by increasing levels of natural substances called incretins. Incretins help to control blood sugar by increasing insulin release, especially after a meal. They also decrease the amount of sugar your liver makes. People are working to find a suitable method for Linagliptin quantification. In this paper cost effective, a simple and new method has been presented. This HPLC method was validated subsequently to assay the tablet dosage form of Linagliptin.

Table 1: Optimized chromatographic conditions Linagliptin.

Test	Condition
Mobile Phase	Methanol : Buffer Solution (50:50V/V), Isocratic
Diluent	Mobile phase
Column	Column 4.6 mm x 250 mm, 5 µm (Xterra RP18 Preferable)
Column oven	30ºC
Flow rate	1.0 mL/min
Detector	PDA
Injection volume	20 µL
Run time	10

Table 2: Results of specificity.

Sample Name	Peak purity Index	Single Point Threshold	Remarks
Standard solution	1.000000	0.999856	Impurity Not Detected so peak is pure
Sample solution	0.999999	0.999867	Impurity Not Detected so peak is pure
	Observation (No interfering peak)		
Blank solution	No interfering peak		
Placebo Solution	No interfering peak		

Table 3: Percent recovery of Linagliptin from simulated tablet contents.

Sample No	Spiked level (%)	Amount added (mg)	Amount found (mg)	% of Recovery	Mean (%)
1	50	2.3	2.35	102.0	100.4
2	50	2.5	2.52	100.7	
3	50	2.4	2.36	98.4	
4	100	5.6	5.53	98.8	99.3
5	100	5.1	5.03	98.2	
6	100	5.0	5.03	100.6	
7	150	7.2	7.14	99.1	98.9
8	150	7.3	7.15	98.0	
9	150	7.4	7.37	99.6	
Grand average (%)					
% RSD of 09 determination					

Table 4: Relative standard deviation of six determinations of Linagliptin contents (method Precision) in simulated tablet amount.

No. of Sample	% of Assay	Average Result (%)	RSD (%)	ICH limit of RSD (%)	Remarks
Sample-1	99.97	99.13	0.742	NMT 2.0 %	Method Precision of Linagliptin measurements is complied
Sample-2	100.12				
Sample-3	98.84				
Sample-4	98.94				
Sample-5	98.44				
Sample-6	98.48				

Table 5: Relative standard deviation of six determinations of Linagliptin contents (Intermediate Precision) in simulated tablet amount.

No. of Sample	% of Assay	Average Result (%)	RSD (%)	ICH limit of RSD (%)	Remarks
Sample-1	101.78	99.99	1.490	NMT 2.0 %	Intermediate Precision of Linagliptin measurements is complied
Sample-2	101.97				
Sample-3	98.69				
Sample-4	98.74				
Sample-5	99.45				
Sample-6	99.33				

System suitability: Standard solution was injected onto the HPLC system and chromatograms were recorded. The results are summarized in table-6.

Table 6: Data for System Suitability of Linagliptin.

Injection Number	Retention Time (In minutes)	Peak Area	Theoretical Plates	Tailing Factor
01	5.568	1132153	14988.794	1.178
02	5.565	1132938	14983.349	1.176
03	5.567	1132928	15035.286	1.175
04	5.571	1134225	15001.481	1.175

05	5.573	1132539	14965.635	1.175
06	5.571	1133149	15019.778	1.175
Average	5.569	1132989	14999.054	1.176
% RSD	0.056	0.062	0.169	0.090

Table 7: Data for Linearity.

Percent Concentration	Name Of Sample	Concentration in mg/ml	Peak area	Statistical data
30	Linagliptin	0.0075	326113	Corr. Coefficient :0.99973 y-intercept :16616.78111 Slope : 45,871,599.07834
50	Linagliptin	0.0125	564087	
80	Linagliptin	0.0200	887093	
100	Linagliptin	0.0250	1137904	
150	Linagliptin	0.0375	1703558	

Linearity of analytical method was determined by performing studies regression analysis of linagliptin with different concentration. From the plot of the results (**Fig. 3**) the linear regression equation was obtained as: y=45,871,599.07834x – 16616.78111 with different concentration of Linagliptin. And the response was linearly dependant on the concentration of Linagliptin. The linearity of the regression line is also evident from correlation coefficient R2 = 0.99973 **(Table 7)**. It is important to mention here that the proposed HPLC method for Linagliptin estimation was found linear in the range of 0.0075 to 0.0375 mg/mL (**Fig. 3**). LOD and LOQ were determined to be 0.00072 and 0.00219 respectively.

The specificity of the method was reviewed by checking a standard solution of Linagliptin, its tablet, blank sample and placebo (excipients) materials. Sample of standard and tablet showed peak Linagliptin at retention time 5.58 min when run separately in HPLC while blank and placebo did not show any peak at that RT value. These results indicated that Linagliptin could be detected by the present method and it was also able to separate Linagliptin from its excipients quantitatively (**Table 2**). Percent recovery of Linagliptin in the presence and in the absence of excipients was found within the limit of ICH guideline (Table 2)[6], and thus it means that the developed method is selective for quantification of Linagliptin.

Accuracy was assessed using nine determinations over three different concentration levels covering the predetermined range (0.0125-0.0375mg/mL) of analysis. And there were three replicates of each concentration (**Table 3**). From guideline. Thus, it was indicated that the proposed method was accurate for the analysis of the linagliptin.

Repeatability precision was carried out by six independent determinations of a fixed test concentration (0.025mg/mL) of a homogeneous solution (**Table 4**) of Linagliptin. Values of RSD were calculated from these determinations and the obtained RSD value was reviewed to see whether it was within the limit (NMT 2%) of ICH guideline.[6] In the present case, RSD was found as 0.742% (**Table 4**) which was within the limit (NMT 2%) of ICH guideline[6] and hence the repeatability was complied for the present method of analysis of Levofloxacin. Similarly, it was found that the intermediate precision and system suitability criteria were as per ICH guideline[6] (**Table 5**).

These determinations, it was found that the values of recovery for each estimation were within the range (98%-102%) of ICH percentage recovery.[6] The sample solution was allowed to stand at room temperature (20-25ºC) for different time intervals (0, 24 hrs) to see the stability of Linagliptin. The % assay result difference from initial value obtained 1.75, against the ICH limit (NMT 2) which indicated that the working sample solution was stable for at least 24 hours.[6] In the light of validation parameters results, it can be told that the developed method is valid for the estimation of Linagliptin from the eye drop formulation.

CONCLUSION

A HPLC method was developed and validated for the analysis of Linagliptin in tablet dosage form. The developed method is less costly than the methods reported so far.

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