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Original Article

# Exploring Analytical Quality by Design (AQbD) Enabled RP-HPLC Method for Carvedilol

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## ABSTRACT

**Background:** Carvedilol (CDL) is a beta-blocker that helps to lower the risk of heart attack related mortality and is used to treat congestive heart failure and excessive blood pressure. Objectives: The present study deals with the development and validation of a reverse-phase high performance liquid chromatography method for rapid and reliable analysis of CDL by utilizing the Analytical Quality by Design (AQbD) approach. **Materials and Methods:** Critical Analytical Attributes (CAAs) were selected based on predefined Analytical Target Attributes (ATPs). Further screening of Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) having a significant impact on assigned CAAs was done by Taguchi Design. The optimized mobile phase consists of 0.1% Orthophosphoric Acid (OPA) and methanol in a ratio of 35:65% v/v. The flow rate was maintained at 1 mL/min, the column temperature was 30ºC, and the analysis was carried out at 240 nm. Based on the results obtained, fine tuning of the CAAs was done by opting for the $3^2$-Box-Behnken design. **Results:** The area under the curve was found to be linear within the studied range of 5-30 µg/mL ($R^2$=0.999). The retention time was found to be 4.18 min, with a sharp, well-defined peak. The percentage recovery of the drug during the accuracy study was found to be in the range between 97.06 and 99.29%. The detection and quantification limits were found to be 0.61 µg/mL and 1.86 µg/mL, respectively. **Conclusion:** The developed method was found to be precise with a RSD of <1%. The method can be effectively utilized for routine analysis of bulk drug and dosage forms in quality control labs.

**Keywords:** Analytical Quality by Design, Carvedilol, RP-HPLC, Box-Behnken design, Taguchi Design.

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## INTRODUCTION

Production of quality pharmaceutical product is a highest priority of any pharmaceutical industry. To ascertain the quality of any product, highly efficient, precise and accurate analytical method is required. The assessment of product with poor analytical method leads to generation of inaccurate and misleading data. It is highly risky if the analytical method used during product development stage is poorly developed as it affects on the quality attributes of product developed. This may also in turn result into life threatening health hazards to patients consuming these products and spoils the reputation of pharmaceutical industry.

Various pharmaceutical regulatory agencies like USFDA, ICH have taken these issues very seriously and to tackle such crucial issues they have designed some guidelines to the industry. In such effort, ICH has recently implemented ICH Q14 guidelines pertaining to development of analytical procedures and revision of Q2 (R1), Q2 (R2). Further these agencies are emphasizing the use of Analytical Quality by Design (AQbD) approach for development of analytical method.[1]

Initially Quality by Design (QbD) approach was utilized by formulation scientists to imbibe the quality in the product during its development stage itself. Further the need of accurate and precise analytical method faced by industry explored its use in analytical method development and flourished as AQbD approach.[2-7] As compared to traditional time and chemical consuming trial and error approach, AQbD has equipped analytical scientist with simple and rapid tool for development of highly efficient analytical methods. AQbD approach helps analytical scientists to understand various Critical Analytical Attributes (CAA) which affects on performance of analytical results. It accompanies the quality risk management principles with design of experiments to understand the risks and associated interactions among the method variables. It further defines

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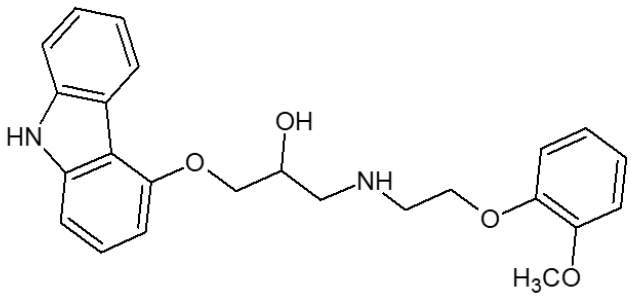
quality target method profile and operable design space where the method will provide the consistent results without significant error. Thus, it will help the analytical scientist to develop a highly robust method for routine analysis of drug and drug products.[8]

Carvedilol is chemically, (2RS)-1-(9H-Carbazol4-yloxy)-3-[[2-(2-methoxy phenoxy) ethyl] amino] propan-2-ol having molecular formula of $C_{24}H_{26}N_2O_4$ and a molecular weight of 406.47 g/ mol.[9]




**Structure of Carvedilol**

Carvedilol, non-selective β-adrenergic blocking agent is indicated for mild to moderate heart failure and hypertension having cardiomyopathic or ischemic origin. In comparison to other β blocker, CDL has least inverse agonist activity which provide additional benefits with respect to morbidity and mortality in CHF.[10]

Reported methods suffer from certain lacuna. To mention few, complex procedures for extraction, time consuming methods, high retention time and use of costlier solvents etc. Further the developed methods have utilized traditional trial and error approach.[11] To the best of our knowledge, there is no study published which explores an AQbD approach to establish an analytical method for CDL analysis. Thus, the aim of the present research work was to develop sensitive, specific, precise, reliable and accurate RP-HPLC method using an AQbD enabled approach for the quick analysis of CDL in bulk and dosage form.

## MATERIALS AND METHODS

### Chemicals reagents and equipment used

Carvedilol (CDL) was obtained from Teva Pharmaceutical Pvt. Ltd., Gujarat, India as a gift sample. All chemicals required for the experiment (HPLC-grade) were procured from authentic sources.

Shimadzu Prominence-i series LC-2030c 3D plus was used to carry out the chromatographic (RP-HPLC) estimation. The system was equipped with Shim-pack GIST C18 column (250 x 4.5 mm i.d., 5 µm,) having guard column C18 (ODS; 4x3.0 mm i.d., Phenomenex, USA). A Lab solutions Version 5.97 SP1software was used. The mobile phase was consisting of 0.1% OPA and MeOH (35:65 v/v) which was forced through the column at a flow rate of 1.0 mL/min, keeping column temperature at 30°C and detection wavelength of 240 nm. The 10 µL sample was injected at each sample time with 10 min of chromatographic run time.

### Preparation of Sample solution

For preparation of CDL stock solution, 10 mg of CDL was dissolved in 10 mL of MeOH. A working sample solution was prepared by serial dilutions of stock solution with mobile phase to get final solution having concentration of 10 µg/mL.

### AQbD enabled RP-HPLC method development

#### *Analytical Target Profile (ATP) and Critical Analytical Attributes (CAA)*

Defining Analytical Target Profile (ATP) is a primary step towards the QbD approach. ATP is nothing but predetermining the various quality attributes of analytical method which are desired by an analyst. This ATP will act as guiding principle for designing the quality and accuracy of the method. While deciding the ATP various factors considered are method application, instrument characteristics, drug characteristics, sample preparation etc. Selection of Critical Analytical Attributes (CAA) helps to meet the desired ATP. CAA's are the attributes of the chromatogram which can be measured and directly correlated with the quality of the developed analytical method. The Tailing factor (Tf), Retention time (Rt), Number of Theoretical Plates (NTPs), peak area, resolution between various peaks, etc. are few examples of CAA.[12,13]

### Risk Assessment studies and Factor screening studies by Taguchi design

Determination of risks and probabilities of failure in the method development is important aspect of QbD which is done by risk assessment study. The risk assessment was established using a Taguchi orthogonal array design with seven components at two levels, where the cause effect relationship between CPP and CMAs was examined. For this study Design expert ® software (Version 7.0.0, Stat-Ease Inc., Minneapolis, MN, USA) was used.[14]

Various critical method variables selected were Injection volume, flow rate, temperature, concentration of organic modifier, and type of organic modifier, type of solvent and % v/v of solvent. Each factor was varied at 2 different levels. Table 1 shows various factors along with their levels.

### Method development and Optimization by Box-Behnken Design (BBD)

On the basis of preliminary study, factors which significantly affected CAA were further screened by using Box-Behnken design. As per the design space 13 different runs were carried out and their effect on CAA was studied by software. Table 2 shows various runs with their experimental conditions. The observed responses were noted and further the data was fed to software to

**Table 1: Taguchi design matrix for screening.**

| Standard trial no. | Type of solvent | % v/v of solvent | Type of organic modifier | Conc. of organic modifier | Temperature | Flow rate | Injection volume |
|---|---|---|---|---|---|---|---|
| 1 | MeOH | 65.00 | OPA | 0.20 | 30.00 | 0.80 | 20.00 |
| 2 | ACN | 65.00 | OPA | 0.05 | 30.00 | 1.00 | 20.00 |
| 3 | ACN | 60.00 | FA | 0.20 | 40.00 | 1.00 | 20.00 |
| 4 | ACN | 60.00 | FA | 0.05 | 30.00 | 0.80 | 10.00 |
| 5 | MeOH | 65.00 | FA | 0.05 | 40.00 | 1.00 | 10.00 |
| 6 | ACN | 65.00 | OPA | 0.20 | 40.00 | 0.80 | 10.00 |
| 7 | MeOH | 60.00 | OPA | 0.20 | 30.00 | 1.00 | 10.00 |
| 8 | MeOH | 60.00 | OPA | 0.05 | 40.00 | 0.80 | 20.00 |
| Factors selected for screening studies | | | | Levels of factors | | | |
| | | | | Low (-1) | | High (+) | |
| Type of solvent | | | | ACN | | MeOH | |
| % v/v of solvent | | | | 60 | | 65 | |
| Type of organic modifier | | | | FA | | OPA | |
| Conc. organic modifier | | | | 0.05 | | 0.2 | |
| Temperature | | | | 30 | | 40 | |
| Flow rate | | | | 0.8 | | 1.0 | |
| Injection volume | | | | 10 | | 20 | |

MeOH: Methanol; ACN: Acetonitrile; FA: Formic acid; OPA: Orthophosphoric acid.

get best fit model like linear, quadratic, polynomial, 2FI etc. and to generate the equations of the model.[15]

## Polynomial equation can be represented in generalized form as

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

Wherein response or dependant variable is denoted as Y. $\beta_0$ is the intercept which is an average arithmetic mean of all quantitative outcomes of 13 runs, $\beta_1$ and $\beta_2$ are linear coefficients; $\beta_{12}$ interaction coefficients between two factors; $\beta_{11,}$ and $\beta_{22}$ quadratic coefficients obtained from the observed response; $X_1$ and $X_2$ are coded levels of independent variables; $X_1X_2$ is interaction term and $X_1^2$ and $X_2^2$ are quadratic terms respectively. The magnitude of coefficients and their signs play an important role in identifying the effect of corresponding independent variable on responses. The effect is directly related to the magnitude of coefficient i.e. higher is the value of magnitude of coefficient more significant is the effect and vice versa. Further sign of the magnitude denotes its positive or negative effect on the studied response. Analysis of Variance (ANOVA) was used to determine the statistical significance of the model. The $p<0.05$ was considered as significant for model and model terms. Both 2D-contour plots and 3D-response surface plots were used to determine the correlation between the independent and dependent variables. Further interaction studies were carried out by overlay plots.

## Method validation

In order to validate the proposed chromatographic method, it was assessed for linearity, specificity, accuracy, precision, robustness, and system applicability in accordance with the ICH Q2 (R1) guidelines.[16]

## System Suitability

The system suitability was assessed by injecting six replicate injections of a standard solution of CDL at a concentration of 15 µg/mL. Various factors like NTP, Rt, PA, and Tf have been determined. RSD values for PA and Rt were less than 2%, which is within the acceptable range.

## Linearity

The linearity of the method was established with six different concentrations of CDL standard solutions ranging from 5-30 µg/mL in triplicate. The plot between concentrations and peak area was obtained to get calibration curve. Further equation of the line was obtained to get slope, intercept and coefficient of correlation ($R^2$).

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

Equations 1 and 2 were used to determine the magnitude of the detection and quantification limits.

**Table 2: $3^2$ Box-Behnken designs along with the observed values for optimization of RP-HPLC method for CDL**

| Run No | Independent Variables | | Dependent variables | | | |
|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ |
| 1 | 65.00 | 10.00 | 4.221 | 406576 | 1.191 | 4441 |
| 2 | 60.00 | 15.00 | 5.595 | 605931 | 1.172 | 5000 |
| 3 | 65.00 | 15.00 | 4.228 | 610665 | 1.206 | 4394 |
| 4 | 55.00 | 20.00 | 8.289 | 801457 | 1.18 | 5632 |
| 5 | 60.00 | 15.00 | 5.597 | 604773 | 1.173 | 4991 |
| 6 | 60.00 | 15.00 | 5.6 | 605588 | 1.174 | 4980 |
| 7 | 60.00 | 15.00 | 5.607 | 605613 | 1.175 | 4943 |
| 8 | 60.00 | 15.00 | 5.602 | 605547 | 1.174 | 4929 |
| 9 | 60.00 | 10.00 | 5.599 | 405319 | 1.115 | 5129 |
| 10 | 60.00 | 20.00 | 5.593 | 808590 | 1.192 | 4878 |
| 11 | 55.00 | 15.00 | 8.297 | 602148 | 1.16 | 5786 |
| 12 | 65.00 | 20.00 | 4.225 | 814187 | 1.217 | 4329 |
| 13 | 55.00 | 10.00 | 8.316 | 404127 | 1.138 | 5963 |

$X_1$: Solvent concentration in mobile phase (% v/v); $X_2$: Injection volume; $Y_1$: Retention time; $Y_2$: Peak area; $Y_3$: Tailing factor; $Y_4$: Number of theoretical plates.

$$LOD = \frac{3.3 \times SD}{\alpha} \quad \text{.....(1)}$$

$$LOQ = \frac{10 \times SD}{\alpha} \quad \text{.....(2)}$$

Where SD represents the standard deviation of response of lowest concentration (intercept) and α is slope of linear calibration curve.

### Precision

To determine the precision of the established method, intra-day and inter-day variations in responses were studied. Intraday precision was carried out by analysing the three different concentrations (5 µg/mL, 15 µg/mL and 25 µg/mL) three times in a day whereas inter-day precision was established with analysis of three different concentrations on three different days. Peak areas were determined and their % RSD values were calculated.

### Accuracy

To determine the accuracy, % recovery studies were performed at three different concentration levels. Briefly, to the bulk powder of drug (10 mg) the tablet powder equivalent to 50%, 100% and 150% of drug was added. The powder mixture is diluted with mobile phase and analyzed to determine the % recovery of the samples.

### Robustness

Robustness of the method was checked by intentionally varying the chromatographic conditions such as flow rate (±0.1 mL/min), mobile phase composition (MeOH: 0.1% OPA (64:36 and 66:34, v/v), and column temperature (±1°C) and studying the effect on each of the CAAs. The system suitability attributes were calculated, including NTP, Tf, % RSD of PA, Rt and % recovery.

### Assay of Marketed tablets

Twenty marketed tablets of Carvedilol (Cardivas, Batch no-SIC 3318A, Mfg. 12/2021, Exp. 11/2024, Sun Pharma. Laboratories ltd.) were accurately weighed and triturated. A powder sample containing 10 mg of CDL was carefully weighed and placed in a 50 mL volumetric flask containing 20 mL of methanol. The mixture was vortex mixed to dissolve the drug completely. Further volume was made with MeOH, which was then filtered through a 0.45 µm membrane filter (Nylon). Suitable dilution of the filtrate with the mobile phase was carried out and CDL content was determined using the developed method.

## RESULTS AND DISCUSSION

### Preliminary method development

Review of available literature has witnessed the utilization of mobile phase compositions like acetonitrile, methanol, formic acid and acetic acid at different flow rate, injection volume, concentration etc.

Present study deals with development of a simple, precise and robust RP-HPLC method for the determination of Carvedilol (CDL). The method was optimized by changing method parameters such as mobile phase composition, organic modifier concentration and column temperature in order to obtain an intense and symmetrical peak with no tailing.

Preliminary studies were carried out by using methanol and water at various proportions. The results were not acceptable as poor resolution with tailing factor of more than 2 was observed. Further studies were conducted by replacing methanol with acetonitrile. The use of acetonitrile and water in different ratios has resulted into poorly resolved broad peaks with tailing. When MeOH

and 0.1% aqueous solution of formic acid was tried as mobile phase well defined sharp peak of CDL was obtained and tailing factor reduced from 2.0 to 1.363. When ACN and 0.1% aqueous solution of formic acid was tried as mobile phase, the CDL peak eluted very near to the negative peak. Further decrease in ACN concentration to separate the CDL peak from negative peak led to increased retention time. Hence further trails were done with MeOH and formic acid. Further increase in the concentration of formic acid up to 0.5% v/v reduced the tailing factor from 1.363 to 1.20 with improved peak shape. It was observed that column temperature plays an important role on tailing factor. When the temperature decreased from 40°C to 25°C, the tailing factor reduced from 1.281 to 1.222. When formic acid was replaced with 0.1% OPA, tailing factor was reduced (1.20) and sharp peak was obtained (Figure 1). When ACN and 0.1% OPA was tried as mobile phase, tailing factor was more than 1.3. Based on the results obtained RP-HPLC method was developed and optimized on Shim-pack GIST C18 column using 0.1% OPA in water and MeOH (35:65 v/v) by maintaining 1.0 mL/min flow rate, 30°C column temperature and 240 nm as detection wavelength.

## Risk assessment and Screening Studies[12]

Risk assessment plays an important role in understanding the association between CMV's and CAA's. Based on risk assessment studies, seven factors were selected and their further screening was done based on Taguchi design. The screening study helps to find out the factors which significantly contribute the experimental results within few experimental trials. To represent the effect of these factors on CAA, half-normal plots and Pareto charts were used (Figure 2). Two factors i.e. concentration of methanol ($X_1$) and injection volume ($X_2$) were mainly contributing factors on the studied responses.

## Factor optimization using $3^2$ BBD

The results obtained through the Design expert software has suggested that quadratic model is a best fit model for correlating the independent and dependant variables. F test value with $p<0.05$ also witnessed the significance of the model. Summary of 4 responses is represented in Table 3. The $R^2$ value for all the responses was ranged between 0.9972-1. The data depicts that response data also fits good to the polynomials. Further confirmation of the best fit was confirmed by closeness of predicted and adjusted $R^2$ value (Difference <0.2). Quadratic equation obtained is represented as equation 1, wherein $b_1$ to $b_{22}$ are the coefficients of interaction terms while $b_0$ is intercept. The 3D-response surface diagrams and 2D-contour plots are as shown in (Figure 3).

## Effect of independent variables on Retention time ($Y_1$)

The quadratic polynomial equation depicting the independent variables effect on retention time is as represented below

$$\text{Retention time } (Y_1) = +5.60 - 2.04X_1 - 0.004833X_2 + 0.007750X_1X_2 + 0.66X_1^2 - 0.002052X_2^2$$

The equation represents that both % v/v of methanol in mobile phase and injection volume have negative effect on the retention time. Further greater value of coefficient of $X_1$

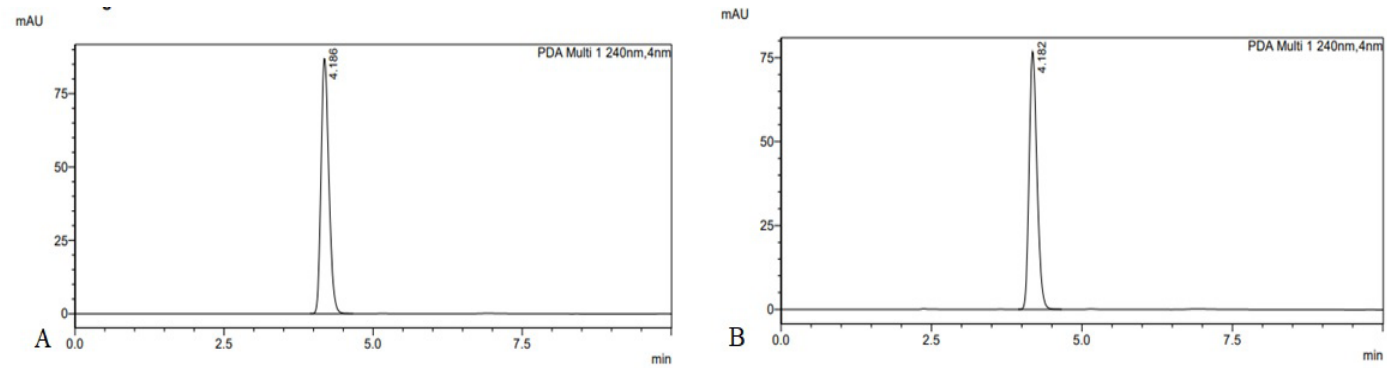



**Figure 1:** RP-HPLC Chromatogram of A) Carvedilol Standard B) Carvedilol Assay.

**Table 3: Analysis of Variance (ANOVA) test results and adequate precision for various regression analysis responses.**

| | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | SD | CV (%) | F Value | *p* Value | Adequacy precision |
|---|---|---|---|---|---|---|---|---|
| Retention time (min) | 1.00 | 1.00 | 1.00 | 0.004 | 0.078 | 250401.1 | <0.0001 | 1312.927 |
| Peak area | 1.0000 | 1.0000 | 0.9999 | 582.32 | 0.09 | 143571.34 | <0.0001 | 1037.993 |
| Tailing factor | 0.8988 | 0.8264 | 0.0690 | 0.011 | 0.94 | 17.22 | 0.0006 | 14.100 |
| Number of theoretical plates | 0.9984 | 0.9972 | 0.9951 | 26.87 | 0.53 | 858.41 | <0.0001 | 89.691 |

witnesses the pronounced effect of % v/v methanol on retention time in comparison to that of injection volume. The combined interaction terms % v/v of methanol, injection volume ($X_1X_2$), have positive effect on the retention time. Table 3 shows the results of ANOVA for retention time data. The value of 250401.42 for Model F represents its significance with such a high value, there is just a 0.01% possibility that noise is influencing the responses. The signal to noise ratio determines the precision of the method. Normally the value of the ratio more than 4 is considered as appropriate. The ratio was found to be 1312.927, indicating that a quadratic model may be used to analyze the results in design space. The 3D-response surface plots, 2D-contour plots and perturbation plots depicting the correlation between retention time and studied independent variables have been shown in Figure 3A, 3E and 4a-A respectively.

## Effect of independent variables on peak area ($Y_2$)

The quadratic polynomial equation depicting the independent variables effect on peak area is as represented below

$$\text{Peak area}(Y_2) = +605700 + 3949.33\,X_1 + 201400X_2 + 2570.25\,X_1X_2 + 296.31\,X_1^2 + 844.X_2^2$$

The equation represents that both % v/v of methanol in mobile phase and injection volume have positive effect on the peak area. Further greater value of coefficient of $X_2$ witnesses the pronounced effect of injection volume on peak area in comparison to that of % v/v methanol. The combined interaction terms % v/v of methanol, injection volume ($X_1X_2$), have positive effect on the peak area. Table 3 shows the results of ANOVA for peak area data. The value of 143571.34for Model F represents its significance. Such a high value could have only 0.01% chance that noise is affecting the results. The signal to noise ratio was found to be 1037.993 which indicate that quadratic model can be suitable to represent the results in design space. The 3D-response surface plots, 2D-contour plots and perturbation plots depicting the correlation between peak area and studied independent variables have been shown in Figure 3B, 3F and 4a-B respectively.

## Effect of independent variables on tailing factor ($Y_3$)

The linear equation depicting the independent variables effect on tailing factor is as represented below

$$\text{Tailing factor}\ (Y_3) = +1.17 + 0.023\,X_1 + 0.024\,X_2$$

The equation represents that both % v/v of methanol in mobile phase and injection volume have positive effect on the tailing factor. The values of coefficient of $X_1$ and $X_2$ have almost same which witnesses that both the factors have equal effect on the studied response. Absence of interaction terms and squared terms indicate that there was no significant interaction between the independent variables in case of tailing factor. Table 3 shows the results of ANOVA for tailing factor data. The value of 17.22 for Model F represents its significance. Such a high value could have only 0.06% chance that noise is affecting the results. The signal to noise ratio was found to be 14.1 which indicate that linear model can be suitable to represent the results in design space. 3D-response surface plots, perturbation plots and 2D-contour plots depicting the correlation between tailing factor and studied independent variables have been shown in Figure 3C, 3G and 4a-C respectively.

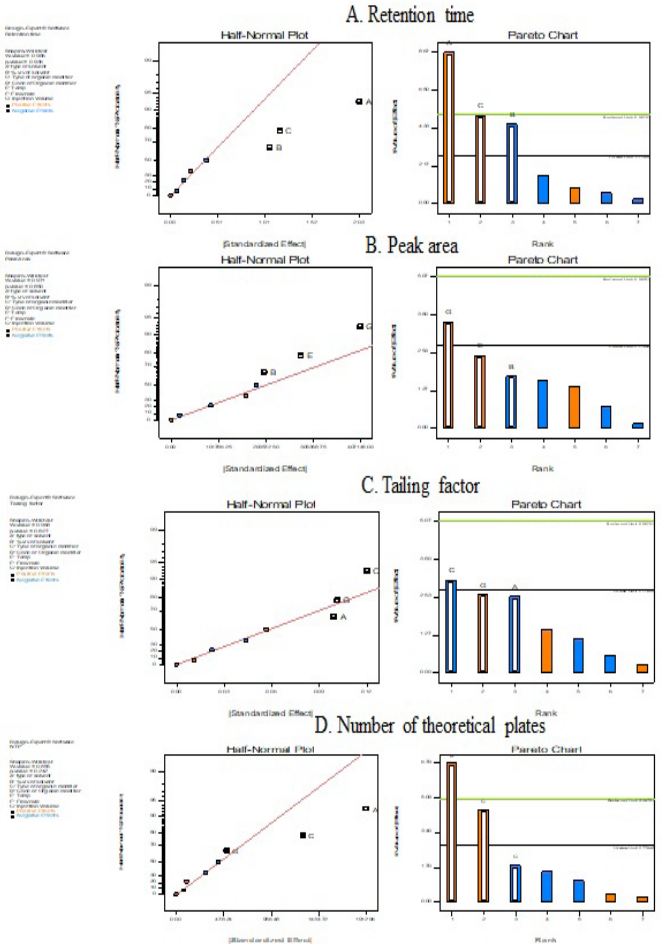



**Figure 2:** A-D Half-normal plots and Pareto charts indicating the factor effects of the responses, i.e., retention time, peak area, tailing factor and number of theoretical plates.

**Table 4: System suitability test parameters (*n*=6) Concentration=15 µg/mL.**

| Compound | Retention time (Rt, min) | RSD % of retention time | Peak area | RSD % of peak area | Theoretical plates (N) | Tailing factor (T) |
|---|---|---|---|---|---|---|
| CDL | 4.18 | 0.09 | 697961 | 0.19 | 4361 | 1.21 |

*n*=number of replicates;RSD=Relative standard deviation.

## Effect of independent variables on number of theoretical plates ($Y_4$)

The quadratic equation depicting the independent variables effect on number of theoretical plates is as represented below

$$\text{Number of theoretical plates}\,(Y_4) = +4973.24 - 702.83\,X_1 - 115.67\,X_2 + 54.75\,X_1X_2\,105.16X_1^2 + 18.66X_2^2$$

The equation represents that both % v/v of methanol in mobile phase and injection volume have negative effect on the NTP. Further greater value of coefficient of $X_1$ witnesses the pronounced effect of % v/v of methanol in mobile phase on $Y_4$ in comparison to that of injection volume. The combined interaction terms % v/v of methanol, injection volume ($X_1X_2$), have positive effect on the $Y_4$. Table 3 shows the results of ANOVA for number of theoretical plate's data. The value of 858.41 for Model F represents its significance. Such a high value could have only 0.01% chance that noise is affecting the results. The signal-to-noise ratio was found to be 89.691, indicating that the quadratic model is appropriate for representing the results in design space. Figure 3D, 3H, and 4a-D exhibit 3D-response surface plots, perturbation plots, and 2D-contour plots demonstrating the correlation between peak area and studied independent variables, respectively.

## Analytical Method Validation

Validation of an analytical method is considered as a prerequisite for its application in day to day qualitative and quantitative analysis.

## System Suitability

System suitability test results have witnessed that there is no significant difference in PA, Rt, NTP and Tf values when it is studied with six replicates of CDL having concentration of 15 µg/mL. The results are as shown in Table 4.

**Table 5: Analytical method validation results.**

| Precision | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analyte | Content (µg/mL) | Intraday (*n*=6) | | Inter day (*n*=3) | | | | | |
| | | | | Day 1 | | Day 2 | | Day 3 | |
| | | Found (µg/mL) | % RSD | Found (µg/mL) | % RSD | Found (µg/mL) | % RSD | Found (µg/mL) | % RSD |
| CDL | 5.00 | 4.84 | 0.14 | 4.84 | 0.13 | 5.72 | 0.03 | 5.22 | 0.66 |
| | 15.00 | 14.10 | 0.07 | 14.10 | 0.06 | 15.93 | 0.03 | 14.78 | 0.09 |
| | 25.00 | 23.59 | 0.13 | 23.58 | 0.01 | 25.54 | 0.01 | 25.43 | 0.16 |
| Recovery studies (*n*=3) | | | | | | | | | |
| Compound | | Contents (mg) | | Quantity added (mg) | | Theoretical amount (mg) | Recovered amount (mg) | Recovery (%) | % RSD |
| CDL | | 10 | | 5 (50%) | | 15 | 14.89 | 99.29 | 0.01 |
| | | | | 10 (100%) | | 20 | 19.80 | 99.02 | 0.07 |
| | | | | 15 (150%) | | 25 | 24.26 | 97.06 | 0.02 |
| Robustness study (*n*=6) | | | | | | | | | |
| Parameter | Modification | CDL | | | | | | | |
| | | % RSD of peak area | | % RSD of retention time | | Theoretical plates | | Tailing factor | Percentage recovery |
| Flow rate | 0.90 mL/min | 0.10 | | 0.04 | | 4694 | | 1.22 | 110.53 |
| | Optimized | 0.47 | | 0.01 | | 4325 | | 1.21 | 100.00 |
| | 1.10 mL/min | 0.05 | | 0.03 | | 3985 | | 1.21 | 93.00 |
| Mobile phase composition | 64% MeOH | 0.05 | | 0.02 | | 4216 | | 1.22 | 101.27 |
| | Optimized | 0.47 | | 0.01 | | 4325 | | 1.21 | 100.00 |
| | 66 % MeOH | 0.02 | | 0.04 | | 4417 | | 1.21 | 101.04 |
| Column temperature | 29°C | 0.05 | | 0.11 | | 4282 | | 1.21 | 101.16 |
| | Optimized | 0.47 | | 0.01 | | 4325 | | 1.21 | 100.00 |
| | 31°C | 0.01 | | 0.01 | | 4354 | | 1.22 | 101.11 |

*n*=number of replicates;RSD=relative standard deviation.

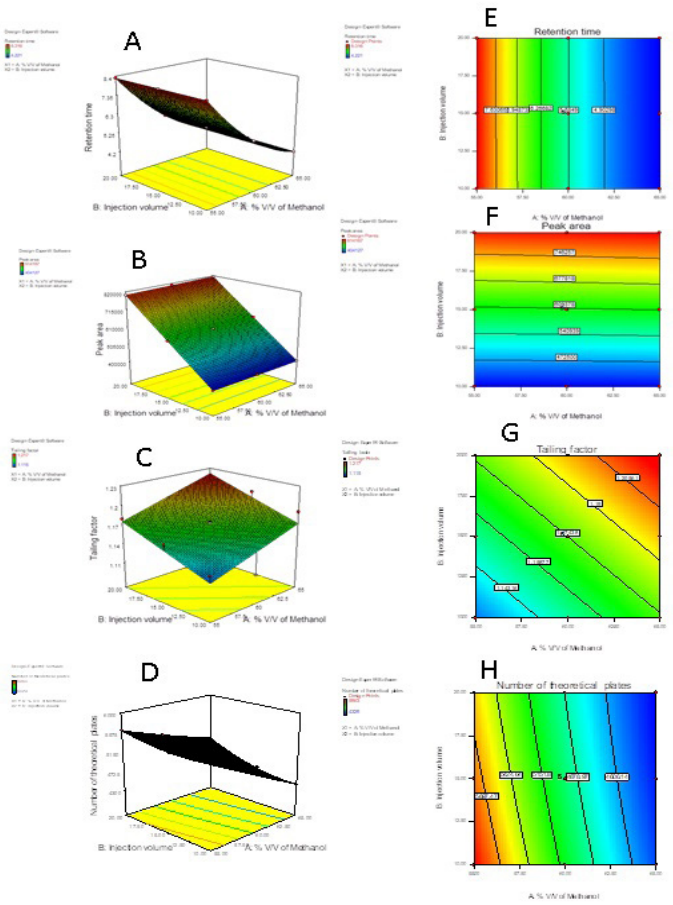



**Figure 3:** 3D-Response surface (A-D) and 2D-contour (E-H) plots showing the effect of %V/V of methanol in mobile phase and injection volume on retention time, peak area, tailing factor and number of theoretical plates. $X_1$=A: Methanol in the mobile phase and $X_2$=B: Injection volume.

## Linearity

ICH guidelines were used to establish the linearity of the proposed analytical method. Linear results were obtained within studied concentration range of 5-30 µg/mL with regression coefficient value of 0.999. Equation of the linear line obtained is y=53574x-57581. The obtained calibration curve is as shown in Figure 5.

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

LOD and LOQ represent sensitivity of the method. LOD and LOQ values of developed method were found to be 0.61 µg/mL and 1.86 µg/mL, respectively. These findings show the method is highly sensitive.

## Precision

The results of precision study have depicted that the % RSD value of peak area lies within 1% during intra and inter day variation. The results of precision study have been as shown in Table 5.

## Accuracy

The accuracy of the method is an indication about its ability to maintain conformity between experimental and theoretical responses. The results of accuracy study are shown in Table 5 The recovered concentration of CDL was found in the range between 99.41-99.82% with % RSD value of <2% which witnesses the accuracy of the method.

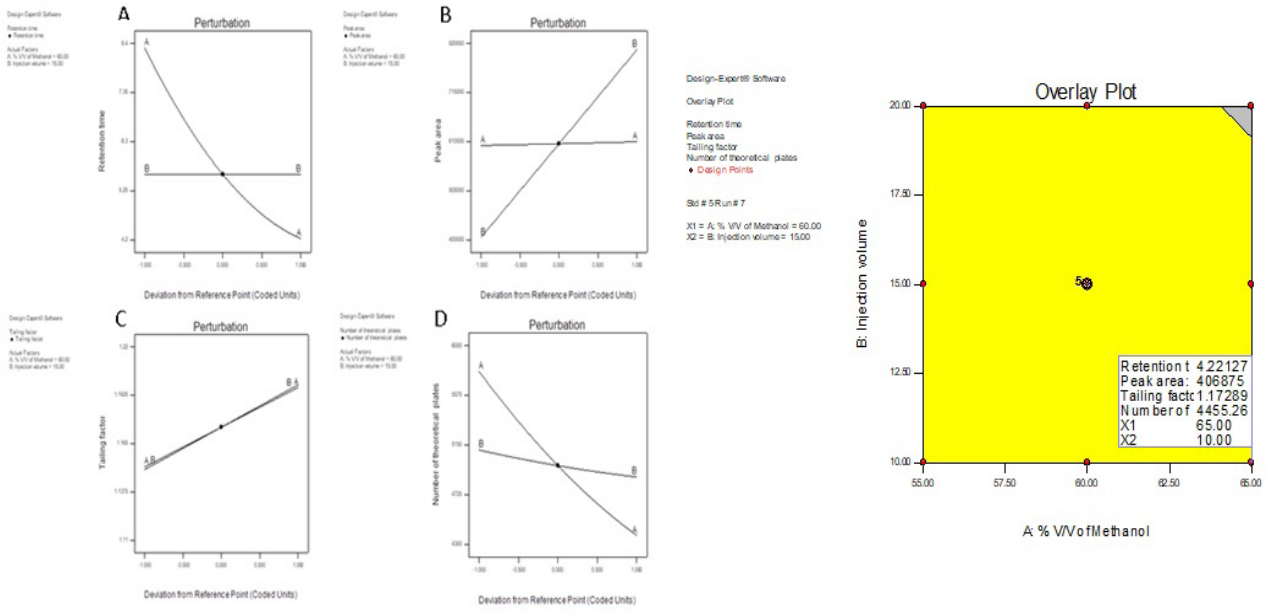



**Figure 4:** a) Perturbation plots (A-D) showing the effect of %V/V of methanol in mobile phase and injection volume on retention time, peak area, tailing factor and number of theoretical plates. A: Methanol in the mobile phase and B: Injection volume. b) Overlay plot showing the optimized chromatographic conditions and their predicted responses.

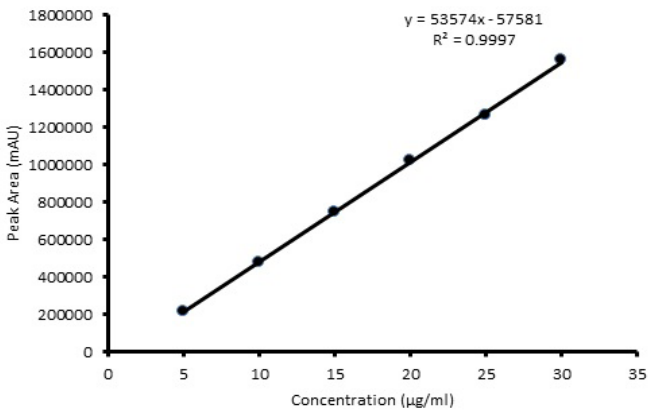



**Figure 5:** Calibration curve of Carvedilol.

### Robustness

In studied CAA, with minor changes in the method parameters, no considerable changes have been observed and degree of % RSD was within <2%. Thus, the robustness of the developed technique was established for minor changes in the method parameters. Table 5 shows the results of the robustness study.

### Assay of Marketed tablets

The assay of the marketed tablet was done by developed method and drug content was found to be 105.60% which complies with prescribed standards (90.0% to 110.0%). The chromatogram for assay study is as shown in Figure 1.

## CONCLUSION

The present study is pertaining to development and validation of simple, reliable and economical RP-HPLC method for analysis of CDL in bulk and dosage form using AQbD approach. The study was started with predefining the ATP. On the basis of ATP, CAAs were selected. The Taguchi design was utilized to screen seven CMAs to further select two most prominent factors. Further optimization of the significant CMAs was carried out by using $3^2$ Box-Behnken Design. The interaction and main effects between studied parameters were further explored with 2D-counter plots and 3D-response surface plots. Various numerical equations were derived from software to get further in-depth insights of interaction effects. The developed method was further validated as per ICH guidelines by utilizing parameters like linearity, system suitability, precision, LOD, LOQ accuracy and robustness. The developed and validated method is suitable to detect and quantify the CDL in bulk and dosage form.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**AQbD:** Analytical quality by Design; **ANOVA:** Analysis of Variance; **ATP:** Analytical Target Attributes; **BBD:** Box-Behnken design; **CAA:** Critical Analytical Attributes; **CMAs:** Critical Material Attributes; **CPPs:** Critical Process Parameters; **CDL:** Carvedilol; **DOE:** Design of Experiment; **ICH:** International Council for Harmonization; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **OPA:** Ortho Phosphoric Acid; **PA:** Peak Area; **QbD:** Quality by Design; **RA:** Risk Assessment; **RSD:** Residual Standard Deviation; **RT:** Retention Time; **NTP:** Number of Theoretical Plates; **RP-HPLC:** Reverse Phase-High Performance Liquid Chromatography; **TF:** Tailing Factor.

## SUMMARY

In the present study, Analytical Quality by Design (AQbD) approach is employed to develop and validate a RP-HPLC method for the rapid and reliable analysis of CDL. Based on the results, the CAAs were optimized using a $3^2$ Box-Behnken design. The optimized mobile phase is composed of 0.1% ortho phosphoric acid and methanol in a 35:65%v/v ratio. The flow rate was kept constant at 1 mL/min, column temperature of 30°C, and the analysis was carried out at 240 nm. Analytical Quality by Design (AQbD) enabled optimized RP-HPLC method was standardized in terms of validating attributes such as system suitability, linearity, LOD and LOQ, precision, accuracy and robustness using ICH: Q2 (R1) guidelines. The area under the curve was determined to be linear within the tested range of 5-30 µg/mL ($R^2$=0.999). The retention time was found to be 4.18 min, with a sharp, well-defined peak. The % recovery of the drug during accuracy study was found to be in the range between 97.06 to 99.29%. The detection and quantification limit were found to be 0.61 µg/mL and 1.86 µg/mL respectively. The developed method was found to be precise with %RSD <1%. The values of precision and robustness parameters was found to be within the required acceptance limit with % RSD <2%. Hence it is concluded that the AQbD based developed RP-HPLC method can be effectively utilized for routine analysis of bulk drug as well as dosage from.

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