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QUALITY BY DESIGN BASED HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION OF LINAGLIPTIN IN TABLET DOSAGE FORM

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ABSTRACT

The current work describes QbD based simple and precise reversed-phase HPLC method development for routine analysis of Linagliptin in bulk drug and pharmaceutical formulations. Chromatographic separation was achieved on a ACE C_{18} column using isocratic elution with mobile phase containing mixture of phosphate buffer pH 7.2 adjusted with OPA 1% solution and methanol in the ratio (70:30v/v), with flow rate 1.0 mL/min and UV detection at 292 nm. The optimization of chromatographic method was carried by Box-Behnken design. The experimental design for the Assay method development utilizes three factors such as Mobile Phase (X1), Flow Rate (X2), and pH (X3) while Assymetry (Y1), Theoretical Plates (Y2), and Retention time (Y3) were used as responses. The developed experimental design was statistically analysed using ANOVA, counter plots and response surface plots. The method was optimized through system suitability test, linearity and assay of Linagliptin. The method was validated as per ICH guidelines for Accuracy, Precision, Ruggedness, LOD and LOQ which showed that proposed method was simple, sensitive, and highly robust for routine analysis of the Linagliptin.

KEYWORDS: Linagliptin, Box-Behnken design, ANOVA, HPLC.

INTODUCTION

Linagliptin (LIN)[1], chemically 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione (figure.1) is a Type 2 Anti diabetic drug which acts by blocking the action of DPP-4, an enzyme that destroys the hormone GLP-1, which helps the body to provide more insulin when it is needed. It stimulates the release of insulin and inhibits the release of glucagon, resulting in the decrease levels of circulating glucose. Linagliptin binds tightly but not irreversibly to the DPP-4 enzyme.

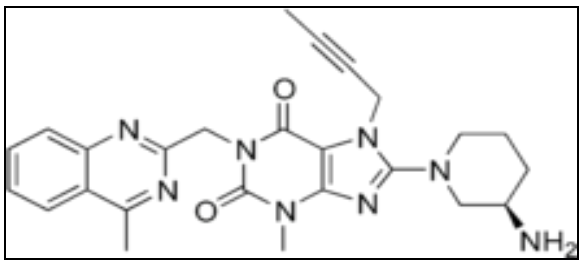

Figure 1.Chemical structure of linagliptin

Marketed formulation of LIN is available only in tablet form. Prior work describes the analytical methods used for determination of LIN in various biological samples such as human plasma, urine and serum by LC-MS[2-3], which necessitates the extensive use of high cost solvents and maintenance of column temperature at controlled conditions to achieve better chromatographic separation. However, the limited number of HPLC methods[4-12] that are available for regular routine analysis for LIN alone or in combination with metformin in pharmaceutical formulations employs the use of high cost solvents that are found to be highly complex and are associated with increasing numbers of process variables, which makes them less acceptable for routine analysis.

On the other hand, HPLC methods require strong optimization of process variables such as mobile phase composition, concentration of the buffer, column temperature and flow rate. Therefore, an attempt was made to develop a new solvent system. However, an extensive search of literature showed that, Badugu et al.,[4] developed the novel method for quantitation of LIN in marketed preparation using Methanol: Water as a solvent, whereas, K. Sujatha et al.,[5] described a novel method using Phosphate Buffer and Methanol that provided effective chromatographic separation. The novelty of present method is QbD driven assay method development using solvent system using Methanol: Phosphate Buffer pH 7.2. The QbD based approach provides deeper understanding of the interaction between the factors and their effect on responses. Hence a robust and less variability in method results is obtained.

MATERIALS AND METHODS

LIN was a generous gift sample from Glenmark Pharmaceuticals, (Mumbai, India) and used as a working standard. The commercially available formulation of LIN i.e. Tradjenta Tablets, (Boehringer Ingelheim India, Pvt. Ltd.) were used for Assay. Potassium Di-hydrogen Orthophosphate and Di-potassium Hydrogen Phosphate was of GR Grade, Methanol was of HPLC Grade. All the solutions were prepared with reverse-osmosis, HPLC grade double distilled water.

The HPLC System was model Shimadzu and was composed of a binary pump, a mobile phase degasser, a UV-VIS detector etc. The mobile phase contains Methanol and Phosphate Buffer (70:30 v/v) with pH 7.2 adjusted with 1% solution of OPA, Flow Rate of 1.0 mL/min and UV Detection was carried out at 292nm. Chromatographic separation was performed at ambient temperature on an ACE C_{18} Column (150 × 4.6 mm, 5µ). The Injection volume was 20 µL. Prior to injection of the drug solution, the column was equilibrate for some time with the mobile phase flowing through the system. The data were acquired, stored and analyzed with Design Expert Software Ver 7.0.

Preparation of standard stock solution

Weighed and transferred accurately about 10 mg of Linagliptin standard in a 50 mL volumetric flask, 35 mL of diluent was added, sonicated to dissolve and diluted up to the mark with diluent. 1 mL portion of this solution was further diluted to 10 mL with diluent (20µg/mL).

Preparation of sample solution

Weighed and powder 20 tablets. An accurately weighed quantity about 182 mg of powdered tablet was transferred in to 25 mL of volumetric flask. 15 mL of diluent was added and sonicated for 30 min, diluted to volume with diluent. A 1 mL of this solution was further diluted to 10 mL with diluent (20 µg/mL; on label claim basis).

Chromatograms of working Standard (LIN) shown in figure 2.

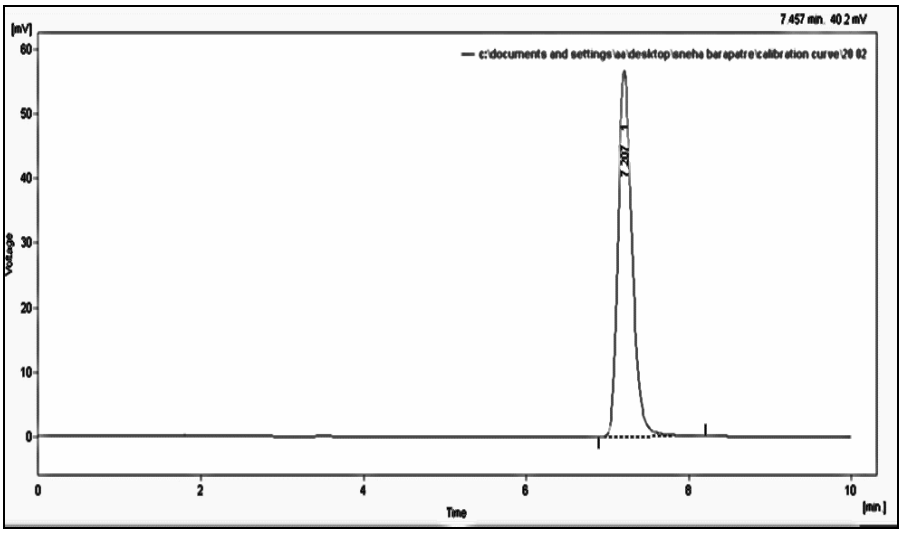

Figure 2. Chromatograms of working standard (lin)

Method development and experimental design

A novel HPLC Method was developed using the mobile phase composition containing Methanol-Phosphate Buffer (pH 7.2) for achieving chromatographic separation. Earlier reports suggested the use of Acetonitrile, Dipotassium Hydrogen Phosphate, Acetonitrile, Water and methanol, 0.02 M Phosphate Buffer and so forth, as the Mobile Phase. However, use of Methanol instead of other organic solvent is a cost-effective approach for regular routine analysis of Pharmaceutical Formulations. The Experimental Design Optimization method along with statistical analysis of data performed by Design-Expert 7.0 software, Full Version, using the Box-Behnken Design (BBD). The BBD has a advantage of optimization for experiments using 3^k-factorial design (where, k=1,2,3…….) having a least three dependent variables or factors and more than one response as compared to other experimental designs such as Central Composite Design (CCD) and Fractional Factorial Design (FFD). The ratio of mobile phase (Methanol: Phosphate Buffer) composition from 66.5:33.5% v/v, Flow Rate between 0.8-1.0 mL/min and pH between 7.0-7.4 indicating three level was fed in the software and a total of 17 runs were obtained using the design.

Model design optimization

The significance of model so obtained was evaluated by two ways i.e. ANNOVA method and Good fit evaluation.

a) ANOVA

ANOVA is a statistical method based on F-test to estimate the significance of model. It involves subdividing total variation into variation due to Residual error, Main effects and Interactions.

b) Main effects (lack of fit)
The Lack of Fit is one of the components of partition of the sum of squares in an ANOVA which can tell that proposed model is fit or not.

Estimation of lin in pharmaceutical dosage form
The developed HPLC Method was used for determination of LIN in Pharmaceutical Formulations. Marketed Formulations of Lin 5 mg strength was procured from local pharmacy were evaluated for the amount of LIN present in the formulation. Twenty tablets were Weighed and crushed. Average weight determined. An accurately weighed quantity of tablet powder equivalent to 5 mg Linagliptin was transferred in 25 mL of volumetric flask, 15 mL of diluent was added, sonicated for 30 min and volume made up to the mark with diluent. This solution was filtered through whattman filter paper no 1. Transferred 1 mL of this solution into 10 mL of volumetric flask and volume made up to the mark with diluent. The representative chromatogram of sample was recorded. The Chromatograms were obtained for LIN in marketed formulation is represented in Figure 3.

METHOD VALIDATION

The method was validated for

System Suitability
The System Suitability was assessed by six replicate analysis of 20 µg/mL concentration of LIN.

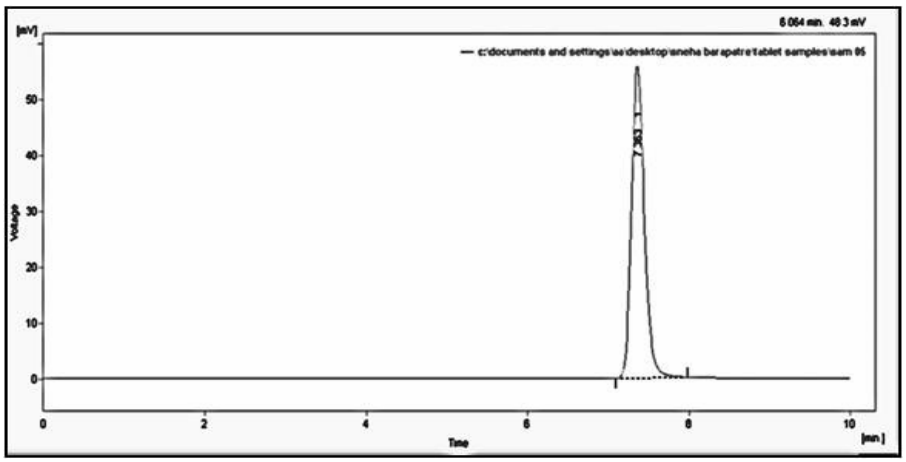

Figure 3: Chromatogram of sample LIN

Linearity
The Linearity of the method was determined by diluting the standard stock solution to 5-30 µg/mL.

Accuracy
Accuracy of the method was determined from the recovery of LIN through 20 µg/mL solution spiked with 50, 100 and 150% extra quantity of LIN.

Precision
Precision was assessed by the measurement of Intraday precision (repeatability) through the assay of three different concentrations on LIN (5-30 µg/mL) at different time intervals in the same day and Interday precision by repetition for Ten days as per ICH Guidelines.

LOD and LOQ
The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined from Slope (S) of the Linearity plot and Standard Deviation of the response to the blank sample by formula,

$$\text{Limit of Detection (LOD)} = \frac{3.3\,\sigma}{S}$$

$$\text{Limit of Quantitation (LOQ)} = \frac{10\,\sigma}{S}$$

Solution Stability
The solution stability study was carried out with sample Linagliptin solution. The solution stability study was performed at Room temperature for 24h and in the refrigerator for 24h. After the specified period results were analysed.

RESULTS AND DISCUSSION

The Suitability of Mobile Phase combination, Flow rate and pH was decided on the basis of Linearity, Assay, System Suitability, lesser time required for analysis (Retention Time), Peak Parameters and ease of preparation. Out of several tried combinations as suggested by BBD, the mobile Phase Composition of Methanol-Phosphate Buffer (70:30% v/v), pH 7.2 and Flow Rate 1.0 mL/min showed efficient chromatographic separation of LIN (20 µg/mL) and Retention time (Rt) at 7.2 min.

Experimental design
A 3^2-factorial design using BBD was applied for observing the effect of Three independent factors such as Mobile Phase Composition (% v/v of Methanol) (X_1), Flow Rate (mL/min) (X_2) and pH (X_3) on three responses

such as Assymetry (Y_1), Theoretical Plates (Y_2) and Retention time (Y_3) as parameters for calculation of proposed method. The Chromatographic conditions and Ranges fixed for selected factors are given in Table 1.

Table 1: Selection of Independent Factors and their Levels

Factor	Name	Units	Type	Low	High	Actual	Actual	Low Coded	High Coded
A	MP	%	Numeric	66.50	73.50	-1.000	1.000	70.000	2.401
B	Flow Rate	mL/min.	Numeric	0.80	1.20	-1.000	1.000	1.000	0.137
C	pH	pH	Numeric	7.00	7.40	-1.000	1.000	7.200	0.137

The sum of total 17 runs were obtained for the fixed variables by selecting a three center repetitions which are generally carried out in order to know the experimental error variance and to test the predictive validity of the model. Each combination of Mobile Phase Composition, Flow Rate, and pH suggested by BBD were finally run on the system; observed for the responses such as peak area and retention time and represented in Table 2.

All Experiments were performed in randomized order to minimize the effects of uncontrolled factors that may introduce a bias on the response. Among the various models, the Quadratic model was suggested by the design with the maximum least square regression for response Y_1, response $Y_2$and response Y_3as compared to other models.

Table 2: Box Behnken Experimental Design Using Factors and Their Responses

Std. Runs	Mobile Phase (%)	Flow Rate (mL/min.)	pH	Asymmetry	Theoretical Plates	R.T.
1	66.50	0.80	7.20	0.757	6740	9.973
9	70.00	0.80	7.00	1.083	9034	7.67
17	70.00	1.00	7.20	1.000	10182	7.453
10	70.00	1.20	7.00	1.119	8705	5.36
8	73.50	1.00	7.40	1.095	9830	5.730
5	66.50	1.00	7.00	1.106	7621	7.61
6	73.50	1.00	7.00	1.091	10446	6.19
15	70.00	1.00	7.20	1.000	10182	7.453
4	73.50	1.00	7.20	0.940	8065	5.077
3	66.50	1.20	7.20	0.878	7046	6.98
14	70.00	1.00	7.20	1.000	10182	7.453
12	70.00	1.20	7.40	0.981	8668	6.123
2	73.50	0.80	7.20	0.844	7939	7.11
16	70.00	1.00	7.20	1.000	10182	7.453
13	70.00	1.00	7.20	1.000	10182	7.453
7	66.50	1.00	7.40	0.922	8105	8.067
11	70.00	0.80	7.40	0.950	8864	9.16

The model was examined using Lack of Fit test, which indicated insignificant lack of fit value corresponding with higher p-value as compared to the model F-value. Furthermore, the model was validated by the application of Analysis of Variance (ANOVA) to both the responses and variables to examine the significance of model which showed that both the responses achieved significant differences in their values. The Quadratic equation of all model responses Y1, Y2 and Y3 are as follows:

Y_1 (Assymetry) = $+158.341+0.25075AX_1+5.16500X_2-46.71062X_3 -0.0089\ X_1\ X_2-0.067143\ X_1\ X_3-0.0312\ X_2\ X_3-0.0051\ X_1^2- 2.06875\ X_2^2+2.9000\ X_3^2$

Y_2(Theoretical Plates) = $-626175.0+17716X_1+71388.125X_2-7518.125X_3-64.28X_1X_2-392.85X_1X_3+831.25X_2X_3-105015X_1^2-36465.625X_2^2+2359.375X_3^2$

Y_3 (Retention Time) = $+25.04804X_1-0.305X1-6.483X_2+1.389X$

Table 3. Anova Results For Response Y_1 (Assymetry)

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model Significant	0.15	9	0.017	30.91	<0.0001
2	A-MP	1	0.012	21.38	0.0024	

3	B-Flow Rate	0.010	1	0.010	18.29	0.0037
4	C-Ph	1	0.025	46.13	0.0003	
5	AB	1	0.00016	0.28	0.6109	
6	AC	1	0.884	16.03	0.0052	
7	BC	0.00	1	0.000006	0.011	0.9182
8	A2	0.016	1	0.016	29.84	0.0009
9	B2	0.029	1	0.029	52.32	0.0002
10	C2	0.057	1	0.057	102.81	<0.0001
11	Residual	0.0039	7	0.0005		
12	Lack of Fit	0.0039	3	0.0013		
13	Pure Error	0.000	4	0.000		
14	Cor Total	0.016	16			

Table 4. Anova Results For Response Y_2 (Theoretical Plates)

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model Significant	22750000	9	2528000	22.26	0.0002
2	A- MP	57260000	1	57260000	50.42	0.0002
3	B- Flow Rate	1081.13	1	1081.13	0.0095	0.9250
4	C- pH	14365.12	1	14365.12	0.13	0.7326
5	AB	8100.00	1	8100.00	0.071	0.7971
6	AC	302500	1	302500	2.66	0.1467
7	BC	4422.25	1	4422.25	0.039	0.8492
8	A2	6854000	1	6854000	60.35	0.0001
9	B2	8958000	1	8958000	78.88	<0.0001
10	C2	37501.64	1	37501.64	0.33	0.5835
11	Residual	795000	7	113600		
12	Lack of Fit	795000	3	265000		
13	Pure Error	0.000	4	0.000		
14	Cor total	23550000	16			

Table 5. Anova Results For Response Y_3 (Retention Time)

Sr. No.	Source	Sum of Squares	Df	Mean Square	F values	p-Value Prob>F
1	Model Significant	23.21	3	7.74	46.64	<0.0001
2	A- MP	9.14	1	9.14	55.10	<0.0001
3	B- Flow Rate	13.45	1	13.45	81.10	<0.0001
4	C- pH	0.62	1	0.62	3.72	0.0757
5	Residual	2.16	13	0.17		
6	Lack of Fit	2.16	9	0.24		
7	Pure Error	0.000	4	0.000		
8	Cor Total	25.36	16			

From the Table 3 Results of ANNOVA for Response Y1 showed that the predicted values for all factors: Percentage Methanol (X_1), Flow rate (X_2) and pH (X_3) are under satisfactory value with predicted model F-value of 30.91 represented the model is highly significant with model p-valueof 0.0037 indicating there is only 0.01% chance that the model F-value is large due to noise.Similarly Table 4 represents the ANOVA results for Response Y_2 which showed that predicted values for the factor with predicted model F-value 22.26 impling that the model is significant with only 0.02% chance that the model F-value is large due to noise. Similarly, Table 5 for the response Y_3showed that the predicted values for all the factors with prerdicted model f-value of 46.64 impling that the model is significant with only a 0.01% chance that the model F-value is larger due to noise. The model further suggested that predicted values for all three responses are closer to the actual values indicating higher Accuracy as well as Precision for the obtained responses.

The model was evaluated for the effect of individual factors on the responses in the form of Counter plots indicating response surfaces of all three responses Y1, Y2 and Y3 showed in Figure 3,4,5 respectively. The counter plot indicates that the effects showed only on two response i.e Response Y1 (Assymetry) and Response Y2 (theoretical Plates) which showed the

Quadratic response but response Y3 has no effect so it showed the Linear response.

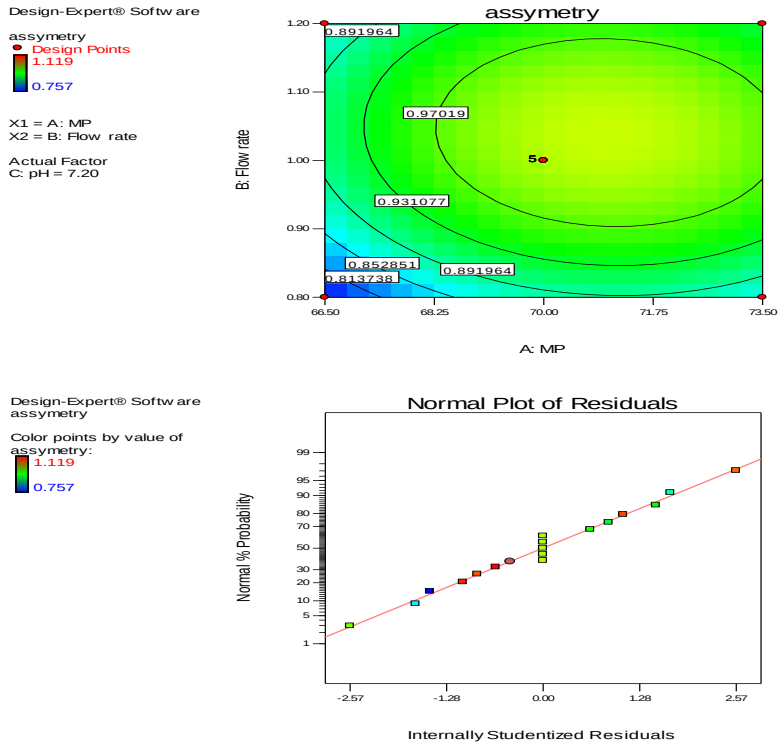

Figure 3. Counter plot and normal plot of residuals for y_1 response (asymmetry)

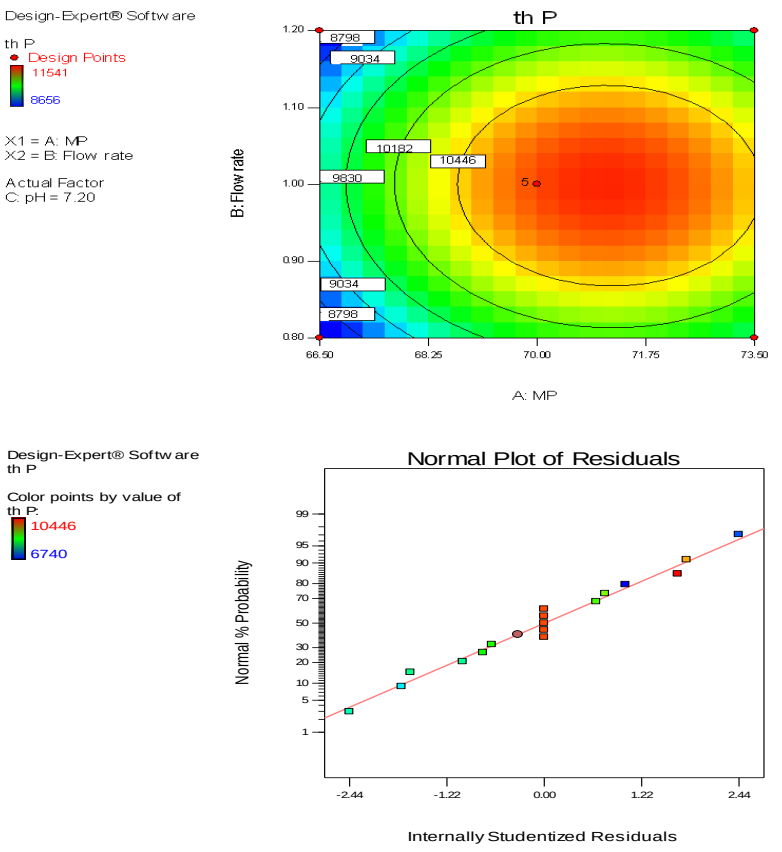

Figure 4. Counter plot and normal plot of residuals for y_2 response (theoretical plates)

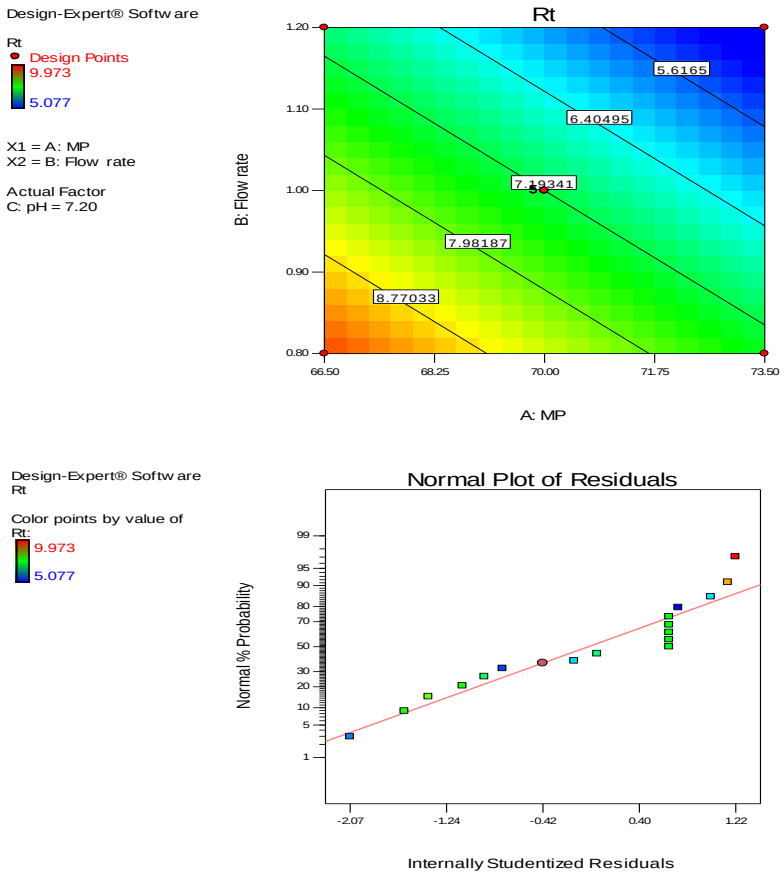

Figure 5. Counter plot and normal plot of residuals for y_3 response (retention time)

Assay

The absence of additional peaks in the chromatogram indicated no interfearence of the formulation excipient used in the Tablet. The developed method showed good chromatographic separations with a mean percentage recovery from tablet of 99.167%.

Linearity

The data obtained was plotted as peak area against concentration, which showed that the coefficient of correlation (r^2) value of 0.998.

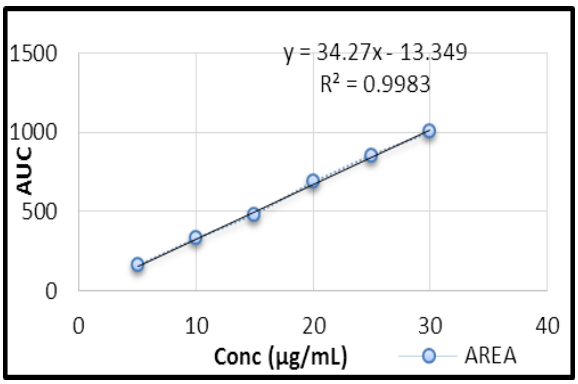

Figure 6: Plot of Linearity Curve for LIN

System suitability study

The SD and RSD (%) were determined for both peak area and retention time is tabulated in Table 6. The tailing Factor and USP Plate count were found to be 1.08 and 10231.33 respectively.

Table 6 System Suitability Study

Linagliptin (20 µg/mL)		
	Peak Area (mV)	**Retention Time (min)**
Mean (n=6)	590.272	7.3798
SD		
%RSD	0.64	

Accuracy

The method showed the percentage recovery was between 99.32-99.96% and RSD value was 0.43% respectively (Table 7).

Precision

The method showed good precision and RSD (%) for repeatability and intermediate precision was 0.44 and 0.63% respectively, which were within the NMT 2% limit. The precision data is summarized in Table 8 and 9.

Table: 7 Accuracy of The Proposed HPLC Method

Drug	Level (%)	Amount Recovered (mg)	Recovery (%)	±SD	RSD (%)
LIN	50	2.4866	99.32	0.4299	0.43
	100	4.9905	99.96		
	150	7.4858	99.73		

Table: 8 Intraday Precision Data for Lin

Time	Weight of Tablet taken equivalent to (mg)	AUC (mV)	% Label Claim
0 h.	182.0	620.977	99.9668
3 h.		615.933	99.1491
6 h.		620.168	99.8342
Mean			99.6500
±SD			0.4388
% RSD			0.44

Table: 9 Interday precision, value of % label claim is 0.6459 for SD and % RSD is 0.63 Data for Lin

Days	Weight of Tablet taken equivalent to (mg)	AUC (mV)	% Label Claim
Day 1	182.0	632.519	101.83
Day 3		634.460	102.14
Day 7		635.025	102.23
Day 10		641.734	103.31
Mean			102.38
±SD			0.5585
% RSD			0.56

DL and QL

The DL and QL values were found to be 0.841 and 2.7775 µg/mL indicated the higher sensitivity of the developed method.

Solution Stability

The results of solution stability study after 24h, %RSD was found to be 0.71.

CONCLUSION

The developed HPLC Method using Methanol:Phosphate Buffer combination as Mobile Phase showed good chromatographic separation. Upon Validation Linearity, Precision, Accuracy, System Suitability, Ruggedness, LOD, LOQ and Solution Stability were proved to be convinient and effective for the quality control as well as routine analysis of LIN in Pharmaceutical dosage Form. The measured signal showed to be Precise, Accurate and Linear over the concentration range 5-30 µg/mL with the co-relation coefficient 0.998, along with Retention Time 7.3798 min makes it economical due to Lower solvent consumption. Application Of 3^2-factorial design using BBD showed that a special attention is required for strict monitoring of the aforementioned two factors during chromatographic testing (i.e. Asymmetry and Theoretical Plates). Thus, the developed method is Rapid, Simple, and Selective for routine Analysis of LIN in Bulk as well as in Pharmaceutical Formulations.

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