International Journal of Scientific Research in Chemistry (IJSRCH)
© 2021 IJSRCH | Volume 6 | Issue 5 | ISSN : 2456-8457

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Linagliptin and Metformin HCL In Its Bulk and Tablet Dosage Form Using Quality by Design Approach

Shivam S. Lale*, Milind P. Wagh

Assistant Professor, MET's Institute of D. Pharmacy, Nashik), Dr. Milind P. Wagh Professor, Pharmaceutics, MVP's COP, Nashik, Maharashtra, India

*Corresponding author : E-mail- laleshivam13@gmail.com

Article Info
Volume 6, Issue 5
Page Number : 01-24

Publication Issue :
September-October-2021

Article History
Accepted : 10 Oct 2021
Published : 30 Oct 2021

ABSTRACT

The present study describes a simple, accurate, precise and cost effective reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for determination of Linagliptin and Metformin HCl in bulk marketed tablet formulation. Optimization was done by response surface methodology, applying a three level Box-Behnken design. Three factors selected were flow rate, column length and methanol concentration in mobile phase. The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, range, accuracy and robustness.

The separation was carried out using three columns with different length. Detection was done using UV detector at 250 nm. The developed method employed mobile phase methanol : phosphate buffer (pH 4.6) (75:25), temperature 25˚c and flow rate 0.8 ml/min, which was optimized with the help of design expert software. High linearity of the developed method was confirmed over concentration range of 400-600 µg/mL for Metformin HCl & 1.5-3.5 µg/mL for Linagliptin with correlation coefficient of 0.9979 & 0.9968. The percentage RSD for precision and accuracy of the method was found to be less than 2%. Peaks were obtained at retention times of 2.6 & 5.6 min. respectively for Metformin HCl & Linagliptin. The proposed method can be successfully used to determine the drug contents of marketed formulation.

Keywords : QbD, Metformin HCl, Linagliptin, Box-Behnken design

I. INTRODUCTION

Trajenta Duo (Company- Boeringer Ingelheim) tablets were purchased from local pharmacy for the study. WATER's HPLC system with manual injection, UV detector & binary pump was used. All solvents were used of HPLC grade. pH of mobile phase was adjusted

by using Ortho-phosphoric acid. Design Expert v 10 was used for DOE.

II. METHODS AND MATERIAL

Development of HPLC Method by QbD Approach and its Optimizations

Selection of analytical wavelength

Standard stock solution of MET & LIN was diluted with diluent to obtain final concentration of 10µg/ml. Solution was scanned using UV-Visible Spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra were overlaid. The wavelength selected was 250 nm.

Selection of mobile phase

The APIs of MET & LIN were injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation of both drugs. After several permutation and combination, it was found that the Methanol and Phosphate buffer of pH 4.6 gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase takes as per design, as it gave good peak shape of MET & LIN with minimal tailing.

Development of Analytical RP-HPLC Method with Design Space and Control Strategy determination by optimization study:

All the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version 10; State-Ease Inc., Minneapolis, MN, USA).

Design of experiments (DOE-1): Thus, 3^3 randomized response surface designs with a Box-Behnken design were used with 17 trial runs to study the impact of three factors on the two key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all 3 possible combinations. The flow rates (X1), Column Length (X2), mobile phase compositions (X3) were selected as independent variables and retention time (RT) and Resolution were selected as dependent variables. The resulting data were fitted into Design Expert 10 software and analysed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, temperature, mobile phase composition on dependent variables. The probable trial runs using 3^3 Box-Behnken designs are as shown in table no 9.2.

Table No. – 1 : 3^3 Box Behnken designs of DOE

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A:MOBILE PHASE COMPOSITION (% Me)	B:FLOW RATE (ml/min)	C:COLUMN LENGTH (mm)	RT of I Drug (min.)	RT of II Drug (min.)	Resolution
1	50	0.8	100	2.6	4.6	1.81
2	75	1	100	2	3.6	1.45
3	75	0.8	150	2.6	5.6	2.72
4	100	0.6	150	4.1	6.1	1.81
5	75	0.8	150	2.6	5.6	2.72
6	100	0.8	250	2.8	5.4	2.36
7	50	1	150	2.1	3.8	1.54
8	75	0.6	250	3.7	5.9	2

9	75	0.8	150	2.6	5.6	2.72
10	75	1	250	2.1	4.3	2
11	75	0.8	150	2.5	5.8	3
12	100	1	150	1.8	3.6	1.63
13	75	0.6	100	3.4	6.1	2.45
14	75	0.8	150	2.6	5.6	2.72
15	50	0.8	250	3	4.5	1.36
16	50	0.6	150	3.2	5.4	2
17	100	0.8	100	2.2	4.5	2.09

Table No. – 2 : Levels selected

Level of Variable	**Levels of Factors**		
	Flow Rate (mL/min)	Column length (mm)	Mobile Phase Composition (M:B)
Low Level (-1)	0.6	100	50: 50
Medium Level (0)	0.8	150	75: 25
High Level (1)	1.0	250	100: 00

Application of proposed method for analysis of marketed formulation

Standard stock solution:

For MET, Std. stock solution was prepared by adding accurately weighed 50 mg API in 100 solvent to make final concentration of 500 ppm. And for LIN, 10 mg of API was dissolved in 100 ml solvent from which 2.5 ml was further diluted up to 100 ml to get concentration of 2.5 ppm.

Sample solution preparation:

Accurately weighed tablet powder equivalent to 50 mg of MET was transferred in a 100 ml volumetric flask and methanol was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with methanol. The solution was filtered through whattman filter paper No.42.

Procedure:

Equal volumes of standard and sample solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of MET & LIN was calculated by comparing a sample peak with that of standard.

Amount of drug in tablet was calculated using following formula-

Concentration in test solution (mg/ml):

$$CT = (AT \times CS) \div AS$$

Assay (%):

$$\%\ Assay = (CT \times 100) \div CS$$

Where,

AT= Area of test solution

AS= area of standard solution

CT= Concentration of drug in test solution

CS= Concentration of drug in standard solution

System Suitability Test:

System suitability is a Pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution.

Validation

Validation of method for analysis of MET & LIN

The developed method was validated as per ICH guidelines.

A) Linearity:

Determination

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

Preparation of standard stock solution

For MET, Std. stock solution was prepared by adding accurately weighed 100 mg API in 100 solvent to make final concentration of 1000 ppm. And for LIN, 10 mg of API was dissolved in 100 ml solvent to get concentration of 100 ppm.

Preparation of linearity solution:

For MET, Linearity was performed by diluting standard stock solution. From stock solution aliquots of 4, 4.5, 5, 5.5, 6 ml diluted to 10ml with diluent such that the final concentration of MET in the range of 400 to 600 µg/ml.

Preparation of linearity solution:

For MET, Linearity was performed by diluting standard stock solution. From stock solution aliquots of 4, 4.5, 5, 5.5, 6 ml diluted to 10ml with diluent such that the final concentration of MET in the range of 400 to 600 µg/ml.

Table No. – 3 : Dilution table for linearity (MET)

Sample	Linearity stock solution- Transfer (ml)	Final volume (ml)
Linearity – 60%	4	10
Linearity – 80%	4.5	10
Linearity – 100%	5	10
Linearity – 120%	5.5	10
Linearity – 140%	6	10

Preparation of linearity solution:

For LIN, Linearity was performed by diluting standard stock solution. From stock solution aliquots of 1.5, 2, 2.5, 3, 3.5 ml diluted to 100 ml with diluent such that the final concentration of MET in the range of 1.5 to 3.5 µg/ml.

Table No. – 4 : Dilution table for linearity (LIN)

Sample	Linearity stock solution- Transfer (ml)	Final volume (ml)
Linearity – 60%	1.5	100
Linearity – 80%	2	100
Linearity – 100%	2.5	100
Linearity – 120%	3	100
Linearity – 140%	3.5	100

B) Accuracy (recovery)

The accuracy of an analytical method is determined by applying the method to analysed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Preparation of standard stock solution

For MET, Std. stock solution was prepared by adding accurately weighed 100 mg API in 100 solvent to make final concentration of 1000 ppm. And for LIN, 10 mg of API was dissolved in 100 ml to get concentration of 10 ppm.

Procedure for Preparation of sample Solution:

Prepare the standard solution by taking stock solution equivalent to 80%, 100%, and 120%, each in triplicate. Inject each preparation into the HPLC system.

Table No. – 5 : Concentration table for accuracy

Sample	Concentration of Std. (µg/ml)	Concentration of For. (µg/ml)	Total Concentration (µg/ml)
Accuracy – 80%	200	250	450
Accuracy – 100%	200	300	500
Accuracy – 120%	200	350	550

Table No. – 6 : Concentration table for accuracy

Sample	Concentration of Std. (µg/ml)	Concentration of For. (µg/ml)	Total Concentration (µg/ml)
Accuracy – 80%	1.25	1	2.25
Accuracy – 100%	1.25	1.25	2.5
Accuracy – 120%	1.25	1.5	2.75

Procedure:

Injected standard preparation and sample preparations of recovery solutions into the HPLC and measure the peak responses for the MET & LIN peaks.

Amount of drug Recovered was calculated using following formula

Calculation:-

$$Conc.found = \frac{AT \times CS}{AS}$$

Where,

AT= Area of test solution

CS= Conc. of Std.

AS= Area of Std.

$$\%\ Recovery = \frac{(Observed\ Conc. \times 100)}{Added\ Conc.}$$

C) Precision:

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation.

Method precision:

Determination:

Prepare five different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution.

Preparation of standard stock solution:

For MET, Std. stock solution was prepared by adding accurately weighed 50 mg API in 100 solvent to make final concentration of 500 ppm. And for LIN, 10 mg of API was dissolved in 100 ml solvent from which 2.5 ml was further diluted up to 100 ml to get concentration of 2.5 ppm% assay values and RSD of assay were calculated.

D) Robustness:

Determination:

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. The sample along with standard was injected under different chromatographic conditions as shown below.

- Changes in flow rate. (±0.10ml/min)

Carry out the following procedure individually by changing following variation in chromatographic conditions.

1) Change in flow rate of mobile phase to 0.7 ml/min.
2) Change in flow rate of mobile phase to 0.9 ml/min.

E) Limit of Detection

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

$$LOD = 3.3\ (SD)/S$$

Where, SD= Standard deviation

S= Slope

F) Limit of Quantitation

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

$$LOD = 10\ (SD)/S$$

Where,

SD= Standard deviation

S= Slope

III. RESULTS

Standard Calibration Curves for MET & LIN

Table No. – 7 : Absorbance values for different concentration of Metformin HCl in methanol (λmax=233nm)

Concentration (ug/ml)	Absorbance
2	0.17
4	0.31
6	0.49
8	0.64
10	0.8

Figure No. – 1 : Beer-Lambert's plot for of Metformin HCl in methanol (λmax=233nm)

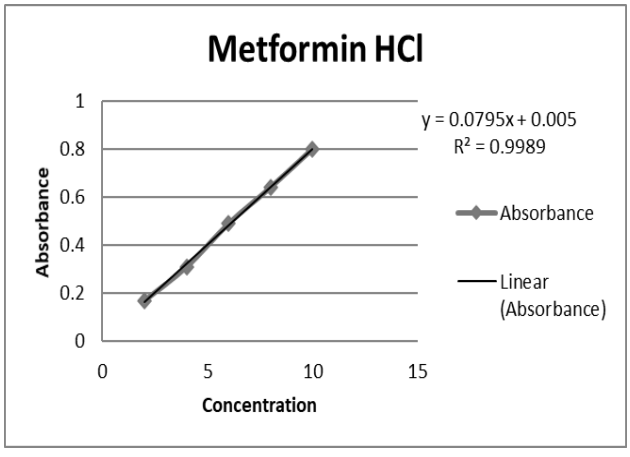
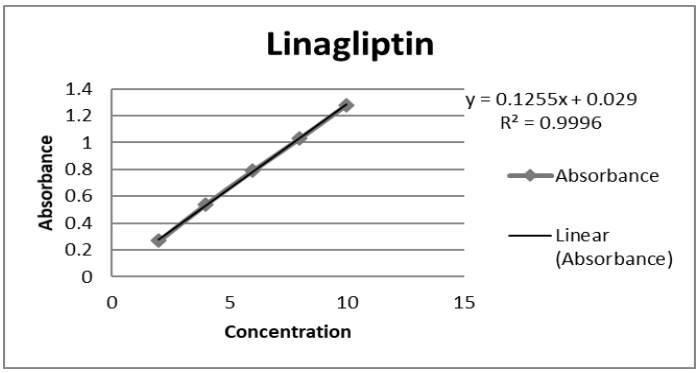


Table No. – 8 : Absorbance values for different concentration of Linagliptin in methanol (λmax=227nm)

Concentration (ug/ml)	Absorbance
2	0.27
4	0.54
6	0.79
8	1.03
10	1.28

Figure No. – 2 : Beer-Lambert's plot for Linagliptin in methanol (λmax=227nm)



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR ANALYSIS OF MET & LIN

A] HPLC Method Development

Table No. – 9 : Layout of Actual Design of DOE

Procedure:

Injected standard preparation and sample preparations of recovery solutions into the HPLC and measure the peak responses for the MET & LIN peaks.

Amount of drug Recovered was calculated using following formula

Calculation:-

$$Conc.found = \frac{AT \times CS}{AS}$$

Where,

AT= Area of test solution

CS= Conc. of Std.

AS= Area of Std.

$$\% \, Recovery = \frac{(Observed \; Conc. \times 100)}{Added \; Conc.}$$

C) Precision:

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation.

Method precision:

Determination:

Prepare five different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution.

Preparation of standard stock solution:

For MET, Std. stock solution was prepared by adding accurately weighed 50 mg API in 100 solvent to make final concentration of 500 ppm. And for LIN, 10 mg of API was dissolved in 100 ml solvent from which 2.5 ml was further diluted up to 100 ml to get concentration of 2.5 ppm% assay values and RSD of assay were calculated.

D) Robustness:

Determination:

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. The sample along with standard was injected under different chromatographic conditions as shown below.

- Changes in flow rate. (±0.10ml/min)

Carry out the following procedure individually by changing following variation in chromatographic conditions.

3) Change in flow rate of mobile phase to 0.7 ml/min.
4) Change in flow rate of mobile phase to 0.9 ml/min.

E) Limit of Detection

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD= 3.3 (SD)/S

Where, SD= Standard deviation

S= Slope

F) Limit of Quantitation

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD= 10 (SD)/S

Where,

SD= Standard deviation

S= Slope

RESULTS

Standard Calibration Curves for MET & LIN

Table No. – 7 : Absorbance values for different concentration of Metformin HCl in methanol (λmax=233nm)

Concentration (ug/ml)	Absorbance
2	0.17
4	0.31
6	0.49
8	0.64
10	0.8

Figure No. – 1 : Beer-Lambert's plot for of Metformin HCl in methanol (λmax=233nm)

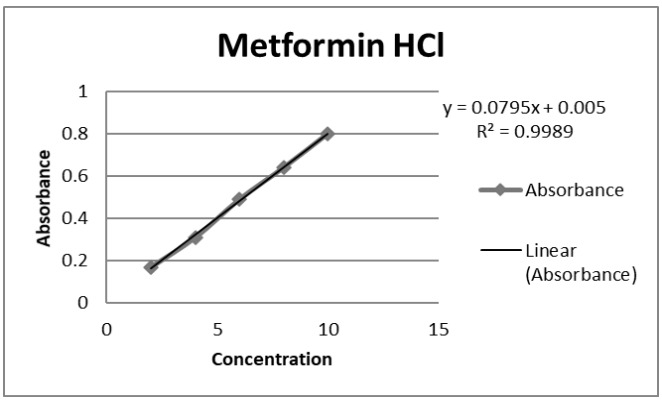
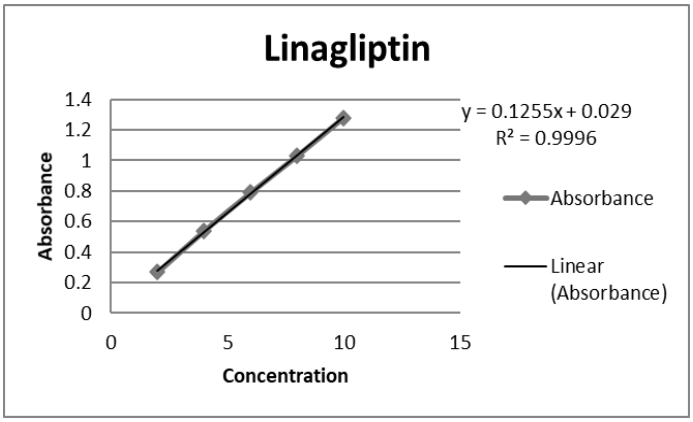


Table No. – 8 : Absorbance values for different concentration of Linagliptin in methanol (λmax=227nm)

Concentration (ug/ml)	Absorbance
2	0.27
4	0.54
6	0.79
8	1.03
10	1.28

Figure No. – 2 : Beer-Lambert's plot for Linagliptin in methanol (λmax=227nm)



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR ANALYSIS OF MET & LIN

A] HPLC Method Development

Table No. – 9 : Layout of Actual Design of DOE

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A:MOBILE PHASE COMPOSITION (% Me)	B:FLOW RATE (ml/min)	C:COLUMN LENGTH (mm)	RT of I Drug (min.)	RT of II Drug (min.)	Resolution
1	50	0.8	100	2.6	4.6	1.81
2	75	1	100	2	3.6	1.45
3	75	0.8	150	2.6	5.6	2.72
4	100	0.6	150	4.1	6.1	1.81
5	75	0.8	150	2.6	5.6	2.72
6	100	0.8	250	2.8	5.4	2.36
7	50	1	150	2.1	3.8	1.54
8	75	0.6	250	3.7	5.9	2
9	75	0.8	150	2.6	5.6	2.72
10	75	1	250	2.1	4.3	2
11	75	0.8	150	2.5	5.8	3
12	100	1	150	1.8	3.6	1.63
13	75	0.6	100	3.4	6.1	2.45
14	75	0.8	150	2.6	5.6	2.72
15	50	0.8	250	3	4.5	1.36
16	50	0.6	150	3.2	5.4	2
17	100	0.8	100	2.2	4.5	2.09

Table No. – 10 : ANOVA table for retention time I

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5.89	9	0.65	17.05	0.0006	significant
A-MOBILE PHASE COMPOSITION	8.882E-016	1	8.882E-016	2.313E-014	1.0000	
B-FLOW RATE	4.93	1	4.93	128.34	< 0.0001	
C-COLUMN LENGTH	0.24	1	0.24	6.38	0.0395	
AB	0.36	1	0.36	9.38	0.0183	
AC	0.000	1	0.000	0.000	1.0000	
BC	4.211E-003	1	4.211E-003	0.11	0.7502	
A^2	5.158E-003	1	5.158E-003	0.13	0.7248	
B^2	0.14	1	0.14	3.75	0.0939	
C^2	2.166E-003	1	2.166E-003	0.056	0.8191	
Residual	0.27	7	0.038			
Lack of Fit	0.26	3	0.087	43.46	0.0016	significant
Pure Error	8.000E-003	4	2.000E-003			
Cor Total	6.16	16				

Final Equation in Terms of Coded Factors:

RT of I Drug =

$$2.6412 - 0.8052 \times B + 0.1750 \times C - 0.3 \times AB - 0.0315 \times BC + 0.0350 \times A^2 + 0.1852 \times B^2 - 0.0262 \times C^2$$

Figure no- 3: Response plot of retention time (min) against flow rate and MPC

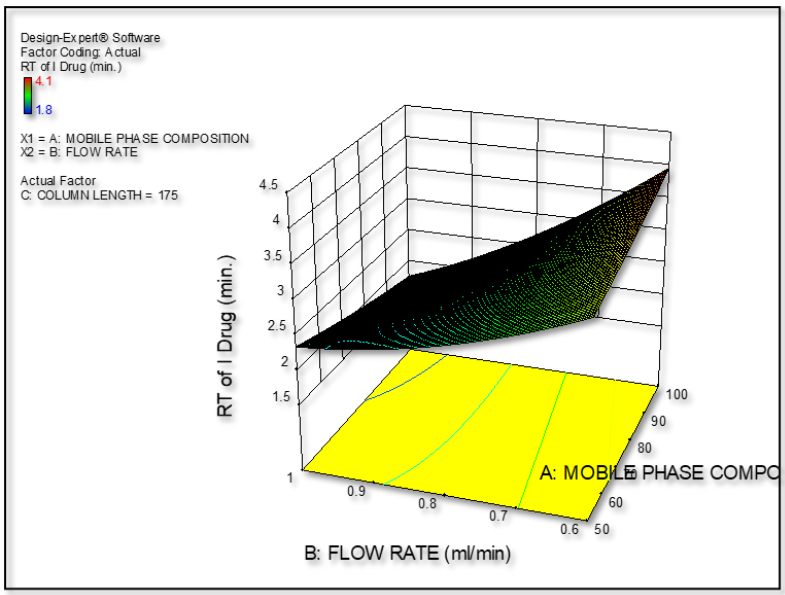


Figure no- 4 : Response plot of retention time (min) against MPC and Column Length

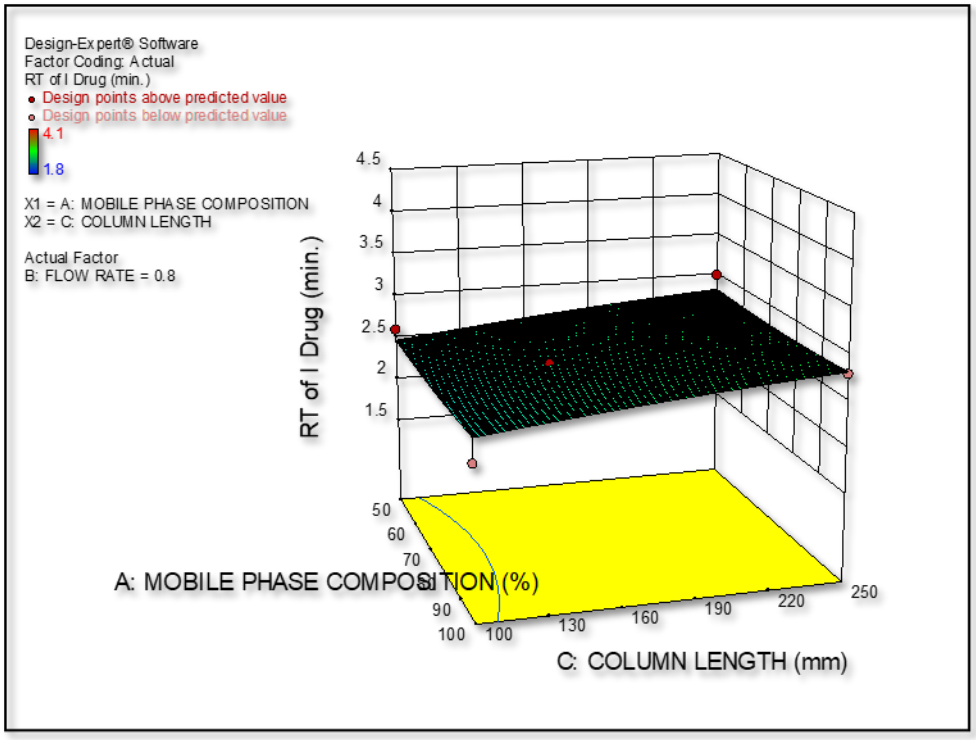


Table No- 11 : ANOVA table for RT II

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	12.03	9	1.34	173.10	< 0.0001	significant
A-MOBILE PHASE COMPOSITION	0.32	1	0.32	40.95	0.0004	
B-FLOW RATE	7.44	1	7.44	963.85	< 0.0001	
C-COLUMN LENGTH	0.21	1	0.21	27.36	0.0012	
AB	0.20	1	0.20	26.23	0.0014	
AC	0.26	1	0.26	33.82	0.0007	
BC	0.19	1	0.19	24.85	0.0016	
A^2	1.37	1	1.37	177.20	< 0.0001	
B^2	0.50	1	0.50	64.92	< 0.0001	
C^2	0.56	1	0.56	72.14	< 0.0001	
Residual	0.054	7	7.720E-003			
Lack of Fit	0.022	3	7.346E-003	0.92	0.5082	not significant
Pure Error	0.032	4	8.000E-003			
Cor Total	12.08	16				

The Model F-value of 173.10 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. "Values of ""Prob > F"" less than 0.0500 indicate model terms are significant. In

this case A, B, C, AB, AC, BC, A++2+-, B++2+-, C++2+- are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. "The ""Lack of Fit F-value"" of 0.92 implies the Lack of Fit is not significant relative to the pure error. There is a 50.82% chance that a ""Lack of Fit F-value"" this large could occur due" to noise. Non-significant lack of fit is good -- we want the model to fit.

Final Equation in Terms of Coded Factors:

RT of II Drug =

$$5.7409 + 0.2039 \times A - 0.9894 \times B + 0.1625 \times C - 0.2250 \times AB + 0.2486 \times AC + 0.2131 \times BC - 0.570 \times A^2 - 0.345 \times B^2 - 0.420 \times C^2$$

Figure no- 5 : Response plot of RT II against flow rate and MPC

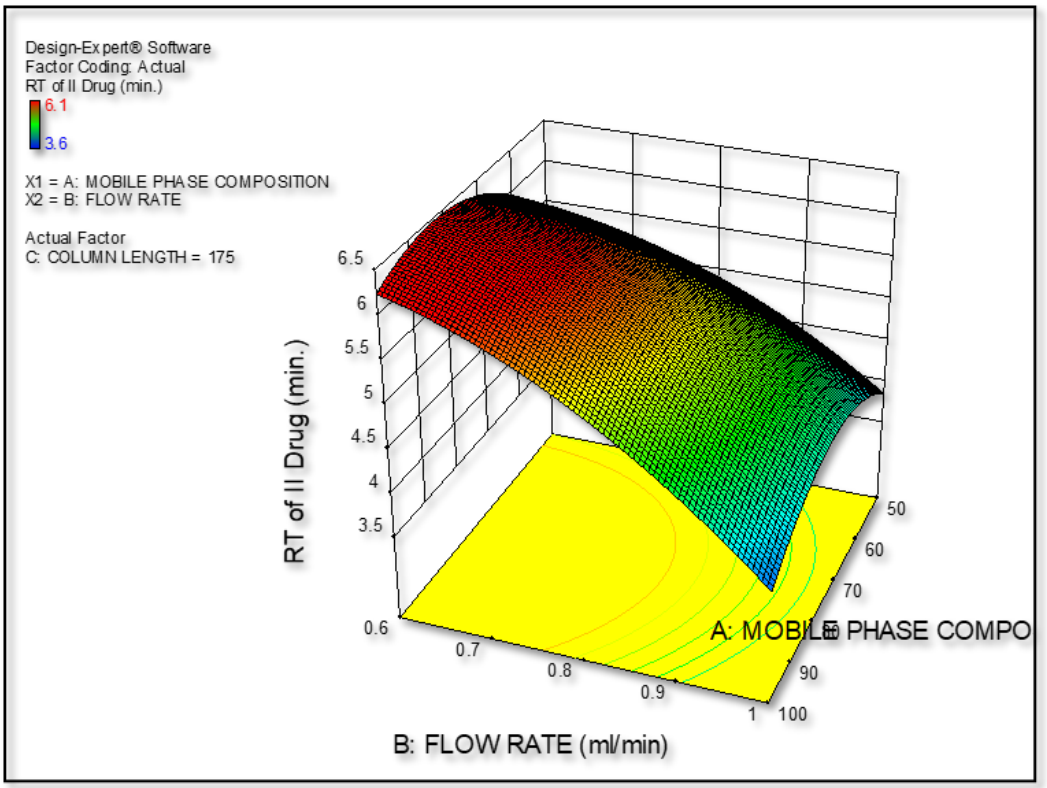


Figure no- 6 : Response plot of RT II against Column Length and mobile phase composition

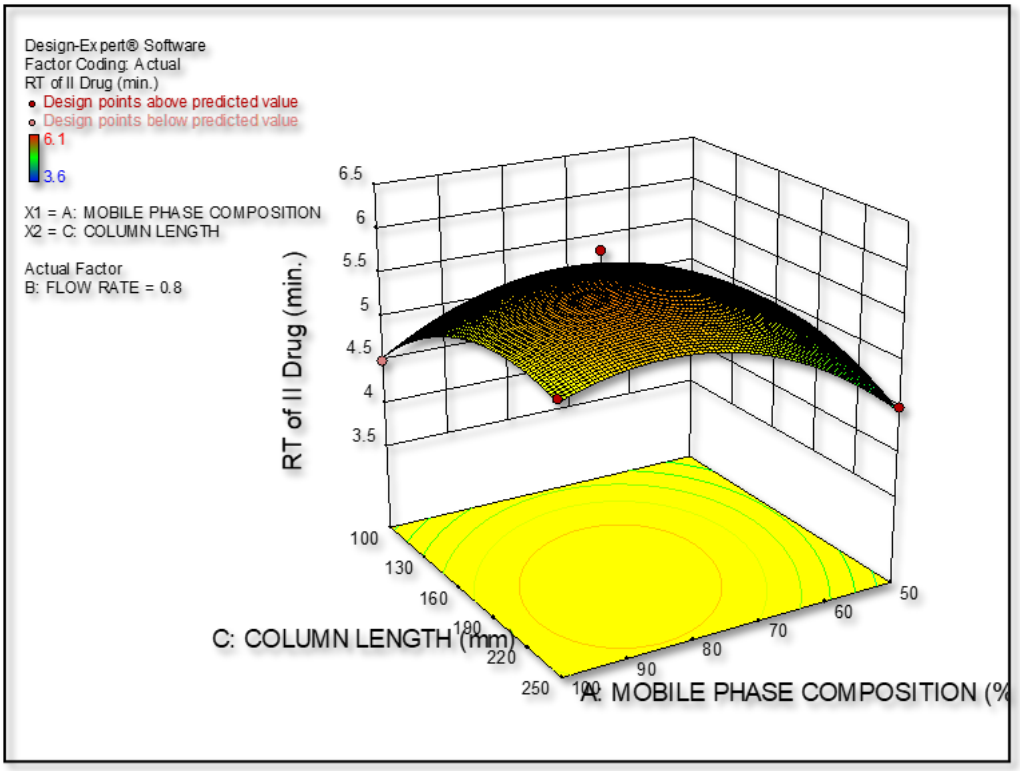


Table no. 12 : ANOVA for response surface Quadratic model RESOLUTION

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3.93	9	0.44	10.79	0.0024	significant
A-MOBILE PHASE COMPOSITION	0.26	1	0.26	6.43	0.0390	
B-FLOW RATE	0.21	1	0.21	5.29	0.0549	
C-COLUMN LENGTH	8.000E-004	1	8.000E-004	0.020	0.8922	
AB	0.020	1	0.020	0.48	0.5090	
AC	0.21	1	0.21	5.28	0.0552	
BC	0.21	1	0.21	5.17	0.0572	
A^2	1.28	1	1.28	31.52	0.0008	
B^2	0.97	1	0.97	24.02	0.0018	
C^2	0.40	1	0.40	9.89	0.0163	
Residual	0.28	7	0.040			
Lack of Fit	0.22	3	0.074	4.69	0.0848	not significant
Pure Error	0.063	4	0.016			
Cor Total	4.21	16				

The Model F-value of 10.79 implies the model is significant. There is only a 0.24% chance that an F-value this large could occur due to noise. "Values of ""Prob > F"" less than 0.0500 indicate model terms are significant. In this case A, A++2+-, B++2+-, C++2+- are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. "The ""Lack of Fit F-value"" of 4.69 implies there is a 8.48% chance that a ""Lack of Fit F-value"" this large could occur due to noise. Lack of fit is bad -- we want the model to fit."

Figure no- 7 : Response plot of Resolution against flow rate and MPC

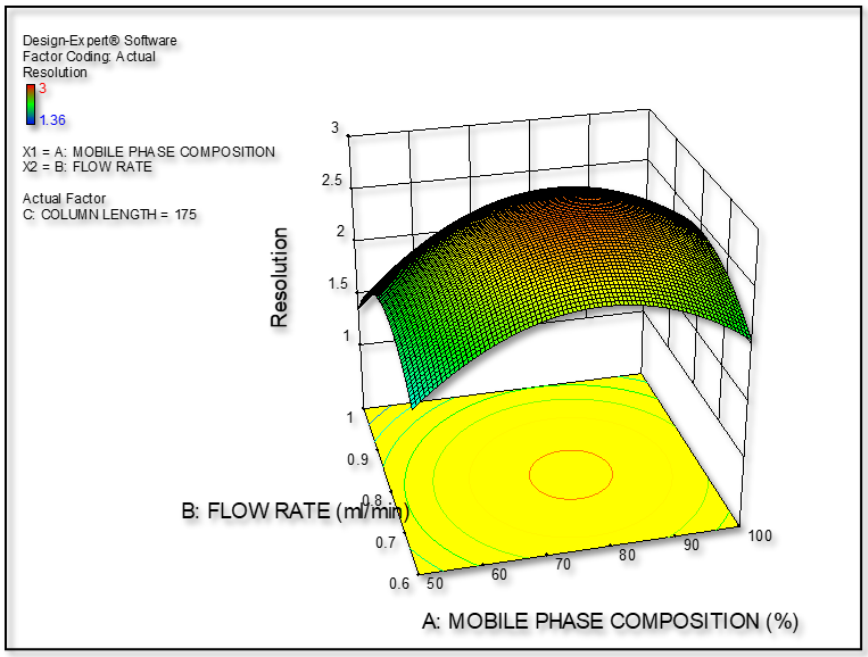


Figure no- 8 : Response plot of Resolution against Column Length and mobile phase composition

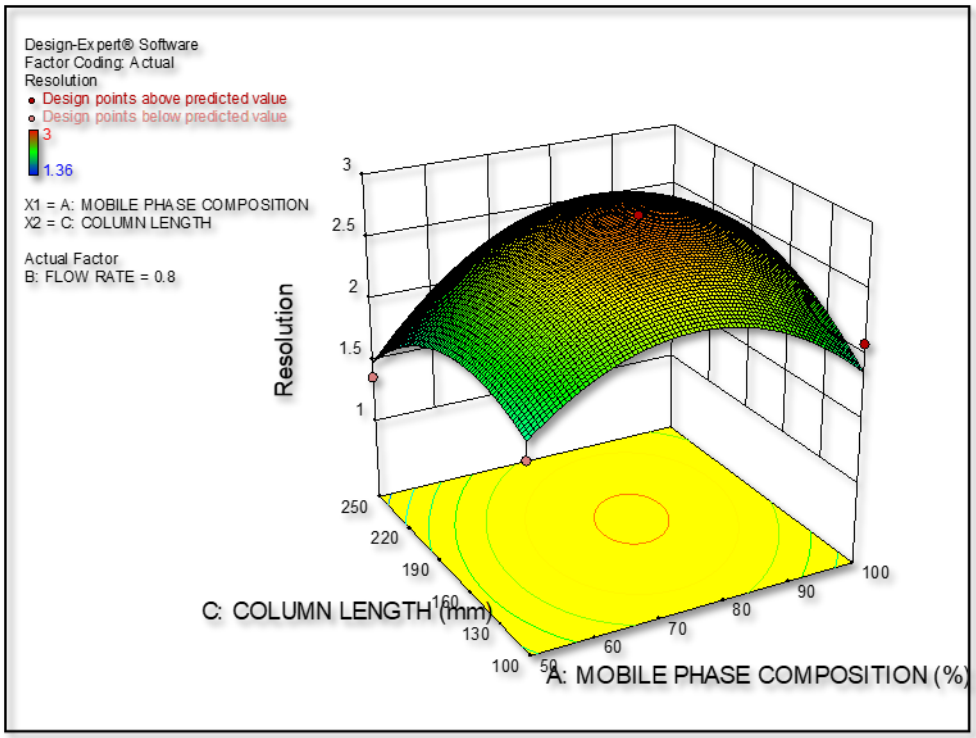


Table no- 13 : Proposed optimised method

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:MOBILE PHASE COMPOSITION	is in range	50	100	1	1	3
B:FLOW RATE	is in range	0.6	1	1	1	3
C:COLUMN LENGTH	is target = 150	100	250	1	1	5
RT of I Drug	none	1.8	4.1	1	1	3
RT of II Drug	none	3.6	6.1	1	1	3
Resolution	is in range	2	5	1	1	3

A) Optimization solution:

Table no- 14 : Result of optimization for DOE

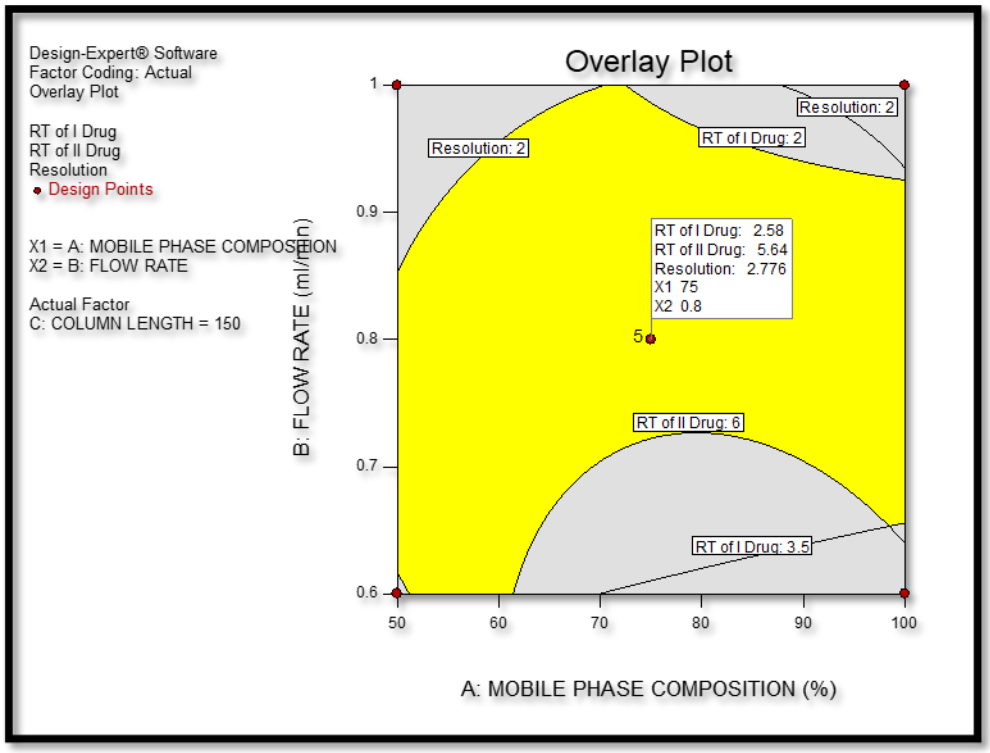
Number	MOBILE PHASE COMPOSITION	FLOW RATE	COLUMN LENGTH	RT of I Drug	RT of II Drug	Resolution	Desirability	
1	56.630	0.868	150.000	2.425	4.900	2.243	1.000	Selected
2	55.008	0.631	150.000	3.205	5.677	2.244	1.000	
3	80.229	0.963	150.000	2.004	4.505	2.268	1.000	
4	100.000	0.600	150.000	3.895	6.132	2.027	1.000	
5	75.000	0.800	150.000	2.580	5.640	2.776	1.000	

B) Developed Method Operable Design Region

Design Space for study DOE:

The graphical optimization done by with the help of Design Expert software provided the base to define the design space as shown in following Figure 9

Figure No. 9 : Design space for DOE



This plot elaborates that the optimized values of both independent variables in the required target range of retention time & Asymmetric factor lie within the yellow region which is the useful optimum region where the design space can be determined whereas the grey colored region is totally restricted to achieve the target response value of dependent variable.

Optimized Method:

Table no- 15: Optimized Method:

Flow rate Ml/min	COLUMN LENGTH	Mobile phase composition (mL)
0.8 mL	150 mm	Methanol: BUFFER (75:25)

C) CONTROL STRATEGY

System suitability test

Table no- 16: System suitability test for MET & LIN

METFORMIN				
Sample Name	**Retention Time (min)**	**Area**	**Plate Count**	**Tailing factor**
Standard 1	2.12	2877534	6285	1.39
Standard 2	2.13	2878541	6187	1.38
Standard 3	2.15	2857565	6104	1.4
Standard 4	2.13	2866543	6084	1.41
Standard 5	2.12	2817564	6114	1.38
MEAN		2859549.4	6154.8	1.392

SD	24998.74399	82.5330237	0.013038405
%RSD	0.874219693	1.34095379	0.936667012

LINAGLIPTIN				
Sample Name	**Retention Time (min)**	**Area**	**Plate Count**	**Tailing factor**
Standard 1	5.87	1956767	4285	1.61
Standard 2	5.83	1967540	4187	1.58
Standard 3	5.93	1976543	4104	1.6
Standard 4	5.98	1967654	4184	1.61
Standard 5	5.86	1965765	4114	1.62
MEAN		1966853.8	4174.8	1.604
SD		7034.216495	72.60647354	0.015165751
%RSD		0.357637995	1.739160524	0.945495691

Acceptance Criteria:

1. %RSD of the five replicate injections is NMT 2.0%.
2. Theoretical plates should be more than 2000.
3. Tailing factor should be NMT 2.

Conclusion:

1. %RSD of the five replicate injections found to be 1.08472586%.
2. Theoretical plates found to be more than 2000.
3. Tailing factor found to be less than 2.

D) METHOD VALIDATION

A) Accuracy :

Table no- 17 : Result and statistical data of accuracy (MET & LIN)

METFORMIN									
Sr. No.	**Conc. Level**	**Conc. (µg/mL) STD stalk solution**	**Conc. (µg/mL) FOR stock solution**	**Area (MET)**	**Conc. Found (µg/mL) MET**	**% Recovery**	**Average % Recovery**	**SD**	**% RSD**
1	80%	250	200	25408421	398.1704848	88.48232996	88.65904792	0.164101007	0.1850922
		250	200	25501546	399.6298288	88.80662863			
		250	200	25467534	399.0968333	88.68818518			
2	100%	250	250	28675334	499.2951677	99.85903354	100	0.214051375	0.2140514
		250	250	28685564	499.4732925	99.89465851			
		250	250	28786543	501.2315398	100.246308			
3	120%	250	300	31764878	608.3993685	110.618067	110.8819808	0.385316102	0.3475011
		250	300	31789476	608.8704991	110.7037271			
		250	300	31967635	612.2828158	111.3241483			
			AVERAGE OF D7-D9 =	28715814					

LINAGLIPTIN									
Sr. No.	**Conc. Level**	**Conc. (µg/mL) STD stalk solution**	**Conc. (µg/mL) FOR stock solution**	**Area (MET)**	**Conc. Found (µg/mL) MET**	**% Recovery**	**Average % Recovery**	**SD**	**% RSD**
1	80%	1.25	1	1663543	1.900028419	84.44570749	84.67495123	0.331346848	0.3913163
		1.25	1	1665091	1.901796479	84.52428794			
		1.25	1	1675543	1.913734311	85.05485826			
2	100%	1.25	1.25	1956767	2.483262737	99.33050947	100	0.579795828	0.5797958
		1.25	1.25	1976547	2.508364824	100.334593			
		1.25	1.25	1976553	2.508372439	100.3348976			
3	120%	1.25	1.5	2256545	3.150070255	114.5480093	114.0819243	1.717458425	1.5054606
		1.25	1.5	2209887	3.084937064	112.1795296			
		1.25	1.5	2275658	3.17675144	115.5182342			
			AVERAGE OF D26-D28 =	1969955.7					

B) Precision

Table no- 18 : Results of Method Precision of MET & LIN

METFORMIN				
Sample Name	**Retention Time (min)**	**Area**	**Plate Count**	**Tailing factor**
Standard 1	2.13	2877534	6285	1.39
Standard 2	2.15	2878541	6187	1.38
Standard 3	2.11	2857565	6104	1.4
Standard 4	2.13	2866543	6084	1.41
Standard 5	2.16	2817564	6114	1.38
MEAN		2859549.4	6154.8	1.392
SD		24998.74399	82.5330237	0.013038405
%RSD		0.874219693	1.34095379	0.936667012

LINAGLIPTIN				
Sample Name	**Retention Time (min)**	**Area**	**Plate Count**	**Tailing factor**
Standard 1	5.89	1956767	4285	1.61
Standard 2	5.91	1967540	4187	1.58
Standard 3	5.88	1976543	4104	1.6

Standard 4	5.93	1967654	4184	1.61
Standard 5	5.93	1965765	4114	1.62
MEAN		1966853.8	4174.8	1.604
SD		7034.216495	72.60647354	0.015165751
%RSD		0.357637995	1.739160524	0.945495691

Acceptance Criteria:

The % RSD for the six determinations shall be NMT 2.0

Conclusion: Precision:

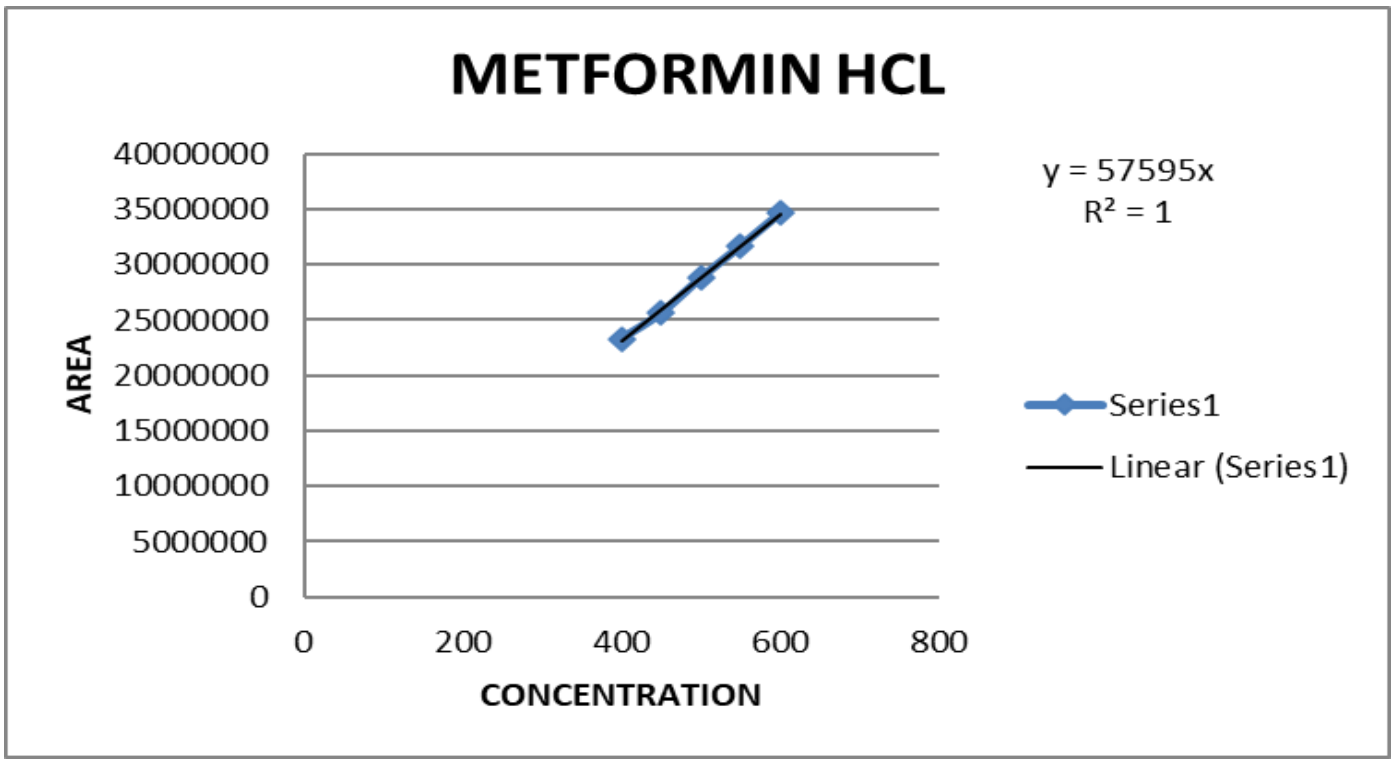
The RSD of method precision is 0.35763 %. Therefore, the HPLC method for the determination of MET & LIN is precise.

C) Linearity:

Table no- 19: Result and statistical data of linearity of MET & LIN

METFORMIN HCl					
Sr.no	**Concentration (µg/ml)**	**RT (min)**	**Area**	**Plate Count**	**USP Tailing**
1	400	2.13	23278453	6581	1.29
2	450	2.13	25587653	6273	1.15
3	500	2.14	28785476	6164	1.21
4	550	2.11	31674635	6481	1.31
5	600	2.14	34657736	6415	1.28
Correlation Coefficient			0.9979		
Slope			57595		

Figure no- 10 : Linearity graph of MET



LINAGLIPTIN HCl					
Sr.no	**Concentration (µg/ml)**	**RT (min)**	**Area**	**Plate Count**	**USP Tailing**
1	1.5	5.9	1185921	4285	1.61
2	2	5.92	1499674	4187	1.58
3	2.5	5.86	1976543	4104	1.6
4	3	5.83	2346537	4184	1.61
5	3.5	5.91	2711283	4114	1.62
Correlation Coefficient			0.9968		
Slope			777739		

Figure no- 11 : Linearity graph of LIN

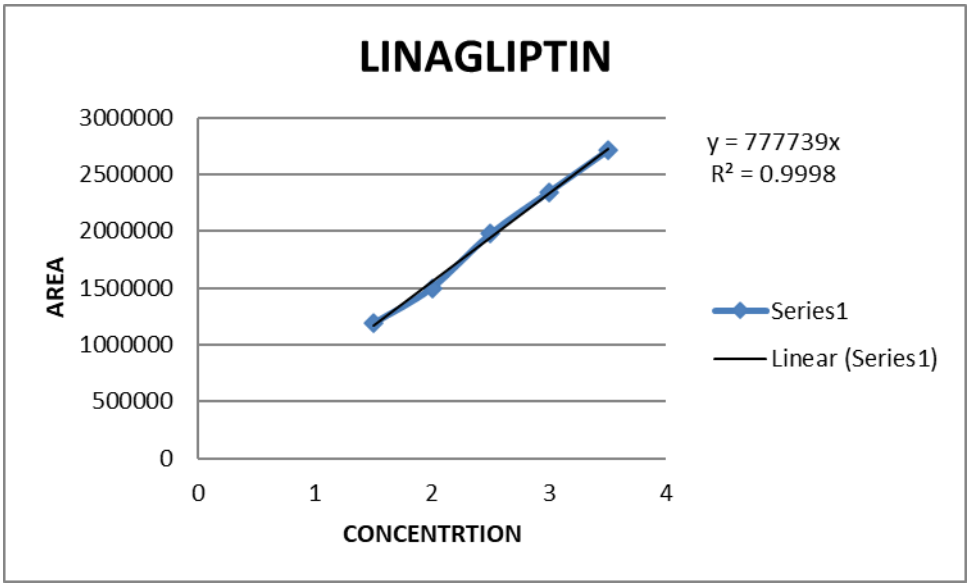


Table no- 20: Result and statistical data of LOD & LOQ of MET & LIN

METFORMIN HCl					
Sr.no	Concentration (µg/ml)	RT (min)	Area	Plate Count	USP Tailing
1	400	3.121	23278453	6581	1.29
2	450	2.932	25587653	6273	1.15
3	500	2.943	28785476	6164	1.21
4	550	2.878	31674635	6481	1.31
5	600	2.895	34657736	6415	1.28
Correlation Coefficient			0.9979		
Slope			57595		
SD			4543		
LOD			0.260298637		
LOQ			0.788783749		

LINAGLIPTIN HCl					
Sr.no	Concentration (µg/ml)	RT (min)	Area	Plate Count	USP Tailing
1	1.5	5.9	1185921	4285	1.61
2	2	5.92	1499674	4187	1.58
3	2.5	5.86	1976543	4104	1.6

4	3	5.83	2346537	4184	1.61
5	3.5	5.91	2711283	4114	1.62
Correlation Coefficient			0.9968		
Slope			777739		
SD			56767		
LOD			0.24086628		
LOQ			0.729897819		

D) Robustness:

Change in flow rate (±10%)

Table no- 21: Data for change in flow rate

Sr. No.	System Suitability parameter	Observations (MET/LIN)			Limits
		As Such	- 10%	+ 10%	
1	Peak area response	28733045/1904918	28737635/1904847	28736542/1904283	% RSD NMT 2.0
2	Theoretical plates	7783/5643	7536/5413	7923/5782	NLT 2000
3	Tailing factor	1.36/1.56	1.41/1.56	1.31/1.56	NMT 2.0
4	Retention Time (Min)	2.13/5.89	1.89/5.53	3.02/6.23	

IV. CONCLUSION

CONCLUSION

- ✓ In this project, as per our objective RP-HPLC method was developed by implementing QbD methodology with mobile phase methanol: water (70:30 v/v). The flow rate used was 0.8 mL /min and UV detection was carried out at 250 nm. The retention time for MET & LIN was found to be 2.6 & 5.6 min respectively.
- ✓ Before method optimization, screening studies were carried out on different mobile phases of varying composition. Based on the results obtained from these studies, suitable mobile phase with appropriate composition was selected and utilised for method development using QbD methodology.
- ✓ Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, risk assessment, design of experiment and validation.
- ✓ The study was done by using 3^3 Box Behnken response surface designs. In this study interaction of 3 factors; flow rate, column length and mobile phase composition vary at 3 levels. Effect of such critical process parameter on critical quality attribute of the method is studied. Responses in terms of retention times and resolution were evaluated throughout all the runs in design.
- ✓ By taking such runs Method Operable Design Region (MODR) also termed as Analytical Design Space (ADS) was developed

- ✓ A desirability function was applied to determine the optimum conditions. Optimum conditions were obtained; the one with higher desirability was selected. Replicates of run having optimized condition were taken to confirm the predicted response with actual response.
- ✓ The RP-HPLC method developed for estimation of MET & LIN was validated as per ICH Q2 (R1) guidelines using various parameters.
- ✓ Linearity for the drugs by the proposed method was determined to study its ability to elicit test results which are directly proportional to the concentration of analyte in the sample response and was found to be in the concentration range of 400-600 µg/mL for Metformin HCl & 1.5-3.5 µg/mL for Linagliptin with correlation coefficient of 0.9979 & 0.9968.
- ✓ The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were established at a signal-to-noise ratio. LOD and LOQ were calculated as $3.3\times\delta/S$ and $10\times\delta/S$ respectively as per ICH guidelines, where δ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. LOD was found to be 0.260 µg/ml and LOQ was found to 0.788 µg/mL for MET and for LIN it was found to be 0.240 µg/ml and LOQ 0.729 µg/mL
- ✓ System suitability test ensures that the analytical system is working properly and can give accurate and precise results. System suitability tests includes tailing factor, number of theoretical plates, area etc. The results of all system suitability parameters were acceptable in their limits defined by official guidelines.
- ✓ The proposed high-performance liquid chromatographic method has also been evaluated for accuracy, precision and robustness and proved to be convenient and effective for the quality control of Metformin HCl & Linagliptin.
- ✓ Moreover, the lower solvent consumption along with the short analytical run time of 10 min leads to a cost effective and environmentally friendly chromatographic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for MET & LIN.

V. REFERENCES

[1]. ICH Q8 (R2), 2009. Pharmaceutical Development, Part I: Pharmaceutical Development., http://www.ich.org/LOB/media//MEDIA4986.pdf.

[2]. ICH Q9, 2005. Quality Risk Management, http://www.ich.orghttp://www.ich.org/LOB/media//MEDIA1957.pdf.

[3]. ICH Q10, 2008. Pharmaceutical Quality Systems., http://www.ich.org.

[4]. ICH Q2 R1, VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY, 1994, http://www.ich.org

[5]. Indian Pharmacopoeia, 2010, Vol. III, The Indian Pharmacopoeia Commission, Ghaziabad, Appendix 3.1.

[6]. Bhatt D. A., Rane S. I., "QbD Approach to Analytical RP-HPLC Method Development and its Validation", Int J Pharm Pharm Sci 3(1), pp. 179-187.

[7]. Mohini Bajaj and Sanju Nanda, "Analytical quality by design (AQbD): new paradigm for analytical method development", International Journal of Development Research, February, 2015, Vol. 5, Issue, 02, pp. 3589-3599.

[8]. Phil Nethercote, Phil Borman, Tony Bennett, QbD for Better Method Validation and Transfer, available at- http://www.pharmamanufacturing.com/articles/2010/060/?Show=all

[9]. S. Karmarkar, X. Yang, R. Garber, A. Szajkovics, M. Koberda, "Quality by design (QbD) based development and validation of an HPLC method for amiodarone hydrochloride and its impurities in the drug substance", Journal of Pharmaceutical and Biomedical Analysis, 100 (2014), pp. 167–174.

[10]. B. Santhosha, A. Ravindranath, C. Sundari, "Validated method for the simultaneous estimation of Metformin Hydrochloride and Vildagliptin by RP-HPLC in bulk and the pharmaceutical dosage form", Int. Res J Pharm. App Sci., 2012; 2(3), pp. 22-28.

[11]. Subhakar Nandipati, V. Krishna Reddy, T. Ravindranadh Reddy, "Development and Validation of RP-HPLC method for Simultaneous Determination of Vildagliptin and Metformin in Bulk and Formulation Dosage", Int. Res J Pharm. App Sci., 2012; 2(3), pp. 44-50

[12]. P. G. Shelke, A. P. Dewani, R. L. Bakal, S. N. Vasu, A.S. Tripathi and A.V. Chandewar, "A validated RP-HPLC method for simultaneous determination of metformin HCL and Linagliptin in pharmaceutical formulation", International Journal of Advances in Pharmaceutical Analysis, Vol. 3 Issue 2 (2013), pp. 37-41.

[13]. A Madhusudhan Reddy, Ayesha Siddika, P Surya Bhaskara Rao, P Manasa, J Siddaiah, P Srinivasa Babu, "formulation and evaluation of sustained release matrix tablets of Metformin Hydrhocloride", Indian Journal of Research in Pharmacy and Biotechnology, Volume 1(2), March-April 2013, pp. 197-200.

[14]. Chandrabatla Varaprasad, Md. Asif, K.Ramakrishna, "RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND LINAGLIPTIN IN TABLET DOSAGE FORM", Rasayan J. Chem. Vol. 8, No.4, October – December, 2015, pp. 426 – 432.

[15]. S. Shirisha, M. Akiful Haque, D. Sireesha, Vasudha Bakshi, S. Harshini, "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin and Linagliptin in Combined Pharmaceutical Dosage Form", IJPRUR, Volume 2 (6), 2014, pp. 491-495.

[16]. Mrs Sheena Moncy, Miss G. Rohini Reddy, Mr. P. Sunil Kumar Chaitanya, Miss G.Priyanka, Miss E. Hima Bindu, "SIMULTANEOUS DETERMINATION OF METFORMIN HYDROCHLORIDE AND LINAGLIPTIN BY RP-HPLC IN BULK AND PHARMACEUTICAL FORMULATIONS", IAJPR, Vol 4, Issue 10, 2014, pp. 4047-53.

[17]. K. Sravana Kumari, B. Sailaja, "ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DIPEPTIDYL PEPTIDASE-4 INHIBITORS: A REVIEW", Int. J. Curr. Res. Chem. Pharma. Sci. 2 (4), 2015, pp. 83- 98.

[18]. Jadhav M. L., Tambe S. R., Implementation of QbD Approach to the Analytical Method Development and Validation for the Estimation of Propafenone Hydrochloride in Tablet Dosage Form, Chromatogr. Res. Int. 2013. Available on http://dx.doi.org/10.1155/2013/676501 23.

[19]. Ramzia I. El-Bagary, Ehab F. Elkady, Bassam M. Ayoub, "Spectrophotometric Methods for the Determination of Linagliptin in Binary Mixture with Metformin Hydrochloride and Simultaneous Determination of Linagliptin and Metformin Hydrochloride using High Performance Liquid Chromatography", Int J Biomed Sci vol. 9, no. 1, March, 2013, pp. 41-47.

[20]. K. Sujatha, J.V.L.N. Seshagiri Rao, "A NEW RP-HPLC METHOD FOR THE ESTIMATION OF LINAGLIPTIN IN TABLET DOSAGE FORMS", IAJPR, Vol 3, Issue 10, 2013, pp. 8376-81.

[21]. Sujan Banik, Palash Karmakar, Md. Anowar Hossain Miah, "Development and Validation of

a UV-Spectrophotometric Method for Determination of Vildagliptin and Linagliptin in Bulk and Pharmaceutical Dosage Forms", Bangladesh Pharmaceutical Journal 18(2), 2015, pp. 163-168.

[22]. Garg L.K., Sait S.S., Krishnamurthy T., Kumar C.P., 2014. Quality by Design (Qbd): A Practical Experimental Design Approach by Blocking and Varying Certain Factors of a Stability-Indicating HPLC Method for Simultaneous Determination of Omeprazole and Ketoprofen. J Liq Chrom Rel Tech, pp. 1-32.

[23]. Connors K.A., 1999. A textbook of Pharmaceutical Analysis, 3rd ed., John Wiley and sons, pp. 373-390.

[24]. Sethi P.D., 2001. "High Performance Liquid Chromatography", Quantitative Analysis of Pharmaceutical Formulations, 1st ed., CBS Publishers and Distributors, New Delhi, 2001, pp. 3-11, 116-120.

[25]. United States Pharmacopoeia, USP35-NF30, pp. 262-265, 2440-2441, 3959-3960.

[26]. Jaiprakash N. Sangshetti, Mrinmayee Deshpande, Zahid Zaheer, Devanand B. Shinde, Rohidas Arote, "Quality by design approach: Regulatory need", Article in press, Arabian journal of Chemistry.

[27]. S. Karmarkar, R. Garber, Y. Genchanok, S. George, X. Yang, and R. Hammond, "Quality by Design (QbD) Based Development of a Stability Indicating HPLC Method for Drug and Impurities", Journal of Chromatographic Science, Vol. 49, July 2011, pp. 439-46.

[28]. www.wikipedia.com, Accessed on 27th Dec. 2015, 4:00 PM.

Cite this article as :
Sh