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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VILDAGLIPTIN BY USING QUALITY BY DESIGN APPROACH

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ABSTRACT
The objective of this study was to develop a new rapid and robust high-performance thin layer chromatographic (HPTLC) method for estimation of Vildagliptin in tablet dosage form using quality by design approach. Chromatography was performed using pre-coated silica gel aluminium plate 60 F_{254}, (10 ×10 cm) as stationary phase and Isopropyl alcohol:Methanol:Ammonia Solution (6:4:0.2, v/v/v) as mobile phase. Detection was carried out at 222 nm. The linear regression analysis data for the calibration plots showed $r^2 > 0.99$ with a concentration range from 50-300 ng/band. A Box–Behnken experimental design with randomized response surface methodology was applied. Band length, saturation time, development distance was set as independent variables while CAAs identified were peak area and retardation factor. The Rf value was predicted for vildagliptin 0.50±0.05 to optimize the chromatographic conditions based on the preliminary trials. The optimized HPTLC method was validated according to International Conference on Harmonization guideline (ICH Q2 R1). The studies successfully demonstrate the use of QbD approach for developing the highly sensitive HPTLC method with enhanced method performance. Developed HPTLC method was successfully applied for routine analysis of Vildagliptin in bulk and tablet dosage form.

KEYWORDS: Vildagliptin, HPTLC, Validation, CAAs, Box -Behnken design.

INTRODUCTION
Vildagliptin is used in the treatment of type 2 diabetes mellitus in adults. Vildagliptin can be used as a monotherapy in patients inadequately controlled by diet and exercise, as duel oral therapy in combination with metformin, sulfonylurea, and thiazolidinedione and as triple oral therapy with metformin and sulfonylurea. Vildagliptin is used in combination with insulin when stable doses of insulin do not provide glycemic control. Chemically vildagliptin is (2*S*)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile an oral antihyperglycemic agent. It is a dipeptidyl peptidase-4 inhibitor class of drug.

QbD is defined in ICH guidelines Q8 (R1) as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". This same QbD principles have been applied to the development of analytical methods, and is termed "Analytical QbD" (AQbD). Analogous to process QbD, the result of AQbD are a well understood, fit for purpose, and robust method that consistently delivers the intended performance throughout its lifecycle.

Analytical method optimization can be done either by sequential optimization methods, using simplex approaches, or by simultaneous optimization strategies, using response surface designs. The main difference between their applications is that sequential method only allows optimization of one response at a time, whereas with response surface designs several factors can be optimized simultaneously. Another difference is that for a response surface design the experimental design domain, defined by the factor levels examined, is expected to contain the optimum, whereas a sequential optimization method can be applied in situations where the experimental domain containing the optimum result is not previously known.

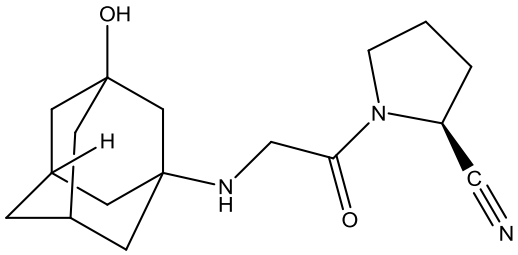

Figure 1: Structure of Vildagliptin.

A few analytical methods have been reported in the literature for the determination of vildagliptin alone or in combination with other drugs in pharmaceutical dosage forms. Which include derivative spectrophotometric methods and methods such as HPLC with ultraviolet (UV) detection, HPTLC, LC-MS, TLC.To the best of our knowledge, this is the first ever study reporting the QbD based development and optimization of HPTLC method of Vildagliptin.

The response surface randomized Box-Behnken design was used for the screening and optimization of the method. A simple, cost effective, precise, accurate, HPTLC method development and validation for estimation of vildagliptin as per International Conference on Harmonization is described in this paper.

MATERIAL AND METHODS

2.1 Chemical, reagents and standards

Vildagliptin standard was procured from Glenmark pharmaceutical Pvt. Ltd., Mukand limited, Malegaon MIDC, Sinnar, India as a gift sample. Commercially available tablets (Galvus®50mg) were procured from the local market. Precoated silica gel 60 F_{254} TLC (Merck, Germany) plates (10x10 cm) were used as stationary phase and a mixture of Isopropyl alcohol, methanol and ammonia solution was used as mobile phase.Solvents of AR grade used for this study were obtained from Merck Specialities Pvt. Ltd. Mumbai and HiMedia Laboratories Pvt.Ltd. Mumbai.

2.2 Instrumentation

Micro syringe (Linomat syringe 659.004, Hamilton-Bonaduz Schweiz, Camag, Switzerland), pre-coated silica gel 60 F_{254} aluminium plates (10 × 10 cm, 250 µm thickness; Merck, Germany), Linomat 5 applicator (Camag, Muttenz, Switzerland), twin trough chamber (20 × 10 cm; Camag, Muttenz, Switzerland), saturation pad (Camag, Muttenz, Switzerland), UV chamber (Camag, Muttenz, Switzerland), TLC scanner III (Camag, Muttenz, Switzerland), WINCATS version 1.4.0 software (Camag, Muttenz, Switzerland) were used in this study. Microsoft excel was also used to treat data statistically.

2.3 Preparation of standard solution

Standard stock solution was prepared by dissolving 50mg of vildagliptin in 10mL of methanol and ultrasonicated for 20 minutes.

2.4 Preparation of sample solution

Twenty tablets were weighed and crushed to obtain fine powder. The average weight of tablets was calculated. An accurately weighed quantity of tablet powder equivalent to about 50mg of vildagliptin was transferred in 10mL volumetric flask, volume was made up to mark with methanol and ultrasonicated for 20 minutes.

2.5 Method Development

Suitable volumes of standard and sample solutions were applied to the HPTLC plates, 10mm from the bottom and 10mm from the side edges in the form of bands (band length 6mm). The mobile phase consisting of Isopropyl alcohol:Methanol:Ammonia solution (6:4:0.2v/v/v) was used in each chromatographic run. Development was carried out in twin trough chambers(10 x 10 cm). The saturation time for the mobile phase was 10min at room temprature (25± 2℃). The development distance was 80mm, which took about 10min. The spots were scanned using the TLC scanner III in the reflectance/ absorbance mode at 222nm and these measurements were operated by winCATS software.

2.5.1 Optimization of Chromatographic Conditions by QBD approach

Analytical target profile(ATP)

The analytical target profile of present work was to develop HPTLC method.

Preliminary investigations

The variouse solvents were tried along with Isopropyl alcohol,toluene,dichloromethane,ethyl acetate, methanol among them isopropyl alcohol and methanol have shown satisfactory results.To remove tailing and quenching ammonia solution was added as third component.

Design of experiments

In order to meet desired ATP, variouse critical analytical attributes(CAAs) were identified, such as peak area,retardation factor.The independent variables selected were band length, saturation time,development distance for further chromatographic optimization of methood.

A Box–Behnken design with three factors, three levels, and 17 runs consisting of five centre points was selected as response surface design to evaluate main, interaction, and quadratic effects of band length, development distance, and chromatographic chamber saturation time on Rf value and peak height(Table2).

Table 1: Coded and actual level of three variables.

Variables	Coded levels of variables		
	-1	0	1
Band length(mm)	4	6	8
Saturation Time(min)	5	10	15
Development distance(mm)	70	80	90

Table 2:Experimental design using Box–Behnken for optimiztion of parameters.

Std	Run	Band Length (mm)	Saturation time (min)	Solvent front (mm)
5	1	4	10	70
9	2	6	5	70
11	3	6	5	90
17	4	6	10	80
8	5	8	10	90
7	6	4	10	90
1	7	4	5	80
12	8	6	15	90
15	9	6	10	80
16	10	6	10	80
10	11	6	15	70
3	12	4	15	80
14	13	6	10	80
4	14	8	15	80
2	15	8	5	80
13	16	6	10	80
16	17	8	10	70

2.8 Analytical method validation

The developed chromatographic method was validated in accordance with the ICH guideline for evaluation of various parameters: linearity, accuracy, precision, limit of detection, limit of quantitization and robustness for Vildagliptin.

Linearity and range

Standard calibration curves were prepared at six different concentration levels in range of 50–300ng/band of vildagliptin. The calibration curves were developed by plotting peak area vs. concentrations of vildagliptin.

Precision

Precision of sample application and measurement of peak area were carried out using three different concentrations 150, 200, and 250ng/band of Vildagliptin. The repeatability and intermediate precision for the determination of vildagliptin was analyzed for the mean, standard deviation, and relative standard deviation (RSD) of the peak area.

Accuracy

Accuracy was evaluated, at three different concentrations spiking of the active ingredient (80%, 100%, and 120%), by adding a known amount of vildagliptin standard to a sample of known concentration and were analyzed daily over a period of 3 days by calculating the recovery of the standards.

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

The LOD and LOQ of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curves of vildagliptin, using the formulae as given below.

LOD = $3.3 \times \sigma \div S$

LOQ = $10 \times \sigma \div S$

Where, σ = the standard deviation of the response of vildagliptin

S = slope of the calibration curve for vildagliptin

Robustness

For robustness study, the effect of deliberate variations in method parameters like the composition of the mobile phase, volume of the mobile phase, time from spotting to development and time from development to scanning were estimated in this study. The effect of these changes on both the Rf values and peak areas was evaluated by calculating the relative standard deviations (RSD) for each parameter.

RESULT AND DISCUSSION

3.1 Selection of detection wavelength

10 µl/ml of Vildagliptin (1000 µg/ml) was applied using Camag Linomat 5 applicator on precoated silica gel 60 F_{254} TLC plates and scanned by camag TLC scanner 3 at 222nm.(figure2)

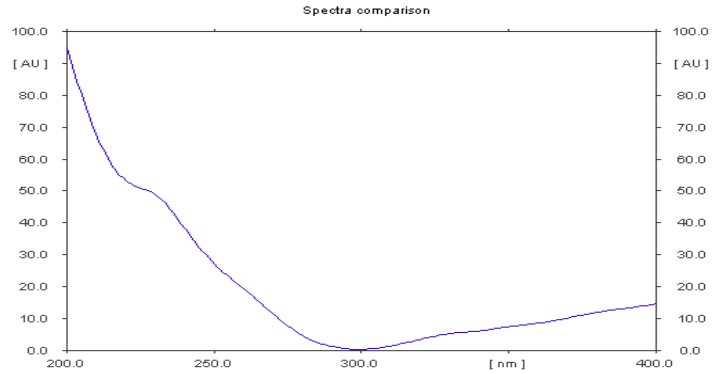

Figure 2 UV spectrum of Vildagliptin.

3.2 Optomization

3.2.1 Preliminary optimization of Chromatographic Conditions-

The selection of appropriate mobile phase was done by using different mobile phases containing solvents of varying polarity at different concentration levels. Different mobile phases like Toluene: Methanol: Ammonia solution, Ethyl acetate: Methanol: Toluene, Ethyl acetate: Dichloromethane: Toluene, Isopropyl alcohol: Methanol: Ammonia solution. Among this mobile phase compositions Isopropyl alcohol: Methanol: Ammonia solution (6:4:0.2v/v/v) was selected.

3.2.2 Optimization of Chromatographic Conditions Using BBD

Experimental runs of design were executed in random order to minimize bias. The model was validated by analysis of variance (ANOVA) using Design Expert 11.1.2.0 software that had been used to develop the experimental matrix. By applying the experimental design, quadratic model was suggested by the software. Polynomial equations were obtained for retardation factor (Rf) and peak area as follows,

For Rf values, R1 = +0.4860+0.0212*A-0.0012*B-0.0350*C-0.0425*AB-0.0200*AC-0.0200*BC-0.1168*A^2-0.0017*B^2+0.008*C^2--- (1)

After applying experimental design, suggested quadratic model was found to be significant with model F-value of 4.04 and p-value is less than 0.05. There was only 3.97% chance that an F-value this large could occur due to noise.

For peak area, R2= +7411.88-60.59*A+458.76*B+815.32*C+795.97*AB+1739.94*AC-172.75*BC--- (2)

Where, A= Band Length, B= Saturation Time, C= Development distance

It was clear that the model fits with the response variables because the model could explain the variability of most of the responses. After applying experimental design, suggested quadratic model was found to be significant with model F-value of 4.40 and p-value is less than 0.05.

Response surface plots were analyzed to visualize the effect of parameters on response. The 3-D response surface plots (figure 3 and figure 4) show the impact of band length, development distance and saturation time on retardation factor and peak area. Chromatographic separation of vildagliptin using optimized chromatographic conditions achieved by means of Box–Behnken experimental design.

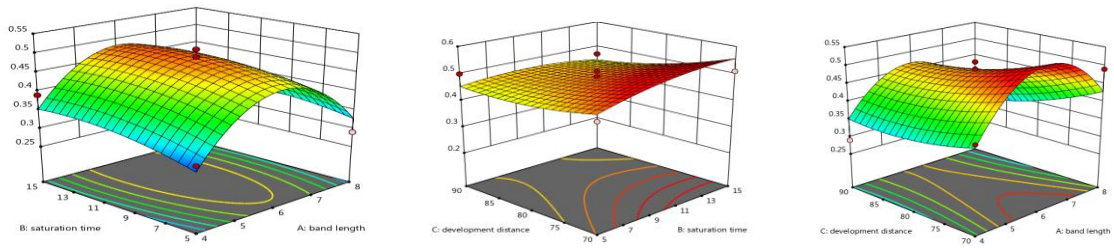

Figure 3:-3-D plots for the effect of CAAs on Rf, namely (A) Band Length, (B) Saturation time, (C) distance plate on Box Behnken design.

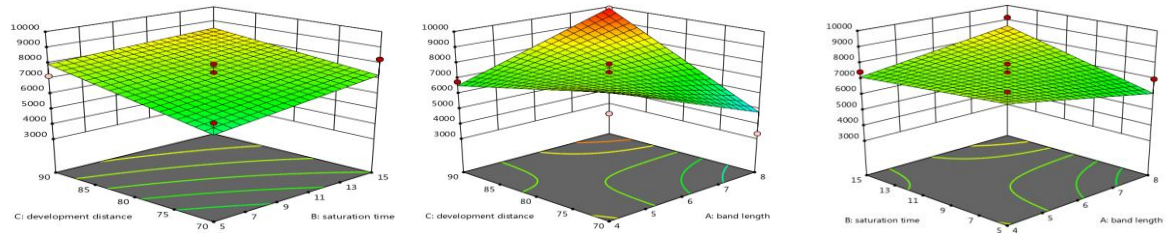

Figure 4:-3-D plots for the effect of CAAs on area, namely (A) Band Length, (B) Saturation time, (C) Distance plate on Box Behnken Design.

3.5 Validation of Method

Linearity

Peak areas were found to have better linear relationship with the concentration than the peak heights. For vildagliptin, the r ² was found to be 0.99785. Calibration graphs were constructed in the concentration range of 50-300ng/band for vildagliptin (Table 3). Validation parameters of Vildagliptin.

Table 3: Linearity studies

Parameters	Vildagliptin
Linearity range	50-300ng/band
Linear regression equation	Y = -490.177+383.360× X
Slope ± SD	383.360
Intercept ± SD	-490.177
Correlation coefficient (r ²)	0.99785
Limit of Detection (LOD)	56.38 ng /band
Limit of Quantification (LOQ)	170.8 ng /band

Precision

Repeatability and intermediate precision of the developed method were expressed in terms of relative standard deviation (RSD) of the peak area. The results showed that the repeatability and intermediate precision of the results at concentration of 400, 600, 800 ng/band for vildagliptin were within the acceptable range (Table 4).

Table 4: Precision studies.

Precision	**Concentration (ng/band)**	**SD**	**%RSD**
Repeatability*	200	54.04	0.74
Intermediate Precision#	150	27.28	0.53
	200	129.75	1.77
	250	55.92	0.60

***** number of determinations for six times

number of determinations for three times at each level

Accuracy/recovery studies

The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines for rivaroxaban. The percentage recovery of vildagliptin at all the three levels was found to be satisfactory (Table 5). For vildagliptin, the % recovery was found between 98.34 and 102.12%.

Table 5: Recovery studies.

Drug	**Recovery level %**	**Initial amount (ng/band)**	**Amount added**	**% Recovery***
Vildagliptin	80	200	160	98.34%
	100	200	200	99.90%
	120	200	240	102.12%

3.3.4. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were found to be 56.38ng/spot and 170.8ng/spot for vildagliptin respectively, indicating the sensitivity of the developed method.

3.3.5. Robustness of the method

The robustness of the method evaluated by assessing the effect of variations in method parameters on peak areas showed low RSD values (less than 2.0%) indicating robustness of the method (Table 6).

Table 6: Robustness study for the developed method.

Parameter	Change	SD	%RSD
Mobile phase composition (±0.1mL)	6:4:0.1	98.25	1.35
	5.9:4.1:0.2		
	6.1:3.9:0.2		
Development to scanning (±5min)	5	136.87	1.88
	10		
	15		
Spotting to development(±5min)	5	45.70	0.62
	10		
	15		
Volume of mobile phase (± 1 mL)	9	75.15	1.03
	10		
	11		

CONCLUSION

An efficient QbD approach was utilized to develop a competent HPTLC method for quantification of vildagliptin. The approach gives better consideration of the factors influencing chromatographic separation and greater assurance in the ability of the methods to meet their desired purpose. Three factors were analyzed to determine their effect with the least number of runs as possible using a Box–Behnken design in conjunction with randomized response surface methodology. The experimental design describes the HPTLC method components including saturation time, development distance, and band length. Their interrelationships are studied and optimized conditions are obtained.

The developed HPTLC method for the estimation of vildagliptin is simple, precise, and robust. Further, the method is found to be accurate and sensitive. The developed method can be used for routine analysis of vildagliptin in bulk and in pharmaceutical formulation.

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