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Development and Validation of RP-HPLC Assay Method for Vildagliptin Using Qbd Approach and Its Application to Forced Degradation Studies

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ABSTRACT

The concept of Quality by design (QbD) has recently gained importance in the area of analytical method development and involves understanding of the critical factors and their interaction effects by a desired set of experiments. So, the present work describes the development of Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method by QbD approach using Design of Experiments and subsequent validation for analysis of Vildagliptin in bulk drug and its pharmaceutical formulation. An efficient experimental design based on systematic scouting of all three key components of the RP-HPLC method (Buffer pH, Organic Phase-% acetonitrile, Organic Modifier-Methanol) is presented. The significance and interaction effects of these parameters on the response variables (Retention time and tailing factor) were evaluated through statistical analysis tools like Analysis of Variance (ANOVA) and plots which revealed the final chromatographic conditions of the method. The developed method was validated and Forced degradation studies were also carried out in order to determine the stability-indicating nature of the method. The chromatographic separation was achieved on Jasco CrestPack RP C18 (250 × 4.6 mm, 5 μ) column using Buffer (pH 6): Acetonitrile: Methanol (70:10:20 v/v) as mobile phase and detection was done using Photo-Diode Array (PDA) detector at 210 nm. The developed method of Vildagliptin is linear over a range of 5-15 μ g/ml having correlation coefficient R^2 value as 0.999. The %RSD for precision and accuracy of the method was found to be less than 2%. Forced Degradation studies revealed that the method was found to be stability-indicating. The results showed that the proposed method is suitable for the precise and accurate determination of Vildagliptin in bulk and its formulation.

Keywords: Vildagliptin, Quality by design approach; RP-HPLC, Validation, Forced degradation studies.

INTRODUCTION

Quality by design (QbD) has become an important paradigm in the pharmaceutical industry since its introduction by the US Food and Drug Administration

(USFDA). [1-2] 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. QbD involves understanding of the critical factors and their interaction effects by a desired set of experiments. This article describes how statistically QbD principles can be put into practice to develop optimized chromatographic conditions for the HPLC method. The experimental runs were conducted as per the Box-Behnken statistical screening design. Under this design, factors such as Buffer pH, Organic

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Phase (% acetonitrile) and Organic Modifier (Methanol) were screened and optimized.

Vildagliptin is an oral antidiabetic drug of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. These DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to reduce hyperglycemia in patients with type 2 diabetes. Vildagliptin acts on the incretin system. An incretin hormone Glucagon-like peptide 1 (GLP-1) is released in the gut wall after food ingestion from the L-cells. This hormone inhibits glucagon secretion and stimulates insulin secretion and rapidly eliminated by DPP-4. [3] Vildagliptin thus inhibits DPP-4, allowing GLP-1 to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas. [4] Chemically it is (2S)-{[(3-Hydroxyadamantan-1-yl) amino] acetyl} pyrrolidine-2-carbonitrile (Fig. 1). The drug is not official in pharmacopoeia. It is White to off-white solid powder that is soluble in water and Dimethyl sulfoxide (DMSO).

Literature survey revealed that few analytical methods such as UV spectrophotometry [3] and HPLC [4-6] methods have been reported for the estimation of Vildagliptin. But since the existing methods are not stability indicating, have less sensitivity, accuracy and precision, the objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC method using QbD approach for the estimation of Vildagliptin in bulk and tablet form. The chromatographic conditions for the proposed method were optimized with the help of design expert 9.0. Furthermore, the developed RP-HPLC method was used for forced degradation study of Vildagliptin in different stress conditions in order to establish inherent stability of the drug. The method was further validated and the results of analysis were validated statistically and by recovery studies. The developed method was found to be simple, precise, accurate, economic and thus can be used for *in-vitro* analysis of Vildagliptin.

MATERIALS AND METHODS

Instrumentation

The analysis of the drug was carried out on a JASCO PU-2089 LC system equipped with Quaternary Gradient HPLC Pump and Photo Diode Array Detector using a Reverse phase HPLC column. The output of signal was monitored and integrated using ChromNAV Chromatogram Software.

Chemicals and reagents

The working standard of Vildagliptin was provided as a gift sample from Cipla Pvt. Ltd., Vikhroli. Mumbai. The marketed formulation i.e. GALVUS tablets containing 50 mg Vildagliptin were procured from local market. Disodium hydrogen phosphate (AR Grade), o-phosphoric acid (GR Grade), HPLC-grade methanol and acetonitrile were purchased from E. Merck, Mumbai, India. HPLC grade water was

obtained by double distillation and purification through milli-Q water purification system.

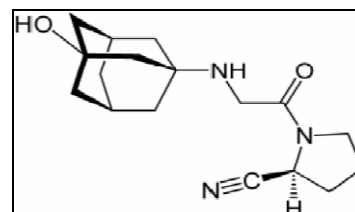


Fig. 1: Chemical structure of Vildagliptin

Table 1: Experimental trials for choice of column

Column	Observation	Inference
C ₈	Poor retention of analyte	Broad and poor peak shape
C ₁₈	Improved retention of analyte	Better peak shape

Table 2: Experimental trials for choice of mobile phase

Mobile Phase composition	Observation	Inference
Water: Acetonitrile	No precision in Retention time. Broad Peak with tailing	Use of buffer required and use of methanol to improve peak shape
Water: Methanol	No precision in Retention time. Better peak shape	Use of buffer and Methanol required.
Water: Acetonitrile: Methanol	No precision in Retention time. Good Peak shape	Use of buffer, acetonitrile and methanol required.

Preparation of Standard Solution

10 mg of working standard of Vildagliptin was accurately weighed and transferred to 10 ml of volumetric flask, added about 4 ml of distilled water (diluent) and sonicated to dissolve. The solution was then cooled to room temperature and the volume was made with diluent to give stock solution of 1000µg/ml (solution A). 1.0 ml of solution A was transferred into a 10 ml volumetric flask and diluted to volume with diluent to give 100µg/ml solution (Solution B). Then, 1.0 ml of Solution B was diluted to 10 ml with diluent to give 10µg/ml solution which was used as the standard solution.

Chromatographic conditions

The isocratic flow rate of Mobile phase was maintained at 1.0 mL/min and the analysis was carried out at ambient column temperature. The injection volume was 20µl. Eluted sample was monitored at 210 nm and the run time was 10.0 min.

Initial method development

Choice of Column

In order to choose the appropriate column, initial experimental runs were carried out as shown in Table 1 and 2. According to the observations of above initial trials and its chromatograms, C₁₈ column was selected for further trials.

Software aided method development

A new Reverse Phase-HPLC method was developed for the determination of Vildagliptin by using QbD approach. A Quality by Design with Design of Experiments approach to the development of an

analytical method mainly involves two phases as follows:

- Screening Phase
- Statistical Analysis and Final Optimization

Screening Phase

A new Reverse Phase-HPLC method was developed for Vildagliptin using Design Expert 9 software. In this software, Box-Behnken statistical screening design was used to optimize the Critical Process Parameters (CPP) or Critical Method Parameters (CMPs) and to evaluate interaction effects of these parameters on the Critical Quality Attributes (CQAs). This Box-Behnken statistical screening design is a 3 factor-2 level design which was specifically selected since it requires fewer experimental runs than other screening designs.

This Screening Phase includes the following steps:

Selection of Critical Method Parameters

Critical Method Parameters are selected number of factors that impact on the analytical technique under development. So, the Critical Method Parameters selected for the study are Buffer pH, Organic Phase (% acetonitrile) and Organic Modifier (Methanol).

Selection of Critical Quality Attributes (CQAs)

Critical Quality Attributes are the responses that are measured to judge the quality of the developed analytical methods. So, the Critical Quality Attributes selected for the study are Retention time and Tailing Factor. These responses were monitored during the experimental trials.

Experimental Trials

As per the Box-Behnken statistical screening design, low, medium and high levels of the critical method parameters were selected based on the preliminary experimentation. So, the Design summary for Box-Behnken screening design is given in Table 3.

Evaluation of all of the above critical method parameters with a Box-Behnken design lead to 12 experimental trials due to permutation and combination of the three parameters. These 12 experimental trials were carried out using the aforementioned chromatographic conditions using the previously selected JASCO CrestPack RP C18 column.

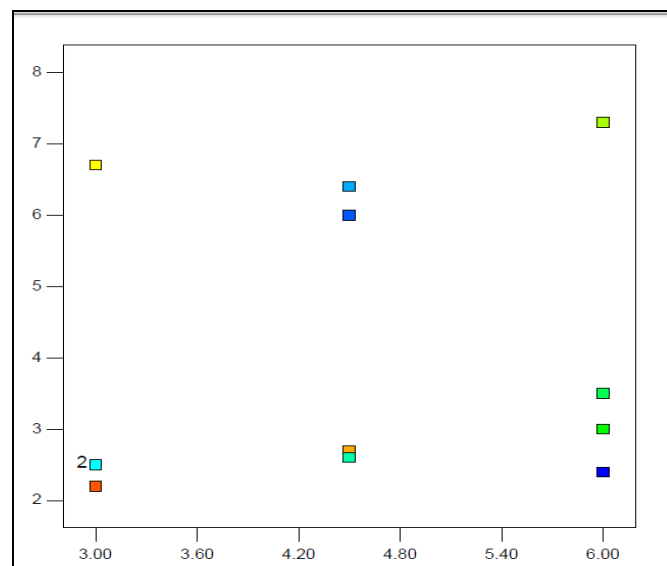
Statistical Analysis and Final Optimisation

The responses obtained after carrying out the above trial runs were fed back to Design Expert software and plots like 3D-response surface plots and Graph plots were plotted. These plots revealed the influence of critical method parameters on the selected quality attributes. The analysis of these plots was used to estimate as to which method parameter gave the most acceptable responses. Thus, based on these observations, the final critical method parameters of the method were determined and the optimized chromatographic conditions were finalized.

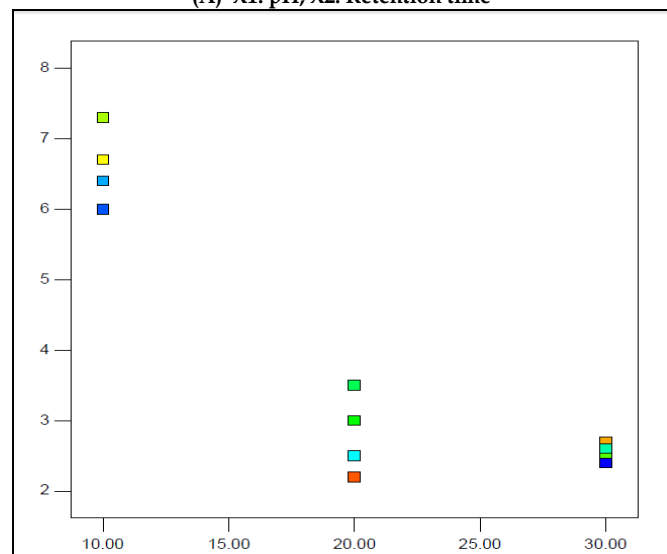
Moreover, the evaluation of statistical analysis tool like ANOVA for each individual response was used to determine the significance of each method parameter selected for the study using the p value (probability).

Validation of the optimized method

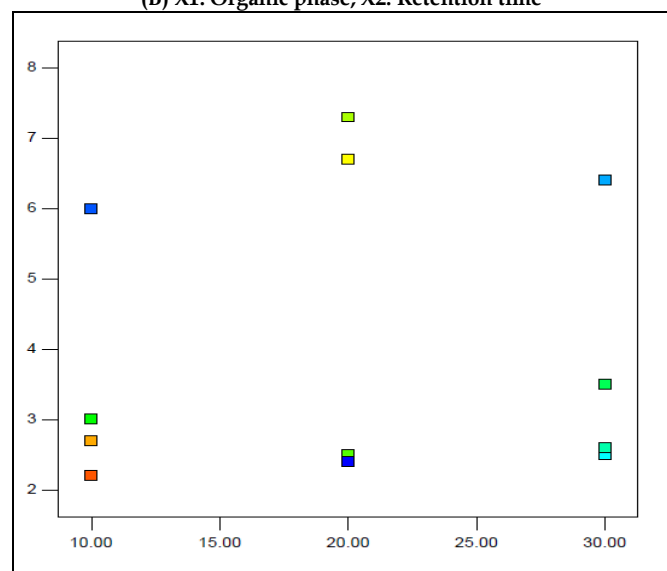
Validation of analytical procedures was performed for Vildagliptin using the following parameters [7]:



(A) X1: pH, X2: Retention time



(B) X1: Organic phase, X2: Retention time

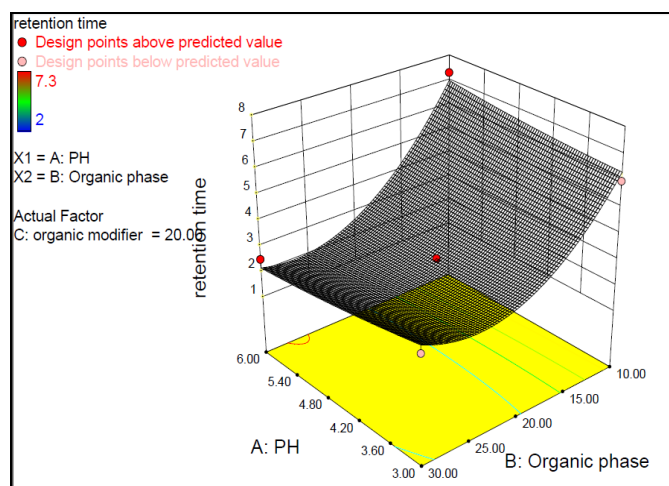


(C) X1: Organic modifier, X2: Retention time

Fig. 2: Graph Plots for Retention Time

Table 3: Design Summary for screening studies

Critical Method Parameters	Type	Low level	Medium level	High level
Buffer pH	Numeric	3	4.5	6
Organic Phase (%acetonitrile)	Numeric	10	20	30
Organic Modifier (Methanol)	Numeric	10	20	30

**Fig. 3: 3D Response-Surface Graphs for Retention Time**

System Suitability

System suitability testing is an integral part of any analytical procedure. System suitability testing was carried out by injecting 6 replicates of 10 µg/ml standard Vildagliptin solution. In this test, system suitability parameters like retention time, number of theoretical plates and tailing factor were evaluated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solution of Vildagliptin using the developed HPLC method. This was done until a signal to noise ratio of NLT 3:1 and NLT 10:1 is maintained for LOD and LOQ respectively.

Specificity

Specificity of the method was determined by recording the chromatogram of standard stock solution of Vildagliptin (10 µg/ml) and blank chromatogram (only diluent). Specificity signifies the identification of analyte, interference from other peaks and peak purity.

Linearity and Range

The Linearity of the method was evaluated in the range of 50% to 150% of the working concentration level i.e. 10 µg/ml for Vildagliptin. The Linearity of response was determined by preparing different concentrations of standard solution i.e. 5 µg/ml (50%), 8 µg/ml (80%), 10 µg/ml (100%), 12 µg/ml (120%) and 15 µg/ml (150%). Then each level was injected six times into HPLC, chromatograms were recorded and peak area was recorded for all the peaks. The calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/ml.

Precision and Accuracy

The Precision is reported in terms of Relative Standard deviation (RSD) over the range of quantitation for a single experiment in which standards are assayed in

replicate (Intraday) and for a series of experiments in which standards are assayed in over several experiments (Interday). Precision of the developed analytical method was tested by injecting three replicate injections of concentration 5 µg/ml, 10 µg/ml and 15 µg/ml (50%, 100% and 150% of the working level). Intraday and interday precision study was carried out by estimating the corresponding responses for the solutions of above 3 concentration levels on the same day and on 3 different days respectively. Accuracy was calculated for the same solutions which were injected for Intraday Precision.

Analysis of marketed formulation

10 units of Galvus Tablets containing Vildagliptin were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg of Vildagliptin was transferred into a 10 ml volumetric flask and sonicated for 20 min with 7 ml of Distilled water (diluent). The resulting suspension was filtered through Whatman 1 filter paper and diluted up to 10 ml with diluent. A suitable aliquot of this filtrate was diluted with diluent in order to obtain a final concentration of 10 µg/ml. A 20 µl of the obtained solution was chromatographed.

Stability Indicating Assay of Vildagliptin [8]

To prove the stability indicating nature of the method, the stock solution of the drug Vildagliptin was stressed under different conditions as follows to promote degradation. The standard solution and the stressed solutions were prepared as follows:

Preparation of Standard Solution

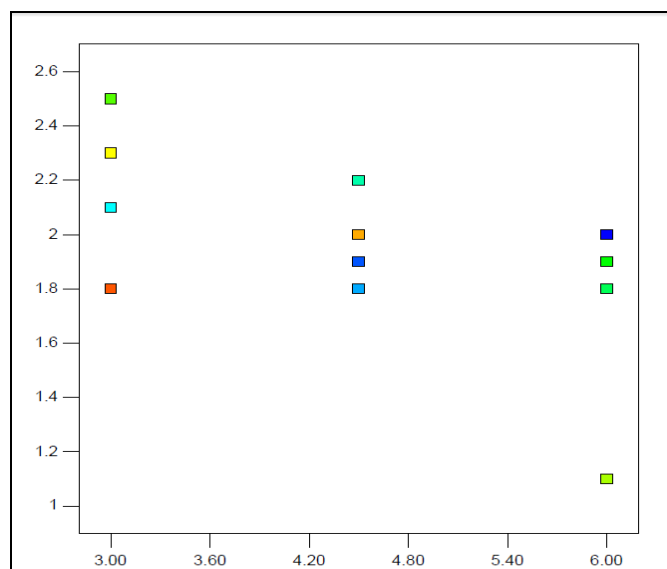
5 mg of working standard of Vildagliptin was accurately weighed and dissolved in 50 ml of Distilled water (diluent) to give a solution of 100 µg/ml. This solution was the untreated standard solution.

Acid Hydrolysis

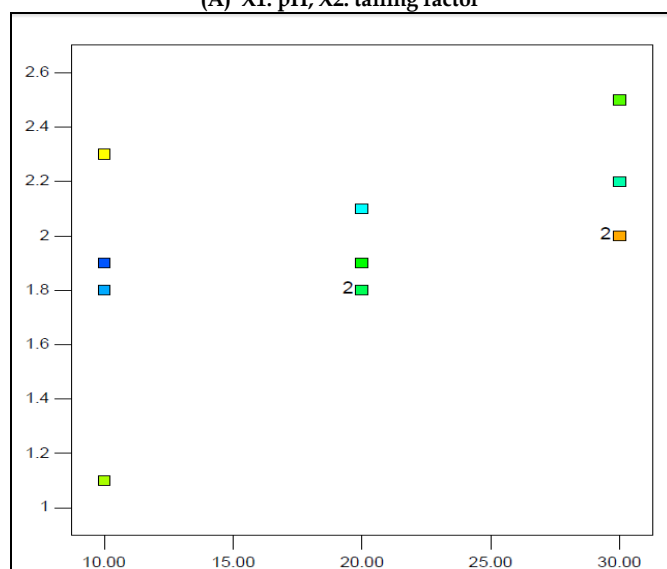
For acid hydrolysis, 5 mg of drug was accurately weighed and transferred to a round bottom flask containing 25 ml of diluent. Then, 5 ml of 1N HCl was added in round bottom flask and this mixture was refluxed on water bath for 2 hours at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to 50 ml volumetric flask, neutralized with the corresponding base and volume was made up with diluent. Finally this solution was loaded into HPLC and the corresponding chromatogram was recorded.

Base Hydrolysis

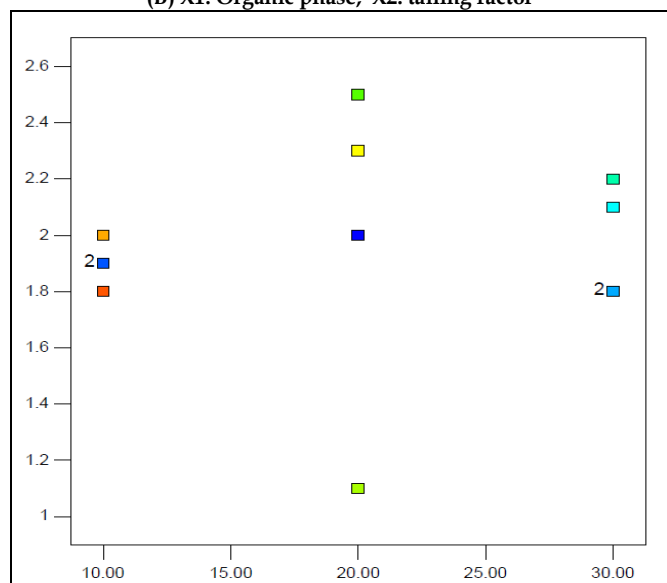
For base hydrolysis, 5 mg of drug was accurately weighed and transferred to a round bottom flask containing 25 ml of diluent. Then, 5 ml of 0.01 N NaOH was added in round bottom flask and this mixture was refluxed on water bath for 30 minutes at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to 50 ml volumetric flask, neutralized with the corresponding acid and volume was made up with diluent. Finally this solution was loaded into HPLC and the corresponding chromatogram was recorded.



(A) X1: pH, X2: tailing factor



(B) X1: Organic phase, X2: tailing factor



(C) X1: organic modifier, X2: tailing factor

Fig. 4: Graph Plots for Tailing Factor

Oxidative degradation

For oxidative degradation, 5 mg of drug was accurately weighed and transferred to a 50 ml volumetric flask

containing 25 ml of diluent. Then, 3 ml of Hydrogen peroxide (6%) was added to this flask and the mixture was kept as such at room temperature for 30 minutes. After 30 minutes, the volume was made up with diluent. Finally this solution was loaded into HPLC and the corresponding chromatogram was recorded.

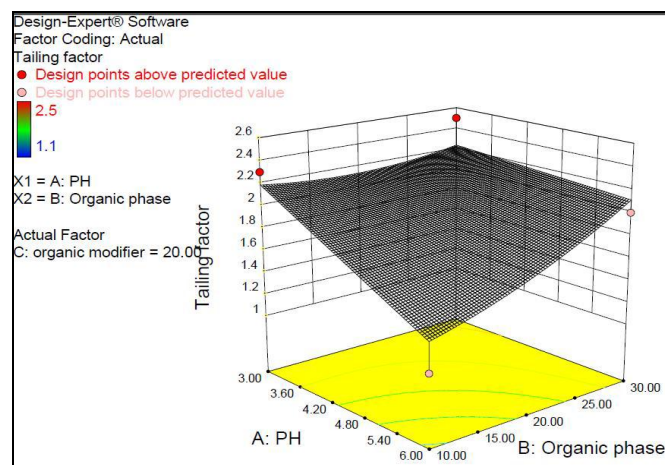


Fig. 5: 3D Response-Surface Graphs for Tailing Factor

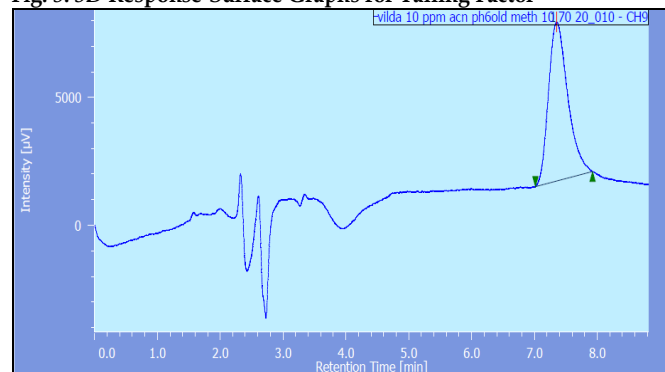


Fig. 6: Representative Chromatogram for Vildagliptin standard solution (10µg/ml)

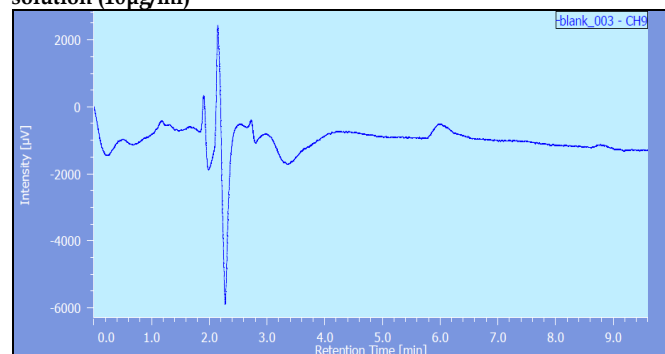


Fig. 7: Chromatogram of Blank run

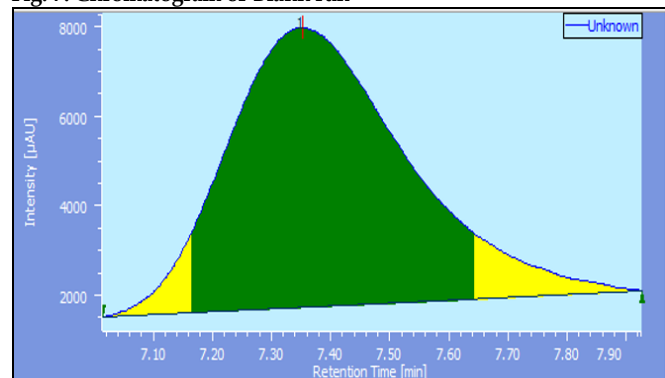


Fig. 8: Peak Purity for Vildagliptin solution (10µg/ml)

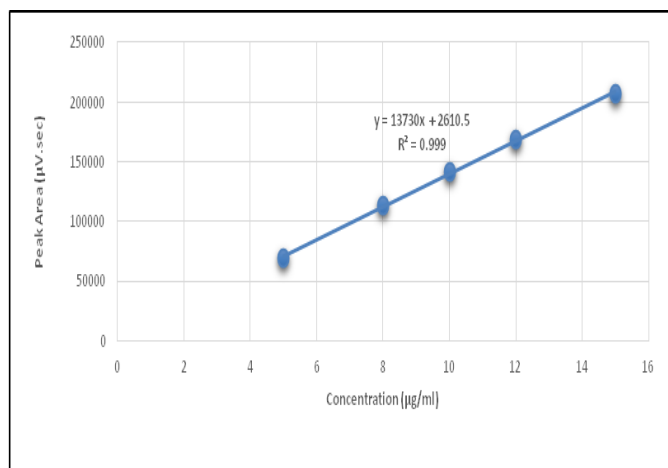


Fig. 9: Calibration Curve for Vildagliptin

Thermal degradation

For thermal degradation study, 5 mg of drug was accurately weighed and transferred to a round bottom flask containing 25 ml of diluent. Then, this solution was refluxed on water bath for 1 hour at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to 50 ml volumetric flask and volume was made up with diluent. Finally this solution was loaded into HPLC and the corresponding chromatogram was recorded.

Photolytic degradation

The photolytic degradation was carried out by exposing drug substance i.e. Vildagliptin (5 mg) under UV light at 290 nm for one week. After 1 week, the drug substance was dissolved in 50 ml volumetric flask containing 25 ml diluent and then the volume was made up with diluent. Finally this solution was loaded into HPLC and the corresponding chromatogram was recorded.

In all degradation studies, % recovery of the drug and % degradation products was calculated.

RESULTS AND DISCUSSION

Choice of Column

Vildagliptin is basic in nature (ionizes in acidic medium), it also has one adamantane backbone containing three condensed cyclohexane rings and a heterocyclic ring containing nitrogen. So, reverse phase chromatography is the best choice. The efficiency of two different reverse – phase columns C8 and C18 were evaluated. C18 column being hydrophobic was preferred for separation of drug because drug retention was a problem on C8 column. Moreover, use of water-acetonitrile and water-methanol lead to poor precision in retention of analyte. This clearly indicated that use of buffer is required in order to control the ionization of analyte.

Development and Optimization of new RP-HPLC method for Vildagliptin using QbD approach^[9-13]

A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

Screening Phase

The first phase of the method development involves the screening of the major effectors of selectivity and peak shape, primarily the buffer pH, organic mobile phase and organic modifier.

This screening phase was carried out using Design Expert 9 software. In this software, Box-Behnken statistical screening design was chosen to optimize the Critical Method Parameters wherein all the parameters were varied simultaneously unlike the conventional OFAT (one factor at a time) approach. The responses obtained after carrying out the 12 experimental trial runs under Box- Behnken design were fed back to DoE software. The values of responses (retention time and tailing factor) are tabulated in Table 4.

Statistical Analysis and Final Optimisation

Statistical Analysis was used to identify the significant influential chromatographic factors and their interaction impact on the two responses i.e. Retention time and Tailing factor. The analysis of 3D-response surface plots and Graph plots were used to estimate as to which method parameter gave the most acceptable responses.

Statistical analysis tool like ANOVA was evaluated for each individual response to determine the most influential chromatographic parameter. Moreover, these statistical analysis tools was used to determine the significance of each method parameter selected for the study. The significance level for probability of null hypothesis was defined at $p \geq 0.05$. Null hypothesis indicates variation in all factors which has no influence on the responses.

The two response variables i.e. Retention time and Tailing factor were statistically evaluated as follows:

Retention time

Retention time is one of the critical quality attribute under experimental design. The effect of most influential chromatographic parameters on Retention time was evaluated using different statistical analysis tools and plots which are described as follows:

Analysis of Variance (ANOVA)

The statistical inference from ANOVA reveals that the p value is significant at alpha (α) = 0.05. If value obtained is less than 0.05, indicating that the model explains a significant portion of variability. Hence null hypothesis can be rejected i.e. the factors have a significant effect on the responses. The ANOVA result for retention time is tabulated in Table 5.

The ANOVA result data reveals that ' p value' for the model is 0.0055 which indicates this model explains significant variability. Also, the Model F-value of 9.28 implies the model is significant. The "Pred R-Squared" of 0.4979 is in reasonable agreement with the "Adj R-Squared" of 0.6932. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.304 in the model indicates an adequate signal.

Moreover, the p value for pH and %acetonitrile is 0.0053 and 0.0008 respectively i.e. less than 0.05 which

indicates that both these parameters have significant effect on Retention time.

Graph Plots

The three graph plots indicate the values of retention time at different levels of pH, % organic phase and % methanol. In Fig. 2 and Fig. 4, the early retention times are neglected as there are chances of merging of early eluting analyte peak with the solvent peaks. Hence, peaks with retention time above 5 mins are only considered. Fig. 3 reveals that the retention time of analyte peak is less than 3 mins at 20% and 30% of organic modifier. Thus, 10% organic phase is suitable as it gives retention time of peak between 5 to 8 mins.

3D Response-Surface Graph

The response surface graph is a 3D plot with pH and %organic phase on X and Y axis respectively and retention time on Z axis. As observed in the Fig, The factor % organic modifier is kept constant as pH and %organic phase are most influential parameters of retention time which was evident from the ANOVA result. The above graph plot indicates that the retention time is above 5 mins at pH 6 and 10% organic phase and at pH 3 and 10% organic phase. While, the retention time is less at other data points, due to which these points are not considered.

Tailing Factor

Tailing factor is another critical quality attribute analysed under experimental design. The effect of most influential chromatographic parameters on tailing factor was evaluated using different statistical analysis tools and plots which are described as follows:

Analysis of Variance (ANOVA)

The statistical inference from ANOVA reveals that the *p* value is significant at alpha (α) = 0.05. If value obtained is less than 0.05, indicating that the model explains a significant portion of variability. Hence null hypothesis can be rejected. The ANOVA result for tailing factor is tabulated in Table 6.

The ANOVA result data reveals that '*p* value' for the model is 0.0128 which indicates this model explains significant variability. Also, the Model F-value of 7.46 implies the model is significant. The "Pred R-Squared" of 0.3962 is in reasonable agreement with the "Adj R-Squared" of 0.5510. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 6.237 in the model indicates an adequate signal.

Moreover, the *p* value for %organic modifier i.e. % methanol is 0.0471 i.e. less than 0.05 which indicates that this parameter has significant effect on tailing factor.

Graph Plots

The three graph plots indicate the values of tailing factor at different levels of pH, % organic phase and % methanol. As per USP, The tailing factor of any peak should not be more than 2. Generally, exact symmetrical peaks have tailing factor of 1. So, closer the value of tailing factor to 1, more symmetrical is the peak.

In the above plots, it is clearly evident that the tailing factor is closer to 1 at pH 6, 10% organic phase (acetonitrile) and 20% organic modifier (methanol).

3D Response-Surface Graphs

The response surface graph is a 3D plot with pH and %organic phase on X and Y axis respectively and tailing factor on Z axis. As observed in the Fig, The factor % organic modifier is kept constant to 20%. The above graph plot indicates that the tailing factor is closer to 1 when pH is 6 and organic phase is 10%. While, the tailing factor is more than 2 at other data points which is not within the acceptance limits.

Thus, the final optimized critical parameters predicted from the above plots are tabulated in Table 7.

The predicted result for the response variables estimated with 95% CI was found to be Retention time as 7.21 mins and tailing factor as 1.36.

Final Optimised Chromatographic Conditions

The final chromatographic conditions developed using Quality by Design approach is tabulated in Table 8.

Validation of the optimized method

Once the chromatographic conditions were set, method validation was done on Vildagliptin for: System suitability, Specificity, Limit of Detection, Limit of Detection, Linearity, Range, Accuracy and Precision.

Evaluation of System Suitability

The standard solution (10 μ g/ml) was injected six times. The %RSD obtained from six replicate injections was found to be less than 2.0%. Tailing factor was less than 2.0. Theoretical plates were also found to be above 2000. Thus all the parameters evaluated for system suitability were found to be within the acceptance criteria and the system was suitable for analysis of Vildagliptin.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ was obtained by successively decreasing the concentration of Vildagliptin as long as a signal to noise ratio of Not less than 3:1 and 10:1 is maintained respectively. The LOD of Vildagliptin was found to be 200ng/ml. The LOQ for Vildagliptin was found to be 600ng/ml. These values indicate that the method developed is sensitive.

Specificity

Blank (Diluent) and Standard solution (10 μ g/ml) were injected. The method was quite selective for Vildagliptin as there was no other interfering peak around the retention time of Vildagliptin (Fig. 7). Even the baseline did not show any significant peak (Fig. 6). In Fig. 8, the green part of peak purity graph corresponds to high purity of 97.22%. Hence, the standard peak of Vildagliptin was found to be pure at working concentration level. Representative chromatograms for specificity are shown as figures.

Linearity and Range

Linearity was evaluated in the range of 50% to 150% of the working concentration level i.e., 10 μ g/ml (5 μ g/ml-15 μ g/ml) for Vildagliptin. The Linearity was confirmed in the range of 5 μ g/ml - 15 μ g/ml. The Co-efficient of

Co-relation (R²) was found to be 0.999 and the equation of the line was $y = 73636x + 170.3$ as evident from the below calibration curve. Thus, the data shows that the response is found to be linear (Fig. 9). This clearly indicates that an excellent correlation existed between the peak area and concentration of the analyte.

Precision and Accuracy

Precision is reported in terms of Relative Standard deviation (RSD) over the range of quantitation for a single experiment in which standards are assayed in replicate (Intraday) and for a series of experiments in which standards are assayed in over several experiments (Interday). The data for intraday and interday is shown in Table 9 and 10 respectively. Accuracy was calculated for the same solutions which were injected for Intraday Precision. The results for Accuracy are shown in Table 11.

The precision of the method for the standard solution of Vildagliptin shows that the Relative Standard Deviation (RSD) for both intraday and interday falls within the limits i.e. within 2%. Moreover, the accuracy data shows that the % mean recovery of Vildagliptin at each level is within the acceptance criteria of 98.0% - 102.0%.

Stability Indicating Assay of Vildagliptin

The validated HPLC method was used to perform forced degradation studies on Vildagliptin. Forced degradation studies done on Vildagliptin indicated that the drug was degraded by 4.595%, 10.326% and 25.497% when subjected to acid hydrolysis, base hydrolysis and oxidation degradation respectively (Table 12). This shows that Vildagliptin is susceptible to acid hydrolysis, base hydrolysis and oxidation.

While, the drug was found to be stable after thermal degradation and photolytic degradation. The results of forced degradation studies reveal that all the degradation products were fully resolved and do not interfere with the analyte peak which indicates specificity of the method. Thus the method can be employed for monitoring the stability of Vildagliptin in bulk drug.

Moreover, these studies also determine the physical and chemical stability of drug substance and drug product which may be further useful to determine the storage conditions for the drug product. Since, Vildagliptin is susceptible to oxidation at room temperature, Vildagliptin tablets should be stored in a dry place as moisture is a catalyst of oxidation and low-moisture environment may sometimes resolve the problem of oxidation. Another alternative is to use an oxygen scavenger that helps to control the oxygen level within the headspace of a drug's primary packaging. This may help to maintain the drug potency and other properties under extended and variable storage and shelf conditions.

Application on Marketed Formulation

The developed Stability Indicating RP-HPLC method was successfully applied for the estimation of Vildagliptin from the marketed formulation of Galvus

tablets which was found to contain 98.92 % of the Label Claim.

Table 4: Factor screening by Box- Behnken Design

Run	Factor 1: pH	Factor 2: % ACN	Factor 3: Methanol	Response 1: Retention Time	Response 2: Tailing Factor
1	6	10	20	7.2	1.3
2	6	20	30	3.6	1.89
3	6	20	10	3.1	1.79
4	3	30	20	2.52	1.95
5	3	20	10	2.2	1.8
6	4.5	30	30	2.7	2.42
7	3	10	20	6.7	1.9
8	4.5	10	30	6.3	2.3
9	6	30	20	2.3	2.19
10	4.5	30	10	2.8	1.99
11	3	20	30	2.5	2.1
12	4.5	10	10	5.9	1.8

Table 5: ANOVA Result for Retention Time

	Model	Factor 1 : pH	Factor 2: % ACN	Factor 3: % Methanol
Sum of squares	31.93	40.10	31.13	0.15
Mean squares	10.64	40.10	31.13	0.15
F value	9.28	53.50	27.15	0.13
p Value	0.0055	0.0053	0.0008	0.7258
Std. deviation	1.07	-	-	-
R- squared	0.7769	-	-	-
Adj- R squared	0.6932	-	-	-
Predi- R squared	0.4979	-	-	-
Adequate precision	7.304	-	-	-

Table 6: ANOVA Result for Tailing Factor

	Model	Factor 1 : pH	Factor 2: % ACN	Factor 3: % Methanol
Sum of squares	40.23	0.042	0.20	0.22
Mean squares	14.82	0.042	0.20	0.22
F value	7.46	0.72	3.35	8.31
p Value	0.0128	0.4201	0.1044	0.0471
Std. deviation	1.37	-	-	-
R- squared	0.6961	-	-	-
Adj- R squared	0.5510	-	-	-
Predi- R squared	0.3962	-	-	-
Adequate precision	6.237	-	-	-

Table 7: Final Optimisation Result

Critical Method Parameters	Low level	Medium level	High level	Final Level selected
Buffer pH	3	4.5	6	6
Organic Phase (%acetonitrile)	10	20	30	10
Organic Modifier (Methanol)	10	20	30	20

Table 8: Final Optimised Chromatographic Conditions

HPLC Pump	Jasco PU-2089 Plus Quaternary Gradient HPLC Pump
HPLC Detector	Jasco MD-2018 Plus Photo Diode Array Detector
Column	JASCO CrestPack RP C18 (250 × 4.6 mm × 5μ)
Mobile Phase	0.01 M Disodium hydrogen phosphate buffer pH-6: Acetonitrile: Methanol (70:10:20)
Elution	Isocratic
Diluent	Distilled water
Wavelength	210 nm
Flow rate	1 ml/min
Injection volume	20μl

Table 9: Intraday Precision Data of Vildagliptin

Conc. (µg/ml)	Lin 1	Lin 2	Lin 3	Lin 4	Lin 5	Lin 6	Mean	SD	%RSD
5	69321	70423	68038	69895	70441	70500	69769.7	961.9	1.37
10	141052	140882	140800	141759	141520	141869	141313.7	462.2	0.32
15	211012	209859	210792	208159	211323	208395	209923	1367.6	0.65

Table 10: Interday Precision Data of Vildagliptin

Conc. (µg/ml)	Lin 1	Lin 2	Lin 3	Lin 4	Lin 5	Lin 6	Mean	SD	%RSD
5	70216	70552	69923	70517	69978	70543	70304.8	308.47	0.43
10	141227	140923	141396	140891	141450	141569	141242.67	288.52	0.20
15	211452	210975	211235	211396	210985	211556	211266.5	245.02	0.12

Table 11: Accuracy Results

Sr. No.	Levels in µg/ml			Levels in µg/ml		
	5	10	15	5	10	15
	Area			Recovery		
1	69321	141052	211012	4.86	10.08	15.18
2	70423	140882	209859	4.94	10.07	15.09
3	68038	140800	210792	4.77	10.06	15.16
4	69895	141759	208159	4.90	10.13	14.97
5	70441	141520	211323	4.94	10.11	15.20
6	70500	141869	208395	4.94	10.14	14.99
Mean	69769.67	141313.7	209923	4.90	10.10	15.10
SD	961.93	462.16	1367.6	0.068	0.033	0.099
%RSD	1.37	0.32	0.65	1.39	0.32	0.66
Accuracy%	-	-	-	98	101	100.67

Table 12: Result of Forced Degradation Studies

S. No.	Degradation Condition	Retention time of Degradants (mins)	% Degradation
1	Acid Hydrolysis	0.750, 5.465	4.595
2	Base Hydrolysis	4.068	10.326
3	Oxidation	4.128	25.497
4	Thermal Degradation	No Degradants	0
5	Photolytic Degradation	No Degradants	0

Thus, The RP-HPLC assay method developed for Vildagliptin by QbD approach is linear, accurate, precise, reproducible and specific as evident from the validation results. The developed method is also stability indicating and can be conveniently used for quality control to determine the assay in regular Vildagliptin product development, production and stability samples.

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REFERENCES

- Vogt FG, Kord AS. Development of Quality by design analytical methods. *J Pharm Sci.* 2011; 100(3): 797-812.
- Li Y, Terfloth GJ, Kord AS. A systemic approach to RP-HPLC method development in a pharmaceutical QbD environment. *Am Pharm Rev.* 2009; 12(4): 571-583.
- Naveed S, Rehman H, Qamar F, Zainab S. Method development and validation of Vildagliptin using UV spectrophotometer. *Int. J. Pharm Sci & Res.* 2014; 5(10): 714-717.
- Khatun R, Mirazunnabi Md. A Validated Reverse Phase HPLC Method for the determination of Vildagliptin from tablet dosage form. *Int. J. of Pharm. & Life Sci.* 2013; 2(3): 90-98.
- Sultana R, Bachar SC, Rahman F. Development and validation of stability indicating assay method of Vildagliptin in bulk and tablet dosage form by RP-HPLC. *Int. J. of Pharm. & Life Sci.* 2013; 4(4): 2530-2534.

- Ramachandra P, Vasudevan M, Chandan RS. Development and Validation of RP - HPLC Method for the Estimation of Vildagliptin in Pharmaceutical Dosage Forms. *World J Pharm & Pharm Res.* 2014; 3(2): 2125-2132.
- ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1).
- Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-A review. *J Pharm Anal.* 2014; 4(3): 159-165.
- Ayre A, Mane P, Ghude K, Nemade M, Gide P. Implementing Quality by Design-A methodical approach in the RP-HPLC method development process. *Int J Advances in Pharm Anal.* 2014; 4(1): 01-06.
- Reddy CM, Venkatram RH, Jinadataraya H, Kumar PC. Optimization technique as a tool for implementing analytical quality by Design. *Int. J. Drug Dev. & Res.* 2013; 5(3): 439-446.
- Kumar NS, Kumaraswamy R, Paul D. QbD Based RP-HPLC Method for Screening and Analysis of Telaprevir and 7 Other Antiretroviral Agents. *Indian Drugs.* 2015; 52(2): 20-30.
- Debrus B, Lebrun P, Rozet E, Schofield T, Mbinze JK, Marini RD, Rudaz S, Boulanger B. A New Method for Quality by Design Robust Optimization in Liquid Chromatography, LC-GC Europe July 2013.
- Turabi ZM, Khatatbeh OA. Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Form. *Int J Pharm Sci Drug Res.* 2014; 6(2): 154-159.

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