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Application of Quality by Design in the Development of HPTLC Method for Estimation of Anagliptin in Bulk and *in-house* Tablets

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ABSTRACT
This paper comprehends systematic Quality by Design (QbD) based development of Normal-Phase High-Performance Thin-Layer Chromatography (NP-HPTLC) method for qualitative and quantitative estimation of anagliptin in bulk and *in-house* tablets. Chromatographic separation was executed out on aluminum backed Silica gel F_{254} plates using dichloromethane: methanol (9.2:0.8 v/v) as a mobile phase. Densitometry scanning was accomplished at 248 nm. Quality target method profile was defined and critical analytical attributes (CAAs) for the HPTLC method set aside. The mobile phase ratio and saturation time were determinate as critical method parameters (CMPs) and systematically optimized using Central composite design, evaluating for CAAs, namely retention factor (R*f*), Peak-area and Peak-height. Statistical modelization was implemented followed by response surface analysis for comprehending plausible interaction(s) among CMPs. Search for optimum solution was conducted through numerical and graphical optimization for demarcating the design space. The described method was linear. The precision, ruggedness, and robustness values were also within the prescribed limit. The studies successfully demonstrate the utility of QbD approach for developing the highly sensitive HPTLC method with enhanced method performance.
Keywords: central composite design, HPTLC, anagliptin, quality by design, failure mode effect analysis

INTRODUCTION

Anagliptin (AGP), (**Figure 1**) N-[2-[[2-[(2S)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl]amino]-2-methylpropyl]-2-methylpyrazolo[1,5-a]pyrimidine-6-carboxamide, is dipeptidyl peptidase-4 inhibitors. It is used in the treatment of type 2 diabetes mellitus [1].

Dipeptidyl peptidase-4 (DPP-4) inhibitors are promising new class of anti-diabetics. It increases level of incretin such as glucagon-like peptide-1 (GLP-1) and glucose-dependent

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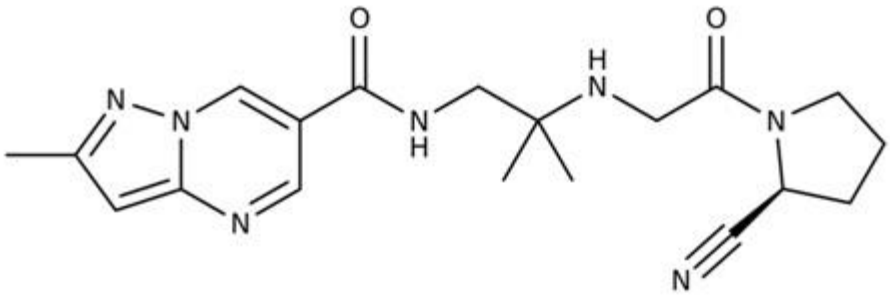

Figure 1. Chemical Structure of Anagliptin

insulinotropic peptide (GIP) which leads to increase glucose-dependent secretion of insulin and decreased blood glucose, haemoglobin A1C and glucagon levels [2-4].

Quality by Design (QbD) concepts is well defined in ICH guidelines Q8 (R1): Pharmaceutical Development [5], Q9: quality risk management [6], and Q10: pharmaceutical quality system [7].

The concept of QbD applied to analytical method development is known now as Analytical Quality by Design (AQbD) [8]. AQbD prevalence in development of a robust and cost effective analytical method which is applicable throughout the lifecycle of the product, to facilitate the regulatory flexibility in analytical method. It means the freedom to change method parameters within a method's design space, referred to as the method operable design region (MODR) [9, 10].

The first step in this process is to define the Quality Target Method Profile (QTMP) or Analytical Target Profile (ATP). QTMP or ATP is a statement that defines the method's purpose which is used to drive method selection, design, and development activities. [11]

After defining the QTMP, the next step is to ascertain the critical analytical attributes (CAAs) similar to Critical Analytical Attributes (CQA) in product development. According to ICH Q8 (R2) "A CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. Next to this is identification of critical method parameters (CMPs) using risk assessment and screening. In general, Ishikawa fishbone diagram can be used for risk identification and assessment. [12]

Further, prioritization exercise is performed by employing initial risk assessment and QRM techniques for identifying the "high-flying few" input variables, termed as Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) from the "probable so many". This process is popularly termed as factor screening. Comparison matrix (CM), Risk Estimation Matrix (REM), Failure Mode Effect Analysis (FMEA) and Hazard Operability Analysis (HAZOP) are the examples of commonly employed risk assessment techniques. The

low-resolution first-order experimental designs (e.g., fractional factorial, Plackett-Burman and Taguchi designs) are highly helpful for screening and factor influence studies. Once the potential and critical analytical method variables are defined with initial risk assessment, then DoE can be performed to confirm and refine critical method variables based on statistical significance. It can be determined per unit operation or combination of selected multiple method variables and their interactions and responses (critical method attributes). This approach provides an excellent opportunity to screen a number of conditions generated from a limited number of experiments. Then, data evaluations by using statistical tools are very important to identify critical method variables and the appropriate optimal ranges for method variables where a robust region for the critical method attributes could be obtained [13, 14].

Literature survey revealed that UV-spectrophotometry method has been reported [15].To our notice, so far no HPTLC method has been reported for the estimation of AGP in tablets. Attempts were, therefore, made to apply AQbD approach to develop simple, robust, sensitive, effective and economical NP-HPTLC method for estimation of AGP in bulk drug and tablets.

EXPERIMENTAL

Chemicals and Reagents

Pharmaceutical grade Anagliptin working standards were obtained as generous gifts from Glenmark Pharm., Nashik, India. Methanol (A.R. Grade) and aluminium backed TLC plates pre-coated with silica gel 60 F_{254} (0.2 mm thick) were purchased from E. Merck Ltd., Mumbai (India).

Instrumentation

The TLC plates were prewashed with methanol and activated at 110^{o}C for 5 min, prior to chromatography. The linear ascending development was carried out in 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) using dichloromethane: methanol (9.2: 0.8 *v/v)* as mobile phase, after saturation of the chamber with mobile phase vapour for 20 min. The development distance was 8 cm. After, chromatography plates were dried in a current of air with the help of air dryer. A Camag HPTLC system containing Camag Linomat 5 sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner-3 with winCATS software version 1.3.0 and Camag twin- trough chamber (20 x 10 cm) were used for the present study. The source of radiation utilized was deuterium lamp emitting a continuous UV-spectrum between 200 to 400 nm.

Preparation of standard solution

Stock standard solution was prepared by dissolving 10 mg of AGP in 100 mL of methanol to get concentration of 0.1 mg/mL.

Table 1. Analytical Target Profile for HPTLC method of AGP

ATP elements	Target	Justification
Target Sample	Bulk drug and drug product	Development and validation of analytical method for any drug is useful for its assay in particular formulation as well as its stability testing.
Type of stationary phase	Normal phase	On the basis of chemical nature of AGP.
Stock standard preparation	Stock solution prepared using bulk drug in methanol	Stock solution is usually prepared with bulk drug in external standard method
Sample preparation	Sample solutions were also prepared in methanol	Sample solutions are usually prepared for the assay of drug in formulation.
Purpose of method	For assay of AGP	The main intent of developed method is the assay of Anagliptin in tablets and it must have the application in routine analysis of drug in tablets.

Selection of analytical wavelength

After chromatographic development bands were scanned over the range of 400 - 200 nm and 248 nm was selected for estimation of drug.

Defining the ATP and CAAs

Analytical target profile is defined for HPTLC method for AGP and shown in **Table 1.** In order to meet the desired ATP, various CAAs were identified, such as Peak-area, Peak-height and retention factor.

Risk assessment studies

Risk assessment studies were performed to identify the CMPs, which possess high risk based on their criticality and influence to affect the CAAs. Besides, risk assessment also furnishes expected interaction(s) among the CMPs and CPPs, estimating the chances of subsequent failure(s), if any. [12]

The first step in the risk assessment was to systematically gather up all the possible factors that could influence method. These factors were organized hierarchically using an Ishikawa or "fishbone" diagram. Further, prioritization studies were carried out for selecting the CMPs/CPPs with high risk by FMEA risk assessment technique.

The outcome of an FMEA are risk priority numbers (RPN) for each combination of occurrence probability, failure mode severity, and possibility of detection, which can be used to rank the risk. FMEA defines the RPN as:

$$RPN = O \begin{pmatrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{pmatrix} \times \begin{pmatrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{pmatrix} S \begin{pmatrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{pmatrix} \times D$$

Table 2. Taguchi design matrix for screening of factors

Run	Mobile phase ratio	Development distance	Relative humidity	Saturation time	Activation time	Time from spotting to chromatography	Time from chromatography to scanning
1	+	+	+	-	-	+	+
2	-	-	+	+	-	+	-
3	-	+	-	-	+	+	-
4	+	-	-	+	+	+	+
5	-	-	+	-	+	-	+
6	+	-	-	-	-	-	-
7	-	+	-	+	-	-	+
8	+	+	+	+	+	-	-

Factors	Levels	
	Low (-)	High (+)
Mobile phase ratio	Dichloromethane : Methanol (9.1:0.9 v/v)	Dichloromethane : Methanol (9.3:0.7 v/v)
Development distance	7.5	8.5
Relative humidity	55	65
Saturation time	15	25
Activation time	8	12
Time from spotting to chromatography	10	30
Time from chromatography to scanning	10	30

where O is the occurrence probability or the likelihood of an event occurring; occurrence probability can be ranked as 5, likely to occur; 3, 50:50 chance of occurring; and 1, unlikely to occur. The next parameter S, the severity, which is a measure of how severe of an effect a given failure mode would cause; these can be ranked as 5, severe effect; 3, moderate effect; and 1, no effect. The final parameter D is the detectability or the ease that a failure mode can be detected, because the more detectible a failure mode is, the less risk it presents to product quality. For D, here rank 1 can be given to parameter which can be easily detectable, 3 as moderately detectable, and 5 as hard to detect. Using this procedure, we have calculated RPN for selecting CMPs.

Factor screening studies

Factor screening was done by using Taguchi design which is widely used screening design. For this seven factors were considered and eight plates were developed and scanned. Factor screening helps to identify CMPs which critically affecting CAAs (i.e., Retention factor, Peak- area and Peak-height). **Table 2** shows the design matrix enlisting the studied factors and the decrypted translation of their respective low and high levels.

The design was analyzed for influence of studied factors on the CAAs. Model fitting was carried out for selecting linear polynomial model by obviating the interaction term(s). As screening is primarily based on the principle of factor sparsity, the Pareto charts were employed for quantitatively identifying the effect of each factor on the selected CAAs [16, 17].

Table 3. Central composite design matrix for optimisation of method parameters

Run	Mobile phase ratio	Saturation time
1	0	0
2	+α	0
3	-α	0
4	+1	+1
5	+1	-1
6	-1	-1
7	0	-α
8	0	+α
9	-1	+1
10	0	0
11	0	0
12	0	0
13	0	0

Factors	Levels				
	-α	-1	0	+1	+α
Mobile phase ratio	Dichloromethane : Methanol (9.06:0.94 *v/v*)	Dichloromethane : Methanol (9.1:0.9 *v/v*)	Dichloromethane : Methanol (9.2:0.8 *v/v*)	Dichloromethane : Methanol (9.3:0.7 *v/v*)	Dichloromethane : Methanol (9.34:0.66 *v/v*)
Saturation time	12.93	15	20	25	27.07

Method development as per the experimental design and statistical analysis

Selection of CMPs actually affecting method performance based on preliminary risk assessment and factor screening pushed forward for further method optimization. Central composite design with α = 1 was used for optimization of selected CMPs, namely mobile phase ratio and saturation time studied at four levels, that is, low, intermediate, high, extremely high and extremely low. **Table 3** summarizes a design matrix consisting of 13 experimental runs as per central composite design including a total of nine experimental runs together with five runs of center point (0, 0). A standard concentration of 300 ng per band was used for all the experimental runs, which were analyzed for CAAs, namely R*f*, Peak-area, and Peak-height.

All the results were analyzed using statistical software Design Expert software version 9.0.6.2. (Stat-ease, Inc., Minneapolis, MN). The experimental data were validated by ANOVA combined with *F*-test. Only the coefficients, which were found to be significant ($P < 0.05$) as per ANOVA analyses, were considered in framing the polynomial equation. Other parameters like lack of fit, coefficient of correlation (r^2) and predicted error sum of squares (PRESS) were also evaluated to check the appropriate model fitting. Response surface analysis was carried out through estimated 2D-contour plots and 3D-response surface. As well as, the model diagnostic plots like normal plot of probability, run plot, residual plot and histogram plot were used to analyze the degree of fitness of the explored data.

VALIDATION OF METHOD

The method was validated by establishing linearity, accuracy, inter-day and intra-day precision of measurement of sample application. The detection limit (DL) and quantification limit (QL) were also determined.

Linearity

Appropriate volumes in the range of 1 - 6 mL were transferred from stock solution into series of 10 mL volumetric flaks and volumes were made up to mark with methanol. From each volumetric flask, 10 µL of solution was applied on HPTLC plate to get concentration in the range of 100 – 600 ng per band. After evaporation of solvents at room temperature for 20 min, chromatography was performed as described above. Calibration curve was established by plotting Peak-area against drug quantity per band. Calibration equations were determined by use of linear regression analysis and correlation coefficients (r^2) were calculated. All measurements were repeated six times.

Repeatability and Intermediate Precision

The precision of the method was confirmed by repeatability and intermediate precision studies. Repeatability studies were executed by analysis of AGP (300 ng per band) six times on the same day. The intermediate precision of the method was checked by analysing three different concentrations 200 ng per band, 300 ng per band and 400 ng per band of AGP for three different days, over a period of week.

Recovery

The accuracy of the experiment was established by over spotting drug standard solution to the pre-analyzed sample solution. The recovery study was performed at three different levels i.e. 80, 100, and 120%. The experiment was repeated three times.

Robustness

For robustness study, the composition of the mobile phase was changed slightly and the effects on the results were examined. Dichloromethane: Methanol in different ratios (9.1:0.9; 9.2:.8; 9.3:0.7 *v/v*) were selected and chromatograms were run. The amount of mobile phase (10 ± 2 mL, i.e. 8, 10, or 12 mL), development distance (8 ± 0.5, i.e. 7.5, 8, 8.5) and duration of saturation (20 ± 5 min, i.e. 15, 20, or 25 min) were varied. Time from application of AGP to the plate to development of the plate and time from development of plate to scanning were also varied (10, 20, or 30 min).

The robustness and ruggedness of the method was assessed at concentration (300 ng per band) for six times.

Detection Limit (DL) and Quantification Limit (QL)

The DL and QL were calculated using the equations

$$DL = 3.3 \times A.S.D/Slope$$

$$QL = 10 \times S.D./Slope$$

The DL was regarded as the amount for which the signal- to-noise ratio was 3:1 and QL as the amount for which the signal-to-noise ratio was 10:1. The DL and QL estimated at concentration range 100– 200 ng per band.

Specificity

The specificity of the method was ascertained by analyzing standard AGP and AGP extracted from tablets. The band for AGP in sample was confirmed by comparing the R*f* and spectra of the band with those obtained from standard. The peak purity of AGP was assessed by comparing spectra acquired at three different positions on the band, i.e. peak -start (S), peak- apex (M), and peak- end (E).

Analysis of Tablet formulation

Due to the unavailability of Anagliptin tablets in the local Indian market, *In-house* tablets were formulated *via* direct compression technique using commonly used excipients containing 100 mg of drug per tablet.

To determine the content of *in-house* prepared tablets of AGP, twenty tablets were weighed and powdered. An amount of powder equivalent of 10 mg of AGP was weighed accurately, transferred into 100 mL volumetric flask containing 50 mL of methanol, sonicated for 20 min, and solution was diluted up to 100 mL with same solvent. The resulting solution was filtered through Whatmann filter paper, extract (3 µL; 300 ng per band) was applied to a TLC plate followed by development and scanning as described above. The analysis was repeated for six times.

RESULTS

Preliminary optimization of mobile phase

The HPTLC procedure was optimized to develop assay method for determination of AGP in bulk and tablet formulation. The drug standard was applied on HPTLC plates and developed with different composition of mobile phases depending on polarity of drug. Mobile phase consisting of Dichloromethane and methanol showed symmetrical peak with low tailing.

Risk assessment studies

All the possible factors that could affect HPTLC method were systematically gathered and organised using Ishikawa or fishbone diagram as shown in **Figure 2.**

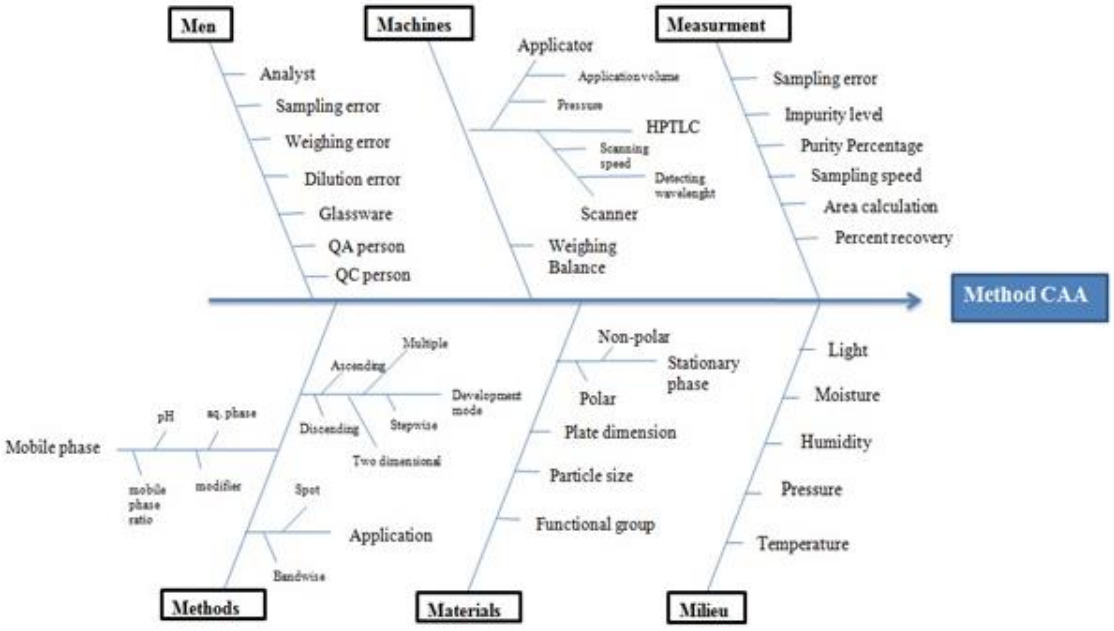

Figure 2. Ishikawa fish-bone diagram showing the CAAs of HPTLC method for AGP

Table 4. Summary of FMEA analysis

Factors	S	O	D	RPN
Mobile phase ratio	5	5	5	**125**
Mobile phase volume	2	2	1	**4**
Development distance	3	2	2	**12**
Relative humidity	2	3	3	**18**
Saturation time	4	3	5	**60**
Activation time	4	2	3	**30**
Time from spotting to chromatography	3	3	4	**36**
Time from chromatography to scanning	3	3	4	**36**
Plate size	2	1	1	**2**

S – Severity; O – Occurrence probability; D - Detectability

Further prioritization studies were carried out by FMEA risk assessment technique and PRN were calculated for nine factors *viz.* Mobile phase ratio, mobile phase volume, development distance, relative humidity, duration of saturation, activation time of pre-washed plates, time from spotting to chromatography, time from chromatography to scanning, and plate size.

In the present study, the greatest RPNs were used to identify the parameters which affect the method performance mostly and thus needed to be studied in more detail. **Table 4** shows listing of the factors considered when doing the FMEA along with their RPN. From FMEA study, five factors identified such as mobile phase ratio, development distance, and relative humidity, duration of saturation and activation time of prewashed plates which were associated with high risk.

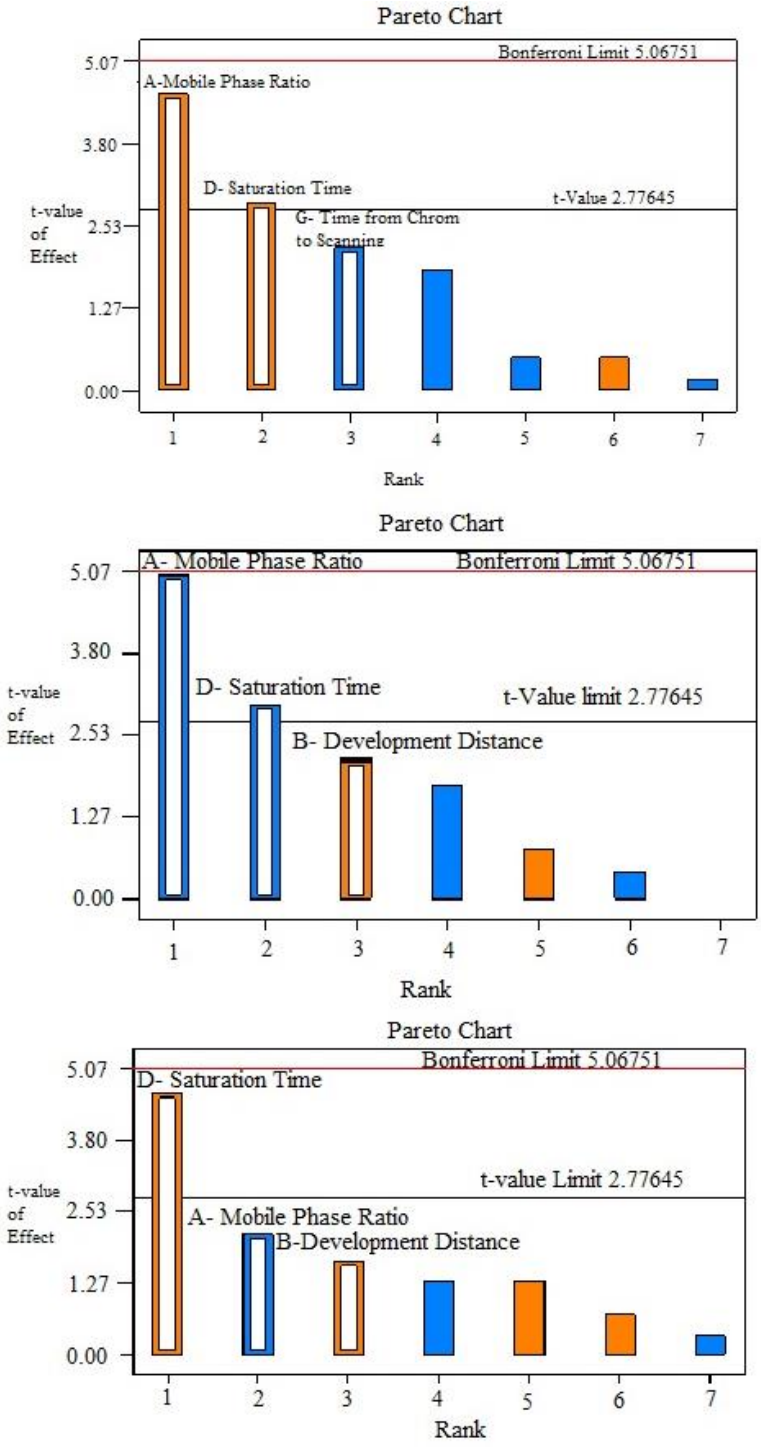

Figure 3. Pareto charts depicting the influence of CMPs on method CAAs for HPTLC method of Anagliptin during screening

Table 5. Summary of results of ANOVA for measured responses

Source	Sum Sq	Df	MS	F- value	P-Value	Model significance
Rf						
Model	0.010	2	0.005231	170.19	<0.0001	Significant
Residual	0.000307	10	0.0000307	-	-	
Total	0.011	12	-	-	-	
Area						
Model	2225.91	2	1112.95	4.48	0.0409	Significant
Residual	2487.02	10	248.70	-	-	
Total	4712.92	12	-	-		
Height						
Model	200.88	2	100.44	17.30	0.0006	Significant
Residual	58.05	10	5.80	-	-	
Total	258.92	12	-	-	-	

Factor screening studies

Taguchi design was used for screening of CMPs. The first-order polynomial equation for response variables was generated and analyzed. The equation 1 shows the coefficients β1 to β7 represent the model terms, and the coefficient β_0 representing the intercept term, Y represents response while $X_1 - X_7$ are factors.

$$\boldsymbol{Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7} \quad (1)$$

The polynomial equation generated during the screening shows that there were absence of significant interaction effect(s) among the factors. **Figure 3** shows the Pareto charts portraying the effect(s) of CMPs on method CAAs. During the screening it was found that the influence of factors viz. mobile phase ratio and saturation time on CAAs was statistically significant ($p < 0.05$), and therefore only these factors were considered during the optimization.

Method development as per the experimental design and statistical analysis

Experimental runs of design were executed in random order to minimize bias. Polynomial equations were obtained for R*f*, Peak-area and Peak - height using design expert software version 9.0.6.2. as described in equations (2) - (4) as follows:

$$\textbf{For } \mathbf{R_f} \textbf{ value: } \mathbf{Y_1 = +0.55 - 0.033\,X_1 + 0.014\,X_2} \quad (2)$$

$$\textbf{For Area: } \mathbf{Y_2 = +2364.08 + 14.14\,X_1 + 8.85\,X_2} \quad (3)$$

$$\textbf{For Height: } \mathbf{Y_3 = +211.92 + 4.98\,X_1 + 0.52\,X_2} \quad (4)$$

where Y_1 (R*f* value), Y_2 (Area) and Y_3 (Height) are responses, X_1 (Mobile phase ratio), and X_2 (Chamber saturation time) are the factors.

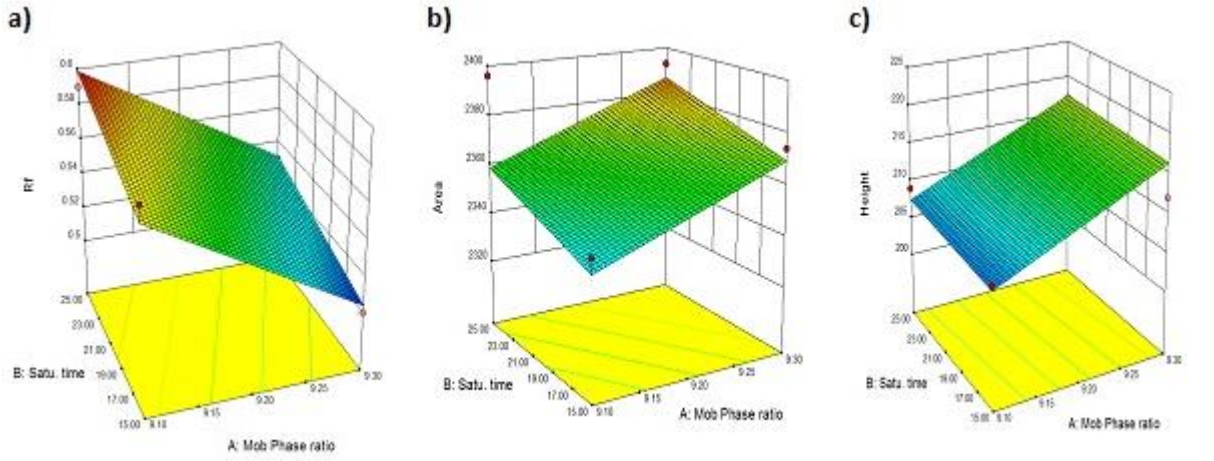

Figure 4. 3D response surface plots showing **a)** influence of mobile phase ratio (X1) and chamber saturation time (X2) on Rf value (Y1) of AGP, **b)** influence of mobile phase ratio (X1) and chamber saturation time (X2) on Peak-area (Y2) of AGP and **c)** influence of mobile phase ratio (X1) and chamber saturation time (X2) on Peak-height (Y3) of AGP.

Table 5 shows the results of analysis of variance (ANOVA) of model for R*f* value, area and height of the chromatogram of AGP. It is confirmed that model was statically significant in its prediction of R*f* value, Peak-area and Peak-height as portrayed by probability value of less than 0.05. All quadratic terms were found statically significant for response R*f* value, Peak-area and Peak-height AGP.

As per the values of coefficients from the polynomial models and their signs (Eqs. (2) - (4)), X_1(mobile phase ratio) have negative effect on responses Y_1 (R*f* value) and positive effect on Y_2 (Peak-area) and Y_3 (Peak- height) while X_2 (chamber saturation time) have positive effect on Y_1 (R*f* value), Y_2 (Peak-area) and Y_3 (Peak-height).

Response surface plots were analyzed to visualize the effect of parameters on response. **Figure 4a** shows the effect of mobile phase ratio (X_1) and chamber saturation time (X_2) on R*f* value (Y_1) of AGP. **Figure 4b** shows the effect of mobile phase ratio (X_1) and chamber saturation time (X_2) on Peak-area (Y_2) of AGP. **Figure 4c** shows the effect of mobile phase ratio (X_1) and chamber saturation time (X_2) on Peak-height (Y_3) of AGP.

Response surface plot depicted saturation time has less influence on R*f* value and Peak-area than mobile phase ratio. While mobile phase ratio and saturation time, both are having near about same influence on Peak-height. When dichloromethane content in mobile phase increases, R*f* value of AGP decreases while increase in saturation times leads to increase in R*f* value as well **Figure 4a.** Peak-area and Peak-height increases with the increase in saturation time and dichloromethane content in mobile phase **Figure 4b** and **4c**.

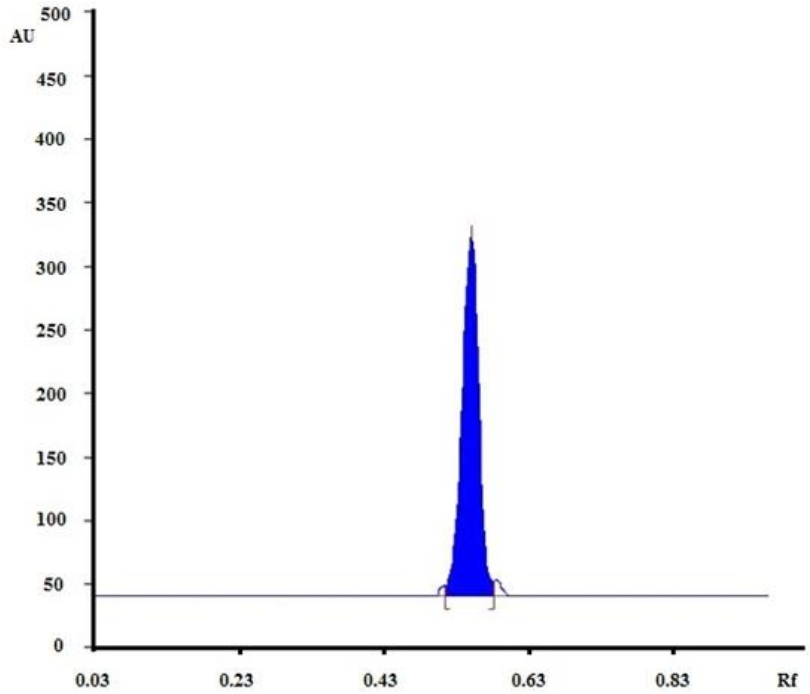

Figure 5. Typical densitogram of AGP (Rf 0.55 ± 0.03) in dichloromethane: methanol (8.2 :0.8 v/v)

Numerical Optimization

A numerical optimization technique by desirability approach was used to obtain optimum conditions for the HPTLC method of AGP.

The numerical optimization suggested the optimized conditions having mobile phase composition containing dichloromethane: methanol (8.2: 0.8 *v/v*) and chamber saturation time of 20 minutes with desirability of 0.990. The typical chromatogram of AGP is shown in **Figure 5**.

Validation of Method

Linearity

Calibration curves were constructed by plotting peak area against concentration per band. A good linearity was obeyed in the concentration range of 100 – 600 ng per band. Linear regression equation was found to be Y = 5.252 X + 767.9. The regression coefficient (r^2 = 0.999) is generally considered as evidence of acceptable fit. All measurements were repeated six times.

Table 6. Intermediate Precision and Repeatability studies

Precision	Concentration (ng/band)	% Amount found	%RSD
Repeatability*	300	99.32	1.02
Intermediate Precision #	200	99.65	1.34
	300	99.18	0.83
	400	99.17	0.95

* number of determinations for six times
# number of determinations for three times at each level

Table 7. Recovery studies

Initial Amount	Amount of drug added (%)	Amount recovered ± SD [ng/band] n=3	% Recovery	%RSD
200	80	157.78 ±1.55	98.61	0.98
200	100	197.645 ± 1.717	98.823	0.869
200	120	238.709 ± 3.117	99.462	1.306

n = number of determinations

Table 8. Robustness studies

Conditions	% RSD
Mobile phase ratio (± 0.1 mL)	1.62
Mobile phase volume (± 2 mL)	1.58
Development distance (± 0.5)	0.68
Saturation time (± 5)	1.02
Time from spotting to chromatography (± 10 min.)	0.87
Time from chromatography to scanning (± 10 min.)	0.92

Repeatability and Intermediate Precision study

The precision of the method was studied as repeatability and intermediate precision. The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% RSD). The results from study of precision are shown in **Table 6.** The established method was found to be precised as the % RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guideline.

% Recovery study

The accuracy of the method is studied to assess that other components in the pharmaceutical formulation do not interfere with analytical method.

When the method was used for extraction and subsequent analysis of AGP in tablet dosage forms after spiking with 80, 100, or 120% excess drug the recovery was found 98.62 – 99.46%, as listed in **Table 7**.

Robustness

The standard deviation of peak areas was calculated for each condition and % RSD was less than 2%. The low values of % RSD are indicative of the robustness of the method. The results of robustness studies are shown in **Table 8**.

Detection Limit (DL) and Quantification Limit (QL)

Detection Limit and Quantification Limit for signal-to-noise ratios of 3:1 and 10:1 were 8.74 ng and 26.51 ng, respectively, which indicate adequate sensitivity of the method.

Specificity

The peak purity of AGP was assessed by comparing the spectra at peak-start, peak -apex and peak- end positions of the spot, i.e., r^2 (*S*, *M*) = 0.996 and r^2 (*M*, *E*) = 0.998. Good correlation (r^2 = 0.99) was also obtained between standard and sample spectra of AGP.

These correlation values indicate the ability of the method to separate and specifically detect AGP from sample solutions.

Analysis of Tablet formulation

Using the proposed chromatographic method, assay of AGP in *in-house* tablets was carried out. The peak at R*f* for AGP was observed in the densitogram of the drug samples extracted from Tablets. There was no interference observed from the excipients used in the formulation of *in-house* Anagliptin tablets. The drug content ± SD was found to be 99.41 ± 1.24 for Anagliptin.

CONCLUSION

The developed HPTLC method for the estimation of Anagliptin is simple, precise, rugged and robust. Further, the method is found to be accurate and sensitive. QbD is successfully implemented for optimization of mobile phase of NP-HPTLC method.

The developed method can be used for routine analysis of Anagliptin in bulk and in pharmaceutical formulation.

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