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RP-HPLC method development and validation of Linagliptin in bulk drug and pharmaceutical dosage form

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ABSTRACT

A reversed phase high-performance liquid chromatographic method was developed and validated for the quantitative determination of Linagliptin. Chromatography was carried out by gradient technique on a reversed-phase C_{18} (4.6 x 100 mm, 5 µm, Make: Phenomenex) with mobile phase mixture of phosphate buffer: methanol (50:50 v/v) used as mobile phase and the pH was adjusted to 3 by using with O-phosphoric acid, at a flow rate of 0.8 ml/min. The chromatogram was recorded at isosbestic point of 238nm. The different analytical performance parameters such as linearity, precision, accuracy, ruggedness, robustness, limit of detection (LOD) and limit of quantification (LOQ) were determined. The linearity of the calibration curve of the analyte in the desired concentration range is good (r>0.9). The recovery of the method was between 98%-102%. Hence the proposed method is highly sensitive, precise and accurate and it can be successfully applied for the reliable quantification of API content in the commercial formulations of Linagliptin.

Key words: Linagliptin, Estimation, RP-HPLC, Validation

INTRODUCTION

Linagliptin, 5 mg tablets are indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus [1]. Linagliptin is described chemically as 1H-Purine-2, 6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-yl)-3, 7-dihydro-3-methyl-1-[(4-methyl-2quinazolinyl) methyl]. The empirical formula is $C_{25}H_{28}N_8O_2$ and the molecular weight is 472.54 g/mol. Its structural formula is shown in fig.1 [2].

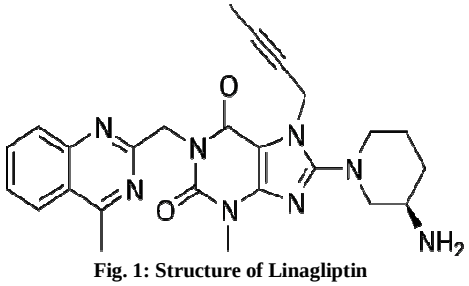

Fig. 1: Structure of Linagliptin

Linagliptin is a competitive and reversible dipeptidyl peptidase (DPP)-4 enzyme inhibitor that slows the breakdown of insulinotropic hormone glucagon-like peptide (GLP)-1 for better glycemic control in diabetes patients [3]. Some literatures revealed the high performance liquid chromatography (HPLC) method for linagliptin [4-7]. In the present study the authors report a rapid, sensitive, accurate and precise RP-HPLC method for the estimation of linagliptin in bulk drug and pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and materials: Linagliptin was obtained as a gift sample from Mylan laboratories. HPLC grade water, acetonitrile and methanol were obtained from Merck. Potassium dihydrogen phosphate and ortho phosphoric acid of HPLC grade were obtained from Fisher.

Instrumentation: Quantitative HPLC was performed on waters liquid chromatograph, with a UV detector equipped with automatic injector with injection volume 20 µl, and 515 pump. A symmetry C_{18} column (4.6 x 100 mm, 5 µm, Make: Phenomenex) was used.

Method development and optimization

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH and flow rate were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of linagliptin in bulk drug and pharmaceutical dosage forms. Mobile phase tried for this purpose were buffer pH 6: acetonitrile (60:40), buffer pH 4: methanol (35: 65), buffer pH 3: methanol (50: 50). The condition that gave best resolution and symmetry was selected. HPLC conditions are given in Table-1.

Table-1 HPLC conditions

PARAMETERS	CONDITIONS
Column (stationary phase)	Symmetry C_{18} (4.6 x 100 mm, 5 µm, Make: Phenomenex) or equivalent
Mobile phase	Phosphate buffer (pH 3) : methanol (50%: 50%)
Flow rate	0.8 ml/ min
Run time	5 (min)
Column temperature	Ambient
Volume of injection loop	20 µl
Detection wavelength	238 (nm)
Drug Rt	2.25 min

Preparation of buffer (pH 3):

7gms of potassium dihydrogen phosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH of the solution was then adjusted to 3 with orthophosphoric acid.

Preparation of mobile phase:

500 ml of the phosphate buffer was mixed with 500 ml of methanol. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Diluent preparation:

Mobile phase was used as Diluent.

Preparation of standard solution:

Standard stock solution of 1000 µg/ml was prepared by dissolving 10 mg of linagliptin in 10 ml of diluent. From this stock solution, 0.3 ml was pippetted out and the volume was made upto 10 ml with the diluent to prepare working standard solution of 30 µg/ml.

Preparation of sample solution:

10 mg equivalent of linagliptin tablet powder was accurately weighed and transferred into a 10 ml volumetric flask. To it, about 7 ml of diluent was added and sonicated to dissolve. The volume was made upto the mark and the solution was filtered through 0.45 µ filter under vacuum. From this stock solution, 0.3 ml was pippetted out and the volume was made upto 10 ml with the diluent to prepare sample solution of 30 µg/ml

Assay:
Inject 20 µl of the standard and sample solution into the chromatographic system and measure the areas for the linagliptin and calculate the % assay by using the formula. The standard and sample chromatograms were shown in fig. 2.

Formula:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.wt}{\text{Label claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim of drug mg/ml**.**

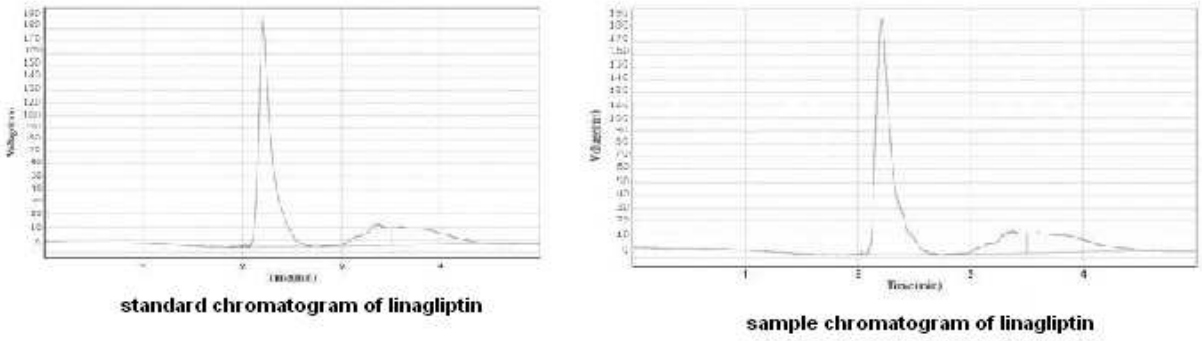

Fig. 2: Standard and sample chromatograms of linagliptin

Method validation:
The method was validated for the following parameters such as linearity, precision, accuracy, limits of detection and quantitation, ruggedness and robustness.
System Suitability:
System suitability was daily performed during entire validation of this method. The results of system suitability were presented in Table 2.

Table 2: System Suitability Parameters

S. No	Parameter	Linagliptin
1	Retention time	2.257
2	Theoretical plates	2658.817
3	Tailing factor	1.287
4	Area	1522658

Accuracy:
The accuracy of an analytical method expresses the closeness of agreement between the value, which is accepted reference value, and the value found. Accuracy studies were done by the standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet. The active ingredients were spiked in previously analyzed tablet powder sample at different concentration levels viz. 50%, 100%, and 150% each of the labeled claim and injected in developed chromatographic conditions in triplicate. The percentage recoveries were then calculated. The recovery data for accuracy studies were shown in Table 3. The accuracy chromatograms were shown in fig 3, 4 and 5.

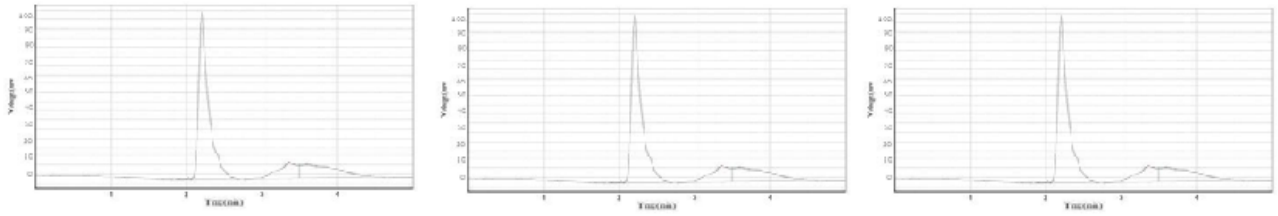

Fig 3: Accuracy chromatograms for 50% solution

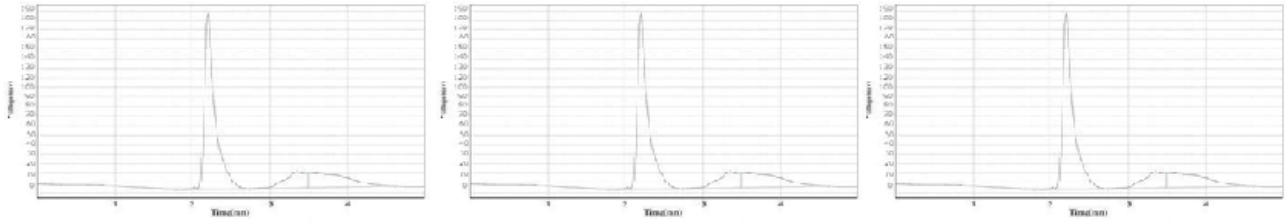

Fig. 4: Accuracy chromatograms for 100% solution

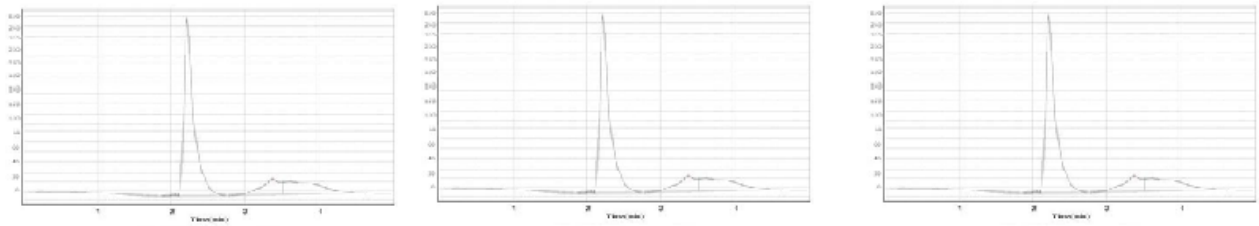

Fig. 5: Accuracy chromatograms for 150% solution

Table 3: Accuracy Result of Linagliptin

% Concentration* (at specification Level)	Area	Amount Added (mcg)	Amount Found (mcg)	% Recovery	Mean Recovery
50 %	2248660	15	14.30397	98.66667	99.7926
100 %	3087025	30	30.82177	101.6667	
150 %	3880773	45	45.47538	99.0444	

Precision:

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\%CV = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

The standard solution was injected for five times and the area was measured for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. Results were reported in Table 4. Chromatograms were reported in fig 6.

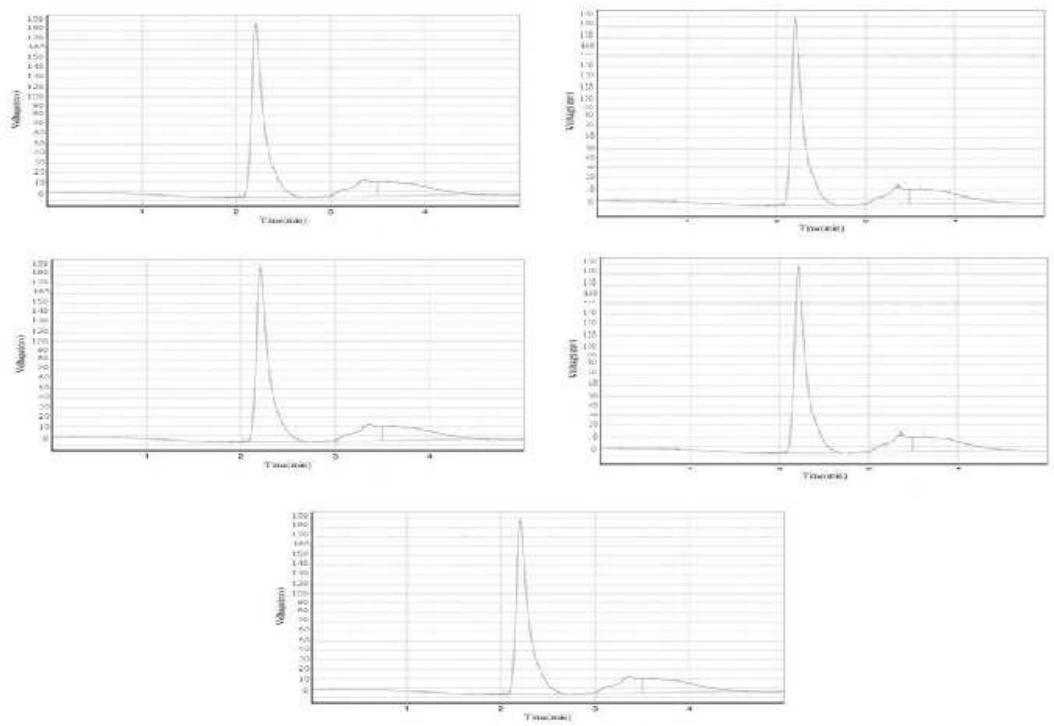

Fig. 6: Precision chromatograms

Table 4: Result of System Precision

Injection	Peak area
Injection 1	1543434
Injection 2	1531289
Injection 3	1495674
Injection 4	1517561
Injection 5	1512189
Average	1520029
Standard deviation	18270.91
% RSD	1.20201

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. Results were reported in Table 5.

Table 5: Result of intermediate system precision

Injection	Peak area
Injection 1	1537347
Injection 2	1526781
Injection 3	1509433
Injection 4	1501556
Injection 5	1563978
Average	1527819
Standard deviation	24632.92
% RSD	1.61229

Linearity:

Aliquots of standard linagliptin stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of linagliptin were in the range of 10-50 µg/ml. Each of these drug solutions (20 µL) was injected into the column, and the peak areas and retention times were recorded.

Evaluation was performed with PDA detector at 215 nm and a Calibration graph was obtained by plotting peak area versus concentration of linagliptin (Fig 7). The linearity Chromatograms were presented in Fig 8. Results were reported in table 6.

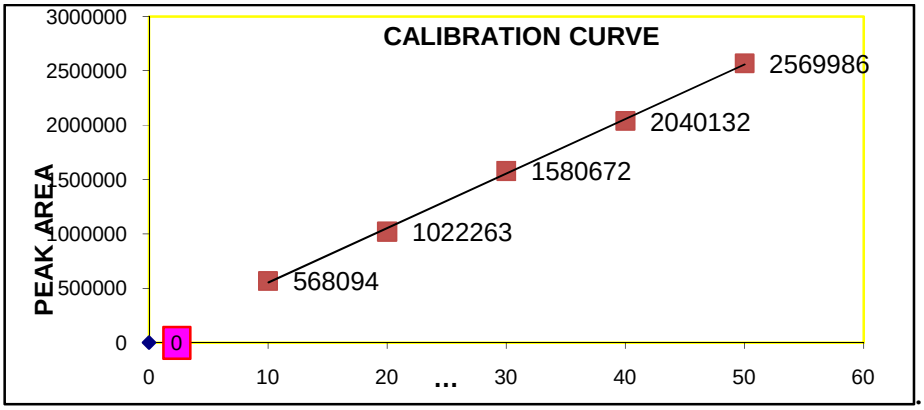

Fig. 7: Calibration Curve for Linagliptin

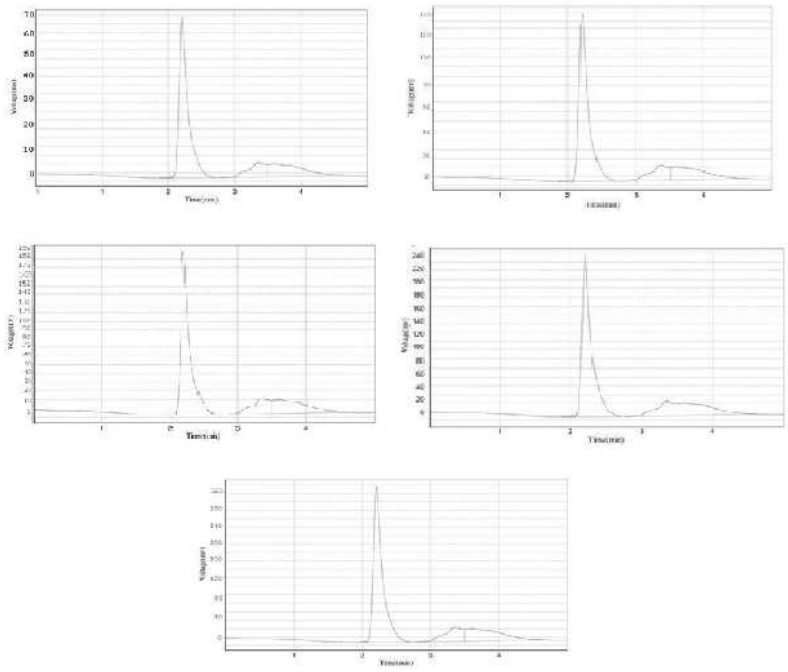

Fig 8: Linearity chromatograms of linagliptin

Table 6: Linearity result of Linagliptin

S.No	Linearity Level	Concentration	Area
1	I	10 µg/ml	568094
2	II	20 µg/ml	1022263
3	III	30 µg/ml	1580672
4	IV	40 µg/ml	2040132
5	V	50 µg/ml	2569986
Correlation Coefficient			0.999546

Limit of Detection [LOD] and Limit of Quantification [LOQ]:

The LOD and LOQ were determined for linagliptin, based on the standard deviation (SD) of the response and slope (S) of the regression line as per ICH guideline according to the formulae given below.

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

Method robustness:

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 7 and 8. The chromatograms for flow rate variation and mobile phase variation were shown in fig 9 and 10 respectively.

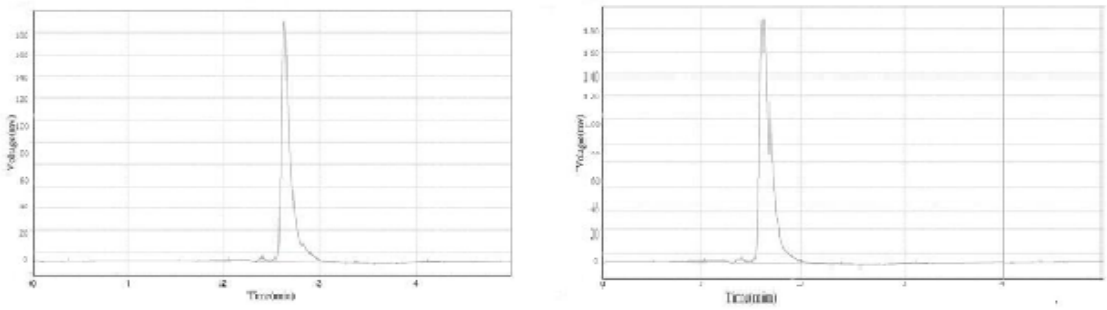

Fig.9: Flow rate variation chromatograms of linagliptin

Table 7: Flow rate variation result of Linagliptin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.7	2790.621	1.277
2	*0.8	2627.451	1.287
3	0.9	2563.609	1.294

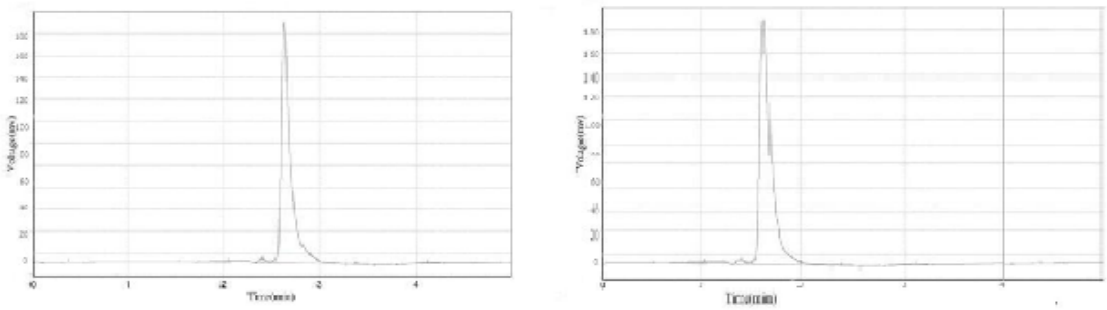

Fig 10: Mobile phase variation chromatograms of linagliptin

Table 8: Mobile phase variation result of Linagliptin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2744.850	1.104
2	*Actual	2627.451	1.287
3	10% more	2538.547	1.388

RESULTS AND DISCUSSION

To optimize the mobile phase, various proportions of buffers with methanol were tested. Mobile phase composition was changed and the method development was started by symmetry C_{18} (4.6 x 100 mm, 5 µm, Make: Phenomenex) column and with a flow rate of 0.8 ml/min, and detection wavelength of 238 nm. Injection volume was 20 µL, and run time was for 5 min. The mobile phase consists of phosphate buffer (pH 3) and methanol. The retention time of linagliptin was found to be 2.25 minutes. The assay result was found to be 100.08%. Quantitative linearity was observed over the concentration range of 10-50 µg/ml. the correlation co efficient was found to be 0.999. The numbers of theoretical plates obtained were 2858.817, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 1.65 and 4.98 µg/ml, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

CONCLUSION

A simple and rapid RP-HPLC method was developed for the estimation of Linagliptin in API and pharmaceutical dosage forms.

Method was developed on Symmetry C_{18} (4.6 x 100 mm, 5µm, Make: Phenomenax).The mobile phase was phosphate buffer (pH 3): Methanol 50: 50 % ratio with a flow rate of 0.8 ml/min. The chromatograms were recorded at 238 nm wave length. The retention time for Linagliptin was found to be 2.257.

The developed method was validated in terms of accuracy, precision, linearity and robustness and results were validated statistically.

Therefore it was concluded that the proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical and can be used for the estimation of Linagliptin in API as well as in pharmaceutical dosage forms.

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