QbD Approach for the Development and Validation of RP-UHPLC Method for Quantitation of Vildagliptin

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ABSTRACT: The present work describes a quality by design (QbD)-based rapid, simple, precise and robust RP-UHPLC method for the routine analysis of vildagliptin in bulk drug and in pharmaceutical dosage forms. Chromatographic separation was achieved by a X-bridge C_{18} column with isocratic elution of mobile phase containing mixture of phosphate buffer (pH 6.8) and acetonitrile in the ratio of 67:33(v/v). The flow rate was 1.0 ml/min and the detection was done at 239 nm with photo-diode array plus (PDA+) detector. The optimization of chromatographic method was carried out by QbD approach using design of experiments (DoE). Two factors utilized for the experimental design of the method were (i) independent variables which comprise percentages of acetonitrile in mobile phase and flow rate and (ii) co-variates which include the retention time, tailing factor and theoretical plates. This design was statistically analyzed by ANOVA, normal plot of residual, box-cox plot for power transform, perturbation, counter plot and 3D response surfaces plots. This was further validated as per the requirements of ICH-Q2B guidelines for linearity, LOD, LOQ, accuracy, precision, specificity and robustness. The results showed that proposed method is simple, sensitive and highly robust for routine analysis of vildagliptin.

Key words: Vildagliptin, UHPLC, quality by design (QbD), development and validation, quantitation

INTRODUCTION

Vildagliptin [(S)-1-[N-(3-hydroxy-1-adamantyl) gly-cyl] pyrrolidine-2-carbonitrile] (Figure 1) is an oral antidiabetic drug belonging to the class of dipeptidyl peptidase-4 inhibitor(DPP-4) and is used in the treatment of type 2 diabetes as second line antihyperglycemic therapy.[1-3] It is an oral incretin enhancer which acts to increase the levels of the incretin hormone glucagon-like peptide-1 (GLP-1) by inhibiting the dipeptidyl peptidase-4 responsible for the quick deactivation of GLP-1. As a result, glucose-dependent functioning of pancreatic islet β and α cells is improved.[4] Though, the drug is not included in official pharmacopeia, it is available in tablet form and given alone or in combination therapy with metformin, sulfonylurea or thiazolidinedione. Galvus® is the innovator product of vildagliptin manufactured by Novartis.[5,6]

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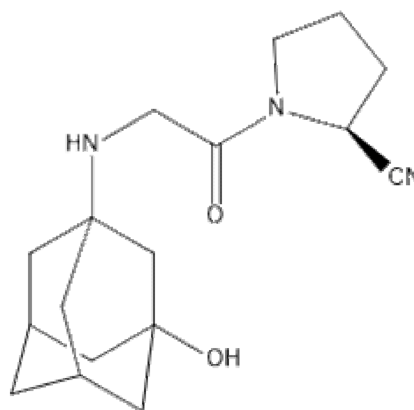


Figure 1. Structure of vildagliptin.

Different methods have been established for the determination of vildagliptin in various matrices like rat plasma[7], dog plasma[8] and in human plasma by using LC-ESI-MS/MS and HPLC[9,10] For estimation of vildagliptin alone and in combination few analytical methods i.e. HPLC, UV spectrophotometric methods have been reported.[11-15] Among these analytical methods, though LC-MS

method is most precise and accurate to analyze vildagliptin, it is not suitable for routine analysis in laboratory or pharmaceutical industries. However, UV spectro-photometry is the easiest method among these techniques but it has lack of accuracy, precision and needs relatively higher amount of analytes to be detected. Based on simplicity and sensitivity, HPLC is the best option to analyze vildagliptin as well as impurities determination. The currently available HPLC methods used either complex mobile phase system i.e. consisting more than two solvents or they have relatively higher retention time, low sensitivity etc. So, an attempt has been taken to develop a new RP-UHPLC method through QbD approach for the estimation of vildagliptin in bulk and tablet form with the aim to overcome these limitations. The concept of quality by design (QbD) has recently gained importance in the area of analytical method development by application of design of experiments approach (DoE). QbD involves understanding of the critical factors and their interaction effects by a desired set of experiments.

MATERIALS AND METHODS

Experimental materials and reagents. Vildagliptin powder (purity >99.92%; Dr. Reddy's Laboratories, India) was collected as a generous gift from Drug International Limited, Bangladesh. HPLC grade acetonitrile (RCI Labscan, Thailand), analytical grade potassium dihydrogen phosphate (Daejung Chemicals & Metal Co. Ltd., Korea) and nano pure water (Evoqua Water Technologies) were used. All other reagents used were of analytical grade. Galvus® tablets labeled to contain 50 mg of vildagliptin were collected from Novartis (Bangladesh) Limited.

Chromatographic conditions. The RP-UHPLC system consisted of Perkin Elmer Flexar series (autosampler, FX-15 binary pump, vacuum degasser, column oven and PDA plus detector) connected to a computer loaded with Chromera Manager Software was used for analysis. The chromatographic separation was achieved using X-bridge C18 (4.6 × 150 mm, 5 µm) column with isocratic elution of mobile phase consisting of mixture of phosphate buffer at pH 6.8 and acetonitrile (67 : 33) at a flow rate of 1 ml/min. The injection volume was 20 µl and the detection was done at 239 nm. All determinations were performed at 25°C.

Stock and standard solutions. Stock solution of vildagliptin (1 mg/ml) was prepared in mobile phase. A working standard solution of 50 µg/ml was prepared from the stock solution by suitable dilution with a mobile phase. Standard solutions over the concentration range of 10-50 µg/ml were prepared from the working standard using the mobile phase.

Sample preparation. Galvus® tablets were crushed to finely grinded powder. A stock sample solution of 1 mg/ml was prepared in mobile phase by transferring a weighed amount of the powder equivalent to 100 mg vildagliptin in a 100 ml volumetric flask containing 50 ml mobile phase. The solution was sonicated (Human Lab Instrument Co. Ltd., Korea) for 10 min and the volume was adjusted to the mark with mobile phase. The solution was then filtered (Whatman filter paper, Grade 1, 110 mm diameter) and stored in suitable container for further use. For assay of tablet, a sample solution of 50 µg/ml was prepared from the stock solution by dilution with the mobile phase and filtered through 0.22µm disc filter (Filter-Bio).

Method development and experimental design. A novel RP-UHPLC method was developed using a mobile phase containing mixture of phosphate buffer at pH 6.8 and acetonitrile (67:33) for achieving chromatographic separation. The experimental design and statistical analysis of data were performed by Design - Expert® software (version 10.0.3.1) using the Box-Behnken Design (BBD) since it requires fewer runs than a central composite design in cases of three or four variables to assess the robustness of the method.[16] The independent variables selected were the acetonitrile concentration in mobile phase (A, %v/v) and flow rate (B, ml/min). Retention time (RT) (R1, min), tailing factor (R2) and theoretical plate count (TP) (R3, N) were considered as covariates or independent variables. A 2-factor, 3-level BBD constructed 9

experimental runs. Dependent and independent variables along with different levels are presented in table 1. The significance of the design was determined by the evaluation of statistical parameters i.e. ANOVA method and Good fit evaluation. The optimization of the method parameters was done on the basis of response surface method.

Method validation

The developed method for the quantitation of vildagliptin was validated as per ICH guidelines.[17]

System suitability test. System suitability was established by injecting 20 µl each for six replicate injections of standard solution (50 µg/ml). The relative standard deviation (% RSD) and mean tailing factor of responses were calculated.

Linearity. Appropriate dilutions of standard stock solution of vildagliptin (10, 20, 30, 40 and 50 µg/ml) were assayed following the developed method. The limit of detection (LOD) and limit of quantitation (LOQ) were determined at the signal to noise ratio of 3:1 and 10:1, respectively.

Precision. Precision of the developed method was assessed by repeatability or intra-assay precision and intermediate precision analyses. Repeatability was determined from six replicate injections of 20 µl each of nominal standard solution (50 µg/ml). The nominal standard solution was analyzed for a period of six days with six replicate injections of 20 µl each on daily basis. The results of both the studies were compared (intermediate precision) and expressed as %RSD of the measurements.

Accuracy. To check for accuracy of the developed method as well as studying the interference of formulation additives on analysis the recovery experiments were carried out by spiking the sample solution with standard drug substance at 80%, 90%, 100%, 110% and 120%. All determinations were carried out in triplicate.

Specificity. Specificity was determined by injecting separately blank, standard and sample solution of vildagliptin in triplicate. The results were confirmed by the peak purity analysis.

RESULTS AND DISCUSSION

A factorial design using BBD was applied for observing the effect of two independent variables, the acetonitrile concentration in mobile phase (A), flow rate (B), on three responses- retention time (R1), tailing factor (R2) and theoretical plate (R3) as parameters for optimization of proposed method. The chromatographic conditions and ranges fixed for selected variables are given in table 1.

Table 1. Selection of independent variables and their levels.

Variables	Name	Unit	Type	Coded values			Actual values		
				Low	Mid	High	Low	Mid	High
A	ACN	%	Numeric	-1	0	1	25	30	35
B	Flow Rate	ml/min	Numeric	-1	0	1	0.8	0.9	1.0

Table2. Box-Behnken experimental design using variables and their responses.

Standard	Run	A: % of ACN	B:Flow rate (ml/min)	Retention time (min)	Tailing factor	Theoretical plate count
8	1	0	-1	2.95	1.372	5112
2	2	0	-1	3.50	1.386	5214
5	3	0	0	2.91	1.372	5112
6	4	1	0	2.96	1.453	5464
7	5	-1	1	3.55	1.291	5079
4	6	-1	0	4.03	1.303	5076
9	7	1	1	2.54	1.431	5272
3	8	1	-1	3.25	1.445	5646
1	9	-1	-1	4.57	1.311	5257

Table 3. ANOVA results for response R1 (RT).

Source	Sum of squares	Df	Mean square	F value	p-value	Significance
Model	2.79	2	1.40	23.39	0.0015	Significant
A- % of ACN	1.92	1	1.92	32.09	0.0013	
B-Flow Rate	0.88	1	0.88	14.70	0.0086	
Residual	0.36	6	0.060			
Cor Total	3.15	8				

Table 4. ANOVA results for response R2 (TF).

Source	Sum of squares	Df	Mean square	F value	p-value	Significance
Model	0.030	2	0.015	387.59	< 0.0001	Significant
A-% of ACN	0.030	1	0.030	765.37	< 0.0001	
B-Flow Rate	3.840E-004	1	3.840E-004	9.81	0.0203	
Residual	2.349E-004	6	3.915E-005			
Cor Total	0.031	8				

Table 5. ANOVA results for response R3 (TP).

Source	Sum of squares	Df	Mean square	F value	p-value	Significance
Model	2.281E+005	2	1.141E+005	9.26	0.0147	Significant
A-% of ACN	1.568E+005	1	1.568E+005	12.73	0.0118	
B-Flow Rate	71286.00	1	71286.00	5.79	0.0429	
Residual	73907.33	6	12317.89			
Cor Total	3.020E+005	8				

Table 6. Summary statistics for response R1, R2 and R3.

Response	R1(RT)	R2(TF)	R3(TP)
Std. Dev.	0.24	6.257E-003	6.257E-003
Mean	3.37	1.37	1.37
C.V. %	7.26	0.46	0.46
PRESS	0.69	5.832E-004	5.832E-004
R-Squared	0.8863	0.9923	0.7553
Adjusted R-Squared	0.8485	0.9898	0.6737
Predicted R-Squared	0.7823	0.9809	0.4748
Adequate Precision	13.433	43.554	8.448

Table 7. The optimized method according to design of experiment.

Method	%ACN	Flow Rate (ml/min)	RT (min)	TF	TP	Desirability
Predicted level	0.643	0.854	2.790	1.315	5291.514	1.000
Actual values	33.25	0.985				
Experimental	33.0	1.0	2.754	1.326	5302.261	
Deviation (%)			0.912	0.591	0.143	

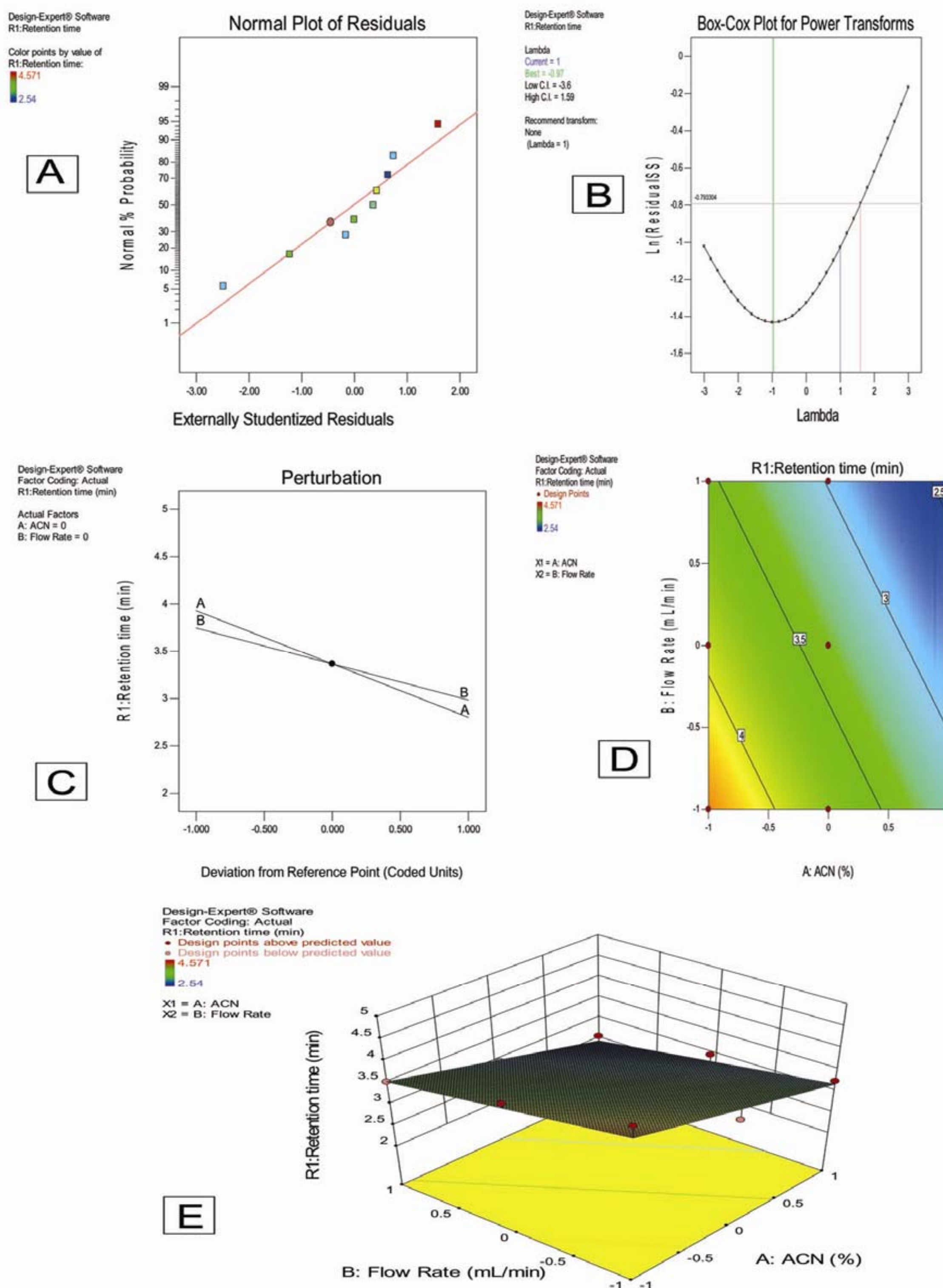


Figure 2. (A)-Normal plot of residual, (B)-Box-Cox plot for power transform, (C)- Perturbation, (D)-Counter Plot, (E)-3D response surfaces effect on R1.

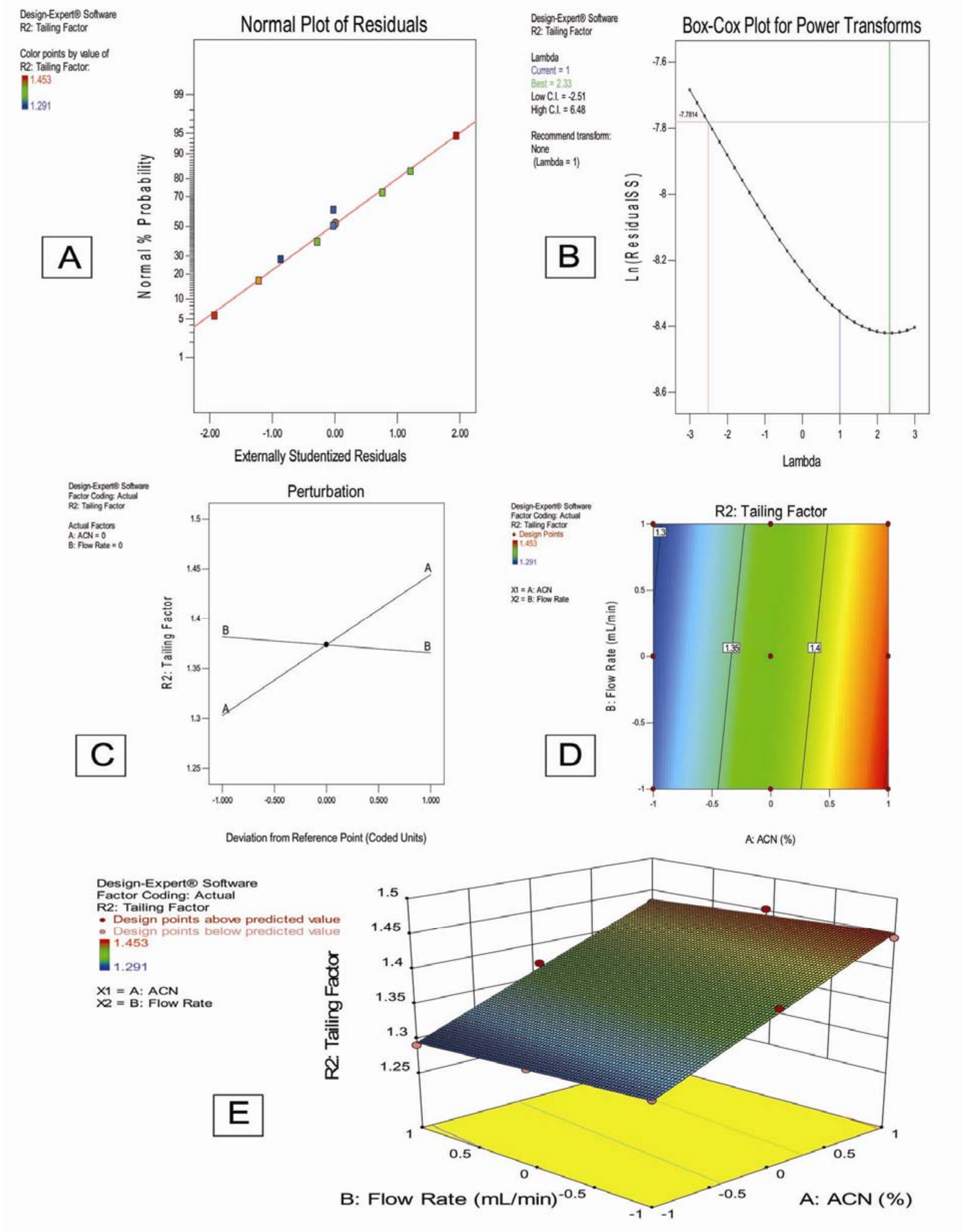


Figure 3. (A)-Normal plot of residual, (B)-Box-Cox plot for power transform, (C)- Perturbation, (D)-Counter Plot, (E)-3D response surfaces effect on R2.

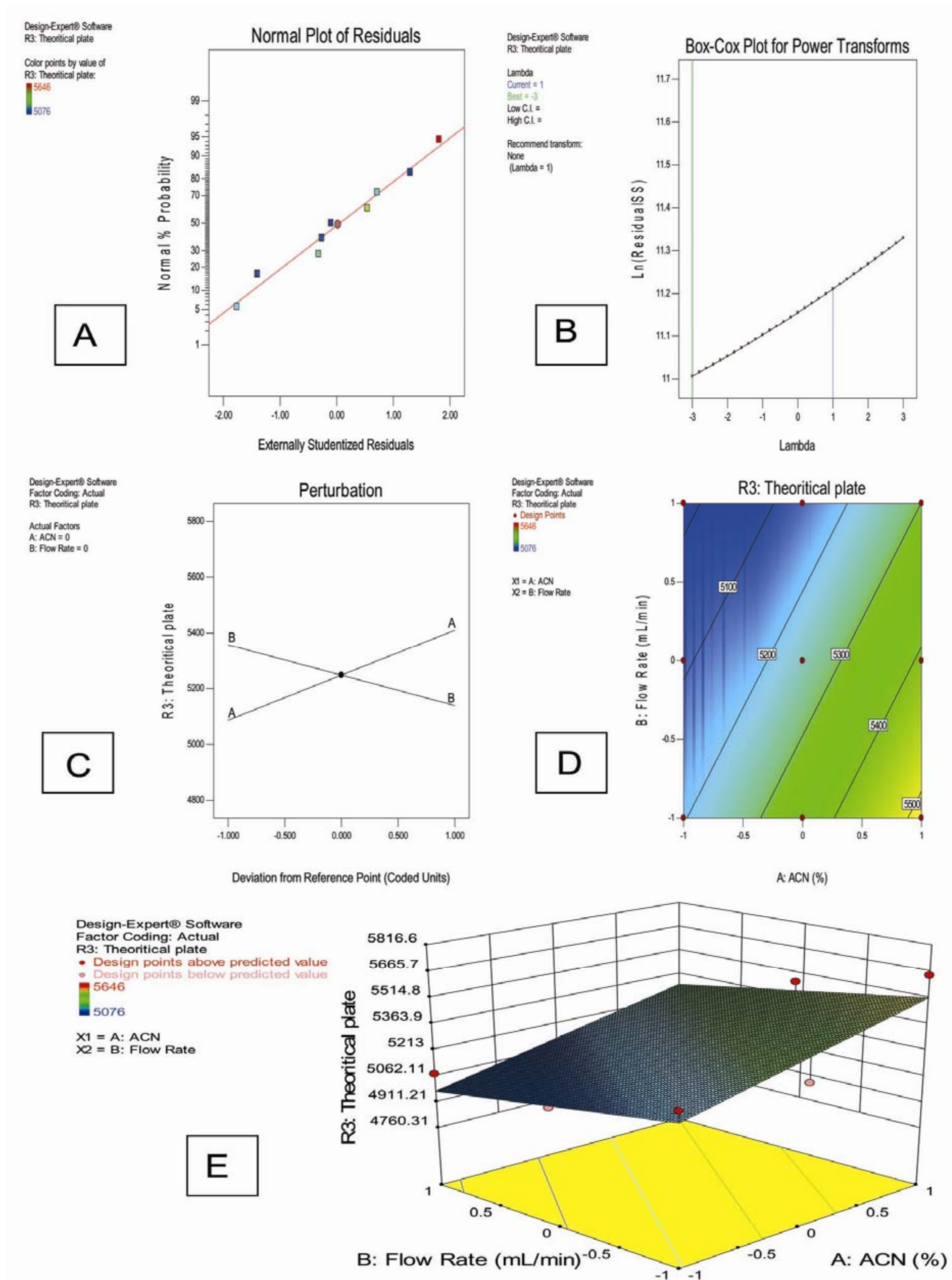


Figure 4. (A)-Normal plot of residual, (B)-Box-Cox plot for power transform, (C) - Perturbation, (D)-Counter Plot, (E)-3D response surfaces effect on R3.

The sum of total 9 runs was obtained for the fixed variables in order to test the predictive validity of the model. Each combination of mobile phase composition and flow rate suggested by BBD were finally run on the system and observed for the responses such as retention time, tailing factor and theoretical plate count as 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 coefficients for all three responses R1, R2 and R3 as compared to other models.

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. Additionally, normal plot of residual indicated all the data were concentrated along the model fit line and there was no observable outlier in the data (Figure 2, 3, 4-A). Furthermore, the model was validated by the application of Analysis of Variance (ANOVA) which showed that the model was significant. The quadratic equation of all model responses R1, R2 and R3 are as follows:

R1 (Retention time) = +3.37-0.57A-0.38 B

R2 (Tailing factor) =+1.37+ 0.071A-(8.00E-003) B

R3 (Theoretical plate) = +5248.0+161.66667A-109.0B

The results of ANOVA for responses R1, R2 and R3 showed that the model F-value of 23.39, 387.59 and 9.26 respectively implies the models are significant. The p-values for the model terms showed that both the variables A and B are significant ($p<0.05$) (Table 3, 4, 5) in all cases.

From the table 6, the predicted R-squared for all responses R1 (0.7823), R2 (0.9809) and R3(0.4748) are in reasonable agreement with the adjusted R-squared values of 0.8485, 0.9898 and 0.6737, respectively i.e. the difference was less than 0.2 in each case. The signal to noise ratio was measured by the adequate precision. The ratio of 13.433, 43.554 and 0.4748 indicate an adequate signal (ratio > 4.0). These models can be used to navigate the design space.

According to the 3D response surfaces and quadratic model equation it is observed that variables A and B both have negative effect on RT (Figure 2) and the variable A has positive effect and B has negative effect on both TF (Figure 3) and TP (Figure 4), hence it shows that the relationship between factors and response is not always linear, when one or more than one factor is altered simultaneously then a factor can result in different grade of responses. The statistical results for RT, TF and TP indicated

Table 8. Accuracy of vildagliptin.

Spike level (%)	% Recovered ± %RSD
80	99.54 ± 0.02
90	100.20 ± 0.04
100	99.88 ± 0.13
110	98.25 ± 0.06
120	101.50 ± 0.03

Table 9. Summary of inter-day and intra-day precision for vildagliptin.

Inter-day	Time (hr)	0	1	2	4	8	12
	Assay (%)	98.56	98.20	99.58	98.94	100.3	101.2
	Mean ± SD	99.63 ± 0.971					
	%RSD	0.975					
Intra-day	Day	1	2	3	4	5	6
	Assay (%)	100.1	99.40	99.21	99.15	98.88	98.75
	Mean ± SD	99.25 ± 0.478					
	%RSD	0.481					

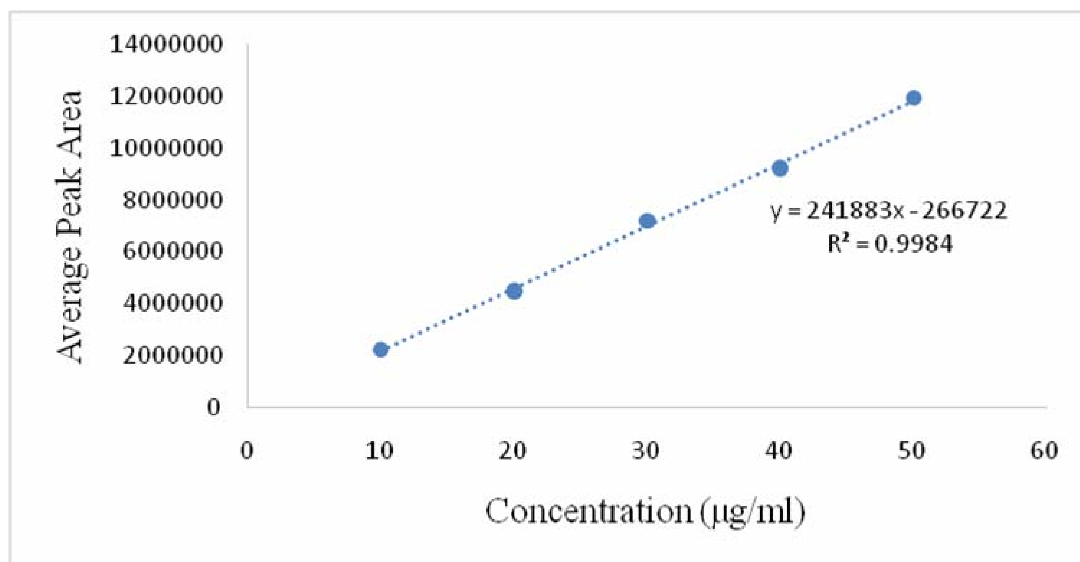
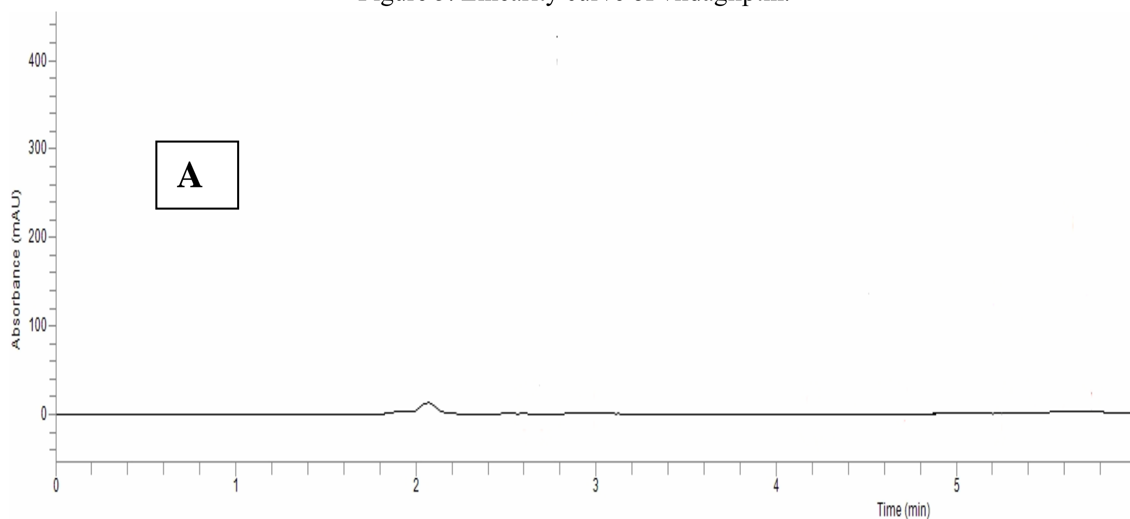
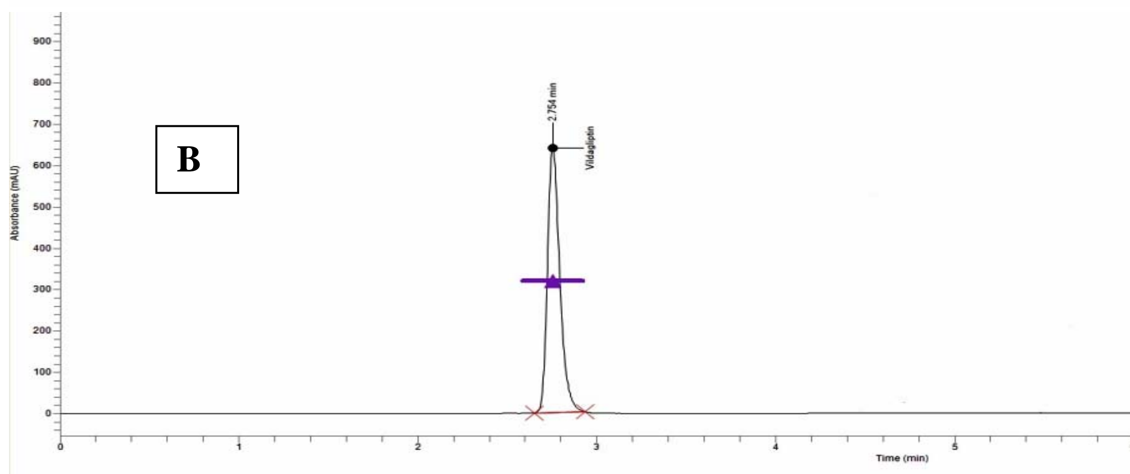


Figure 5. Linearity curve of vildagliptin.





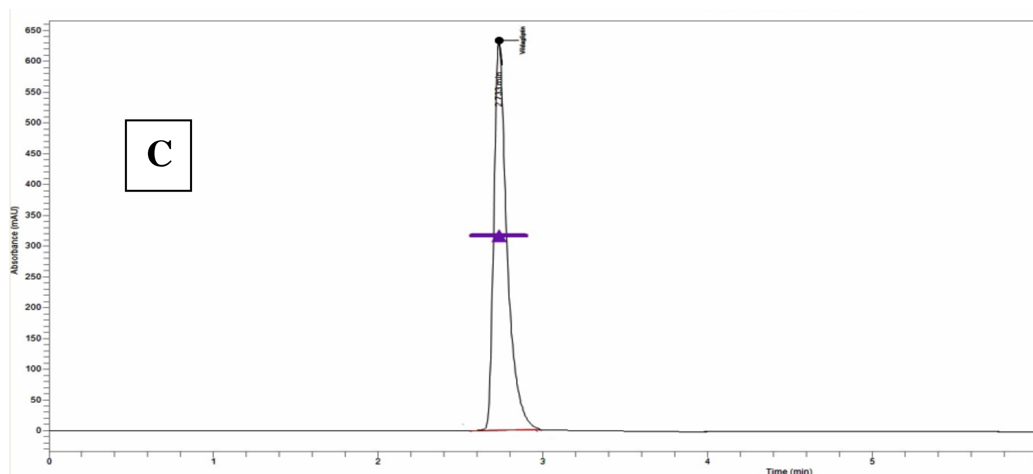


Figure 6. Chromatogram of (A)-Blank, (B)-Standard and (C)-Sample.

that the analytical method was robust since variations in the experimental conditions did not affect on the quantitative analysis of vildagliptin. The experimental results of the predicted method were found to be analogous with the suggested responses and all the results fall within the level of acceptance as shown in table 7 (NMT 2.0%).

Validation of the method

A good linear relationship (R^2=0.9984) was observed between the concentrations of vildagliptin and the respective peak areas. The regression curve was constructed by linear regression and its mathematical expression was Y = 241883X–266722, where Y is the peak area and X is the concentration of vildagliptin (Figure 5). The LOD and LOQ values for the proposed method were found to be 0.01 µg/ml and 0.05 µg/ml, respectively. The method showed that the % RSD of recovery study was not more than 2%. (Table 8). The method was also precise and the % RSD for repeatability and intermediate precision were within the limit (NMT 2%) as shown in table 9. The UHPLC chromatograms recorded for the blank, standard and sample separately revealed that vildagliptin is clearly separated from the response of any interfering peak (Figure 6).

CONCLUSION

The developed RP-UHPLC assay method for vildagliptin by QbD approach was found to be linear, precise, reproducible, accurate and specific. The retention time and very low detection limit of this method indicated its high sensitivity, simplicity and selectivity. The method seems to be suitable for the quality control in the pharmaceutical industry and also for quantitation of drug substances in biological fluid during *in vivo* studies.

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