QbD-Driven Development and Validation of a Robust RP-HPLC Method for Quantitative Estimating Doxycycline Hyclate in Bulk and Novel Nanostructured Lipid Carrier Formulation

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Abstract:

Introduction: The development and validation of a reversed phase high performance liquid chromatography (RP-HPLC) method for the quantification of doxycycline hyclate was done using a quality by design (QbD) methodology.

Methods: The flow rate, mobile phase composition, and column temperature were determined to be the critical process parameters (CPPs) that could have an impact on the CQAs. The CPPs were screened using a Box Behnken design in order to determine which characteristics were most important. The CPPs were then optimized using a central composite design to produce the required CQAs. Linearity, range, accuracy, precision, robustness, Limit of Quantitation (LOQ), and Limit of Detection (LOD) were all verified for the created approach.

Results: With a correlation coefficient of 0.9939, the procedure was found to be linear over the concentration range of 5-30 µg/mL. Less than 2.0% RSD and 98–102% recovery, respectively, are the permitted bounds for accuracy and precision, respectively. Small adjustments to the CPPs were also shown to not affect the method's robustness. The force degradation study also carried out with the maximum degradation in Alkali media.

Conclusion: The developed method is a simple, rapid, and reliable method for the estimation of doxycycline hyclate in bulk drugs and pharmaceutical formulations.

Keywords: Doxycycline Hyclate NLC, RP-HPLC, QbD, Method validation, Force Degradation, Quantitative Estimating.

1. INTRODUCTION

Doxycycline Hyclate[1], a semi-synthetic tetracycline antibiotic, holds a prominent position in the treatment of a wide range of bacterial infections including sexually transmitted infections[2], respiratory infections[3], and skin infections due to its broad-spectrum antibacterial activity[1,4] and favourable pharmacokinetic profile[5]. Doxycycline hyclate Fig. (1) is also used in the treatment of malaria and Lyme disease[6]. This antimicrobial agent is commonly prescribed for the management of respiratory tract infections, skin and soft tissue infections, urinary tract infections, and as prophylaxis for malaria[7, 8].

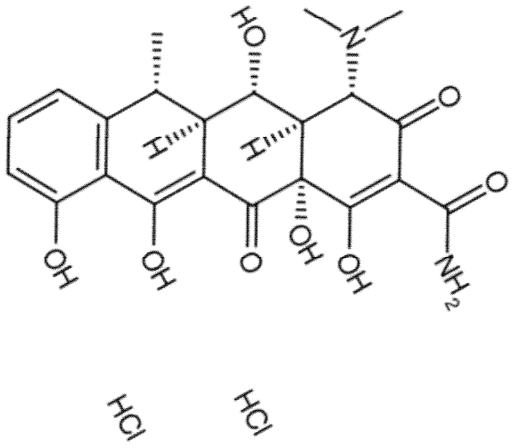


Fig. (1). Chemical Structure of Doxycycline Hyclate

As the global demand for Doxycycline Hyclate-containing pharmaceutical products continues to rise, the need for robust analytical methods for its quantification in drug formulations becomes increasingly essential[9]. The quantification of active pharmaceutical ingredients (APIs) such as Doxycycline Hyclate is pivotal in pharmaceutical analysis, ensuring the quality, safety, and efficacy of drug products[10]. Consequently, the development and validation of accurate and reliable analytical methods for the determination of Doxycycline Hyclate concentrations in pharmaceutical formulations and bulk drug substances are critical for quality control, regulatory compliance, and patient safety[11].

Several analytical methods have been reported for the estimation of Doxycycline Hyclate, including spectrophotometry[4], capillary electrophoresis, and liquid chromatography[12]. Among these techniques, High-Performance Liquid Chromatography (HPLC) has emerged as the method of choice due to its high selectivity, sensitivity, and

versatility, making it particularly well-suited for the analysis of complex pharmaceutical matrices. However, to ensure the consistent accuracy and precision of HPLC-based assays, the development and validation of a well-optimized method are paramount[13, 14].

In recent years, the pharmaceutical industry has embraced the principles of Quality by Design (QbD) as advocated by regulatory authorities like the U.S. Food and Drug Administration (FDA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)[15]. QbD emphasizes the systematic and science-based approach to pharmaceutical development, highlighting the importance of understanding the critical parameters that affect product quality and performance[16]. The application of QbD principles to analytical method development offers a structured framework for achieving method robustness, which is essential for reliable and reproducible results[17].

Traditional RP-HPLC methods are often developed without a systematic approach to understanding the relationship between the critical process parameters (CPPs) and the critical quality attributes (CQAs)[18]. Quality by design (QbD) is a systematic approach to the development and validation of analytical methods[19]. QbD involves identifying the CQAs, CPPs, and the relationship between the CPPs and CQAs. The goal of QbD is to develop a method that is robust and can consistently produce results that meet the desired specifications[20, 21].

Several RP-HPLC methods have been reported for the estimation of doxycycline hyclate in bulk drugs and pharmaceutical formulations. However, most of these methods were not developed using a QbD approach. This research paper aims to present the development and validation of an RP-HPLC method for the estimation of Doxycycline Hyclate, incorporating the principles of QbD. The utilization of QbD in method development offers several advantages, including improved method robustness, reduced risk of method failure, and enhanced method transferability, ultimately ensuring the method's suitability for routine pharmaceutical analysis. Furthermore, this study contributes to the growing body of research on the application of QbD principles in analytical chemistry, demonstrating their effectiveness in achieving reliable analytical methods.

2. MATERIALS AND METHODS

2.1. Chemicals and Regents

Doxycycline hyclate as bulk drug along with its COA was obtained as gift sample from the Leben Laboratories Pvt Ltd Akola (MS). Aloe-emodin was purchase from Yucca

Enterprises Mumbai. HPLC grade methanol was obtained from the Jinendra Scientifics Jalgaon (MS) AR grade phosphate buffer was prepare as per USP and Mili Q water was used to prepare the mobile phase composition and dilutions.

2.2. Instrumentation and chromatographic conditions

A Waters (2695) high performance liquid chromatography (HPLC) system fitted with quaternary solvent system, automatic sampling system, PDA Detector, column oven control system for the column, cooling auto sampler unit was used for the method development. The chromatographic separation of Doxycycline hyclate were performed on water symmetry C18-250 × 4.6 mm with a 5 µm particle size chromatographic column. The mobile phase composition used for the separation was Phosphate Buffer: Methanol (pH 8) in the ratio of 50:50 v/v. The PDA-M20A Prominence diode array detector was used for the detection at 268 nm[22, 23]. The Elution of the Doxycycline hyclate was performed by using isocratic system with flow rate of 0.7 ml/min, column temperature of 50°C ± 2 °C and 10 µl as injection volume.

2.3. Preparation of Calibration Standard Solutions

The calibration standard solutions were made by accurately weighing 10 mg of doxycycline hyclate, transferring it into a clean, dry 10 ml volumetric flask, adding the appropriate amount of previously prepared mobile phase, stirring, and ultrasonically sonicating the mixture for 5–7 minutes to obtain a clear solution. Next, the volume was increased to 10 ml to obtain a stock solution with strength of 1000 µg/ml (Stock 1). The mobile phase was appropriately diluted with the prepared stock solution 1 to yield the six distinct calibration standards. Using these six calibration standards, 5 µg/ml (cal std 1), 10 µg/ml (cal std 2), 15 µg/ml (cal std 3), 20 µg/ml (cal std 4), 25 µg/ml (cal std 5), and 30 µg/ml (cal std 6) a standard curve for the Doxycycline hyclate was created in the range of 5 µg/ml - 30 µg/ml. After passing through a 0.22 µm syringe filter, all of the produced dilutions were ready for further chromatographic analysis[24-26].

2.4. Preliminary Trials for Method Development Studies

The preliminary trial run for the analytical method development of Doxycycline hyclate was carried out by trying the different mobile phase composition with different flow rate and column temperature parameters, Table 1 illustrate all the preliminary trial studies [2] There was total 7 run of Doxycycline hyclate standard carrier out on HPLC system out of 7, In 1-4

run Ammonium Acetate Buffer (pH 4.5) with different concentration of methanol (MeOH) and acetonitrile (ACN) i.e. tri-phasic system was used with flow rate of 1 ml/min & 40ºC column temperature and trial 5 & 6 was run with Monobasic Potassium Phosphate Buffer (pH 8) with different concentration of methanol and acetonitrile with flow rate of 0.8 ml/min & 50ºC column temperature and last run was carried out with biphasic system i.e. Monobasic Potassium Phosphate Buffer (pH 8) with methanol in ratio of (50:50 v/v) at low rate of 0.7 ml/min & 50ºC as column temperature.

2.5. Experimental Design for Analytical Method Development

The critical technique parameters that affected the performance of the analytical method were chosen based on the preliminary trial runs studies. Box Behnken Design (BBD) was the method used in the design process . It was divided into three levels: low level (-1), intermediate level (0), and high level (+1). While Table 2 shows the experimental design matrix according to BBD with 17 experimental designs performed with 5 centre points runs, Table 3 highlights the factors and levels [2].

2.6. Analytical Method Optimization using Quality by Design Approach

The analytical method development optimization of Doxycycline hyclate was carried out using Quality by Design approach (QBD). This approach mainly defines the measurement for the desired quality process and product identification, robustness, separation of analytical peaks; accuracy and precision which are mainly refer as critical analytical attributes (CAAs). This approach also involves the critical method attributes (CMAs) which is focuses on the analytical technique performance viz. injection volume, mobile phase pH, column temperature etc[29, 30].

The design of experiment (DoE) consist of one of the best design called Box Behnken Design (BBD) that mainly consist of dependent and independent variables which have been used to optimize the analytical method of analysis for Doxycycline hyclate. For this research work independent variables of the design were taken that is, Flow rate (A), Mobile phase composition (B) and Column Temperature (C) whereas, dependent variables for design was response 1 [Retention Time (RT)] (Y1), response 2 [Tailing Factor TF)] (Y2) and response 3 [Number of Theoretical Plates (NTP)] (Y3). To analyze the results of total 17 runs polynomial equation was used. The model coefficients with statistical significance <0.05 were considered in framing the polynomial equation. The model was finally ratified by analyzing the parameters like lack of fit analysis test, coefficient of correlations (r^2)[31].

After the model analysis response surface analysis was carried out i.e. response surface methodology (RSM) graphs viz. 2D-contour and 3D response surface plots to determine the relationship between factors and possible interaction effects[32,[2] 33].

2.7. Analytical Method Validation

Analytical method validation is a crucial step in ensuring the accuracy, reliability, and robustness of a method used for the quantification of pharmaceutical compounds like Doxycycline Hyclate. The key parameters typically assessed during method validation, along with references to authoritative guidelines.

According to the ICH Q2 (R1) approved conditions, the developed RP-HPLC technique for Doxycycline Hyclate was validated for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). The force degradation studies of Doxycycline Hyclate to be conducted by ICH Q6A guidelines which includes the various stressful conditions and separation of the pure drug (Doxycycline Hyclate) from its degradation product[34, 35].

2.7.1. Linearity

Linearity evaluates the method's ability to provide results that are directly proportional to the concentration of the analyte within a defined range. It is typically assessed by analyzing samples at different concentrations and plotting a calibration curve[36, 37]. Standard curve for Doxycycline Hyclate were plotted at standard concentration range from 5 to 30µg/ml. The concentration viz. 5, 10, 15, 20, 25 and 30 µg/ml were prepared and evaluated for its area at maximum wavelength of 268 nm. The regression equation was obtained and the unknown concentration of drug was calculated[38].

2.7.2. Repeatability

Repeatability assesses the method's precision when the same operator makes multiple measurements under the same conditions. It is typically expressed as relative standard deviation (RSD)[39]. The repeatability was assessed by spiking a concentration of 10 µg/ml at six times at different time intervals on the same day. The average value, standard deviation and % relative deviation was calculated [40-42].

2.7.3. Inter day Precision

Inter day precision is another critical parameter in the method validation process[43] for quantifying Doxycycline Hyclate using analytical methods. It evaluates the precision and reproducibility of the method when applied on different days, typically by different analysts and using different equipment[44, 45]. The concentration 8 µg/ml, 10 µg/ml and 12 µg/ml were analyzed on the day 1, day 2 and day 3for the inter day precision study. Furthermore, the average value, standard deviation and % relative standard deviation (RSD) were calculated. The limit of % RSD calculation is ≤2%.

2.7.4. Accuracy

Accuracy measures how close the method's results are to the true or reference value. It is typically evaluated through recovery studies, comparing the measured concentration to the known concentration of Doxycycline Hyclate in a sample[46]. The accuracy was measured the percent recovery of analyte at three different concentrations viz. 8 µg/ml, 10 µg/ml and 12 µg/ml. The analyte was injected at all three concentration levels and their % recovery and % RSD was calculated[47, 48].

2.7.5. Limit of Quantitation and Limit of Detection

Limit of Quantitation (LOQ)[49] and Limit of Detection (LOD) [50]were determined by using the slope of the linearity graph of Doxycycline Hyclate and standard deviation of the response to the blank sample[51]. The LOQ and LOD was calculated by using following equation

$$LOQ = 10X\ б/S$$

Where,

б= Standard Deviation

S= Slope of linearity equation

$$LOD = 3.3X\ б/S$$

Where,

б= Standard Deviation

S= Slope of linearity equation

2.7.6. Robustness

Robustness evaluates the method's tolerance to small, deliberate variations in method parameters, such as flow rate, temperature, or mobile phase composition. It demonstrates the

method's ability to remain reliable under minor variations[52-54]. The samples for the robustness were analyzed by the slight changes in the detection wavelength (246 nm, 256nm), flow arte (1.2 ml/min, 0.8 ml/min) and mobile phase composition water: methanol (45:55, 55:45). The effect caused by these changes in parameters was investigated and accordingly the results were analyzed. The robustness study was performed at standard concentration of 20µg/ml and standard deviation and % standard deviation was calculated. The acceptance range for the robustness was the % RSD should be ≤2%[55, 56].

2.8. Preparation of Forced Degradation Samples of Doxycycline Hyclate

A forced degradation study is a type of stability study that is performed to assess the stability of a drug substance or drug product under stress conditions [3]. Stress conditions can include heat, light, acid, alkali, and oxidation[35, 57, 58]. These studies involve subjecting a pharmaceutical compound, such as Doxycycline Hyclate, to various stress conditions to evaluate its stability and identify potential degradation products. The Doxycycline Hyclate samples were individually exposed to acid, base, peroxide, heat, and photolytic stress conditions. The solutions were then examined using high-performance liquid chromatography (HPLC)[59].

2.9. Evaluation of Doxycycline Hyclate in Nanostructured Lipid Carriers (NLC) Formulation

Nanostructured Lipid Carriers (NLC) of Doxycycline Hyclate was formulated and the validated analytical method for Doxycycline Hyclate was developed to evaluate the concentration of Doxycycline Hyclate in developed NLC.

3. RESULTS AND DISCUSSION

3.1. Preparation of Calibration Standard Solutions

Calibration standards and a linearity curve are essential components of quantitative analytical methods, such as those used in High-Performance Liquid Chromatography (HPLC) for the determination of Doxycycline Hyclate. Calibration standards are a set of known concentrations of Doxycycline Hyclate that are used to create a relationship between the concentration of the analyte and the instrument response (peak area in HPLC). This relationship is depicted as a calibration curve, and it is essential for accurately quantifying the amount of Doxycycline Hyclate in test samples[60]. To create the linearity curve, a regression analysis is performed on the data points. A common regression method used is

linear regression. The linear equation generated by this analysis allows you to calculate the concentration of Doxycycline Hyclate in an unknown sample based on its measured instrument response[61, 62]. The Concentrations and respective area was depicted in Table 4 whereas, calibration curve plot was given in Fig. (2).

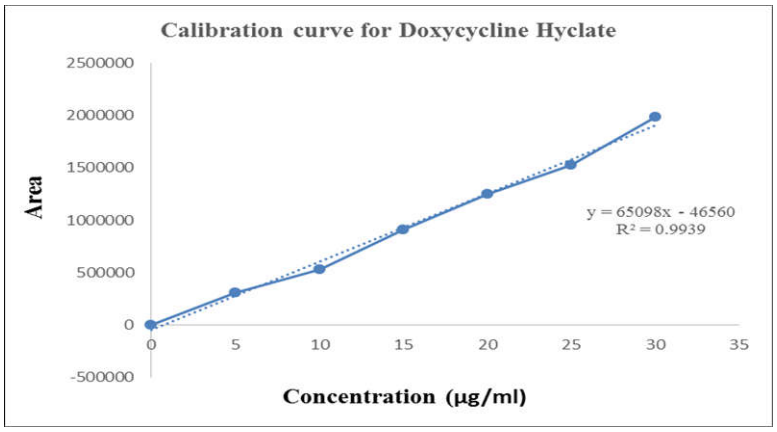
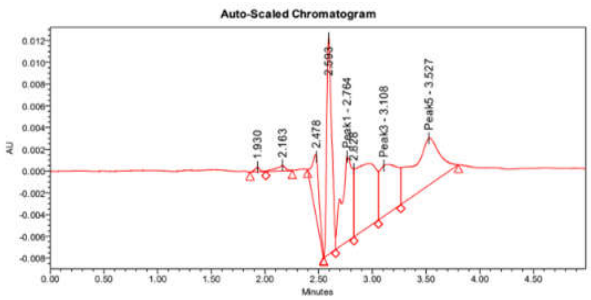


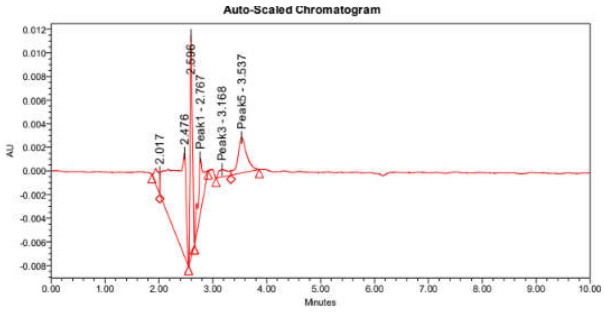
Fig. (2). Calibration Curve for Doxycycline Hyclate

3.2. Preliminary Trials for Method Development Studies

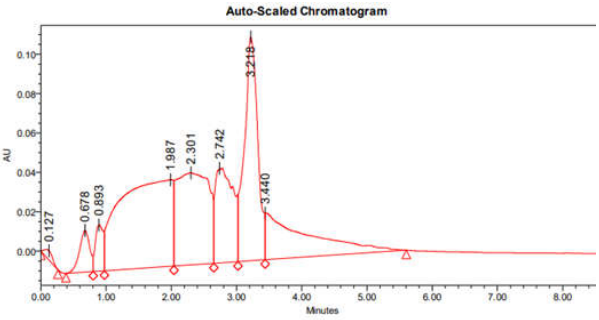
Preliminary trials of HPLC run of Doxycycline Hyclate samples were taken by varying mobile phase composition, flow rate and column temperature. Table 1 describes all the composition with change in parameters. Trial seven was optimized trial in which Monobasic Potassium Phosphate Buffer (pH 8): MeOH (50:50) was used as mobile phase with flow rate of 0.7 ml/min and 50 º C column temperatures. At these parameters the peak was well separated and identified with retention time (RT) of 5.607 minute (Fig. (3)).



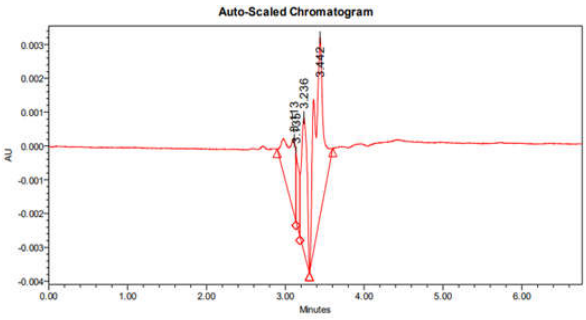
A: Trial 1 chromatogram for Doxycycline Hyclate



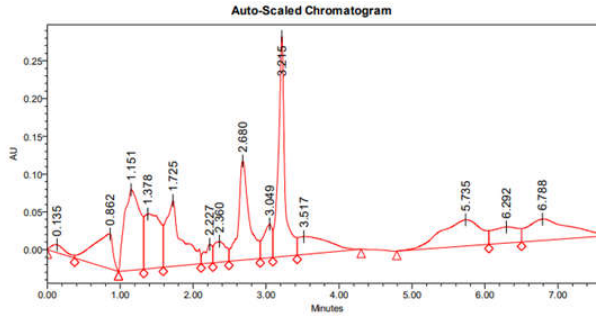
B: Trial 2 chromatogram for Doxycycline Hyclate



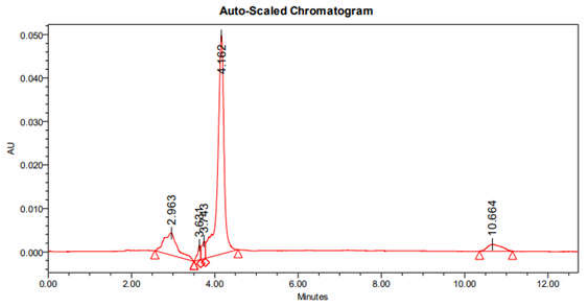
C: Trial 3 chromatogram for Doxycycline Hyclate



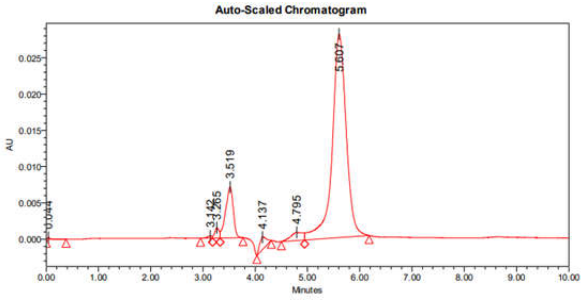
D: Trial 4 chromatogram for Doxycycline Hyclate



E: Trial 5 chromatogram for Doxycycline Hyclate



F: Trial 6 chromatogram for Doxycycline Hyclate



G: Trial 7 chromatogram for Doxycycline Hyclate

Fig. (3). Preliminary Trial Chromatograms of Doxycycline Hyclate for Method Development

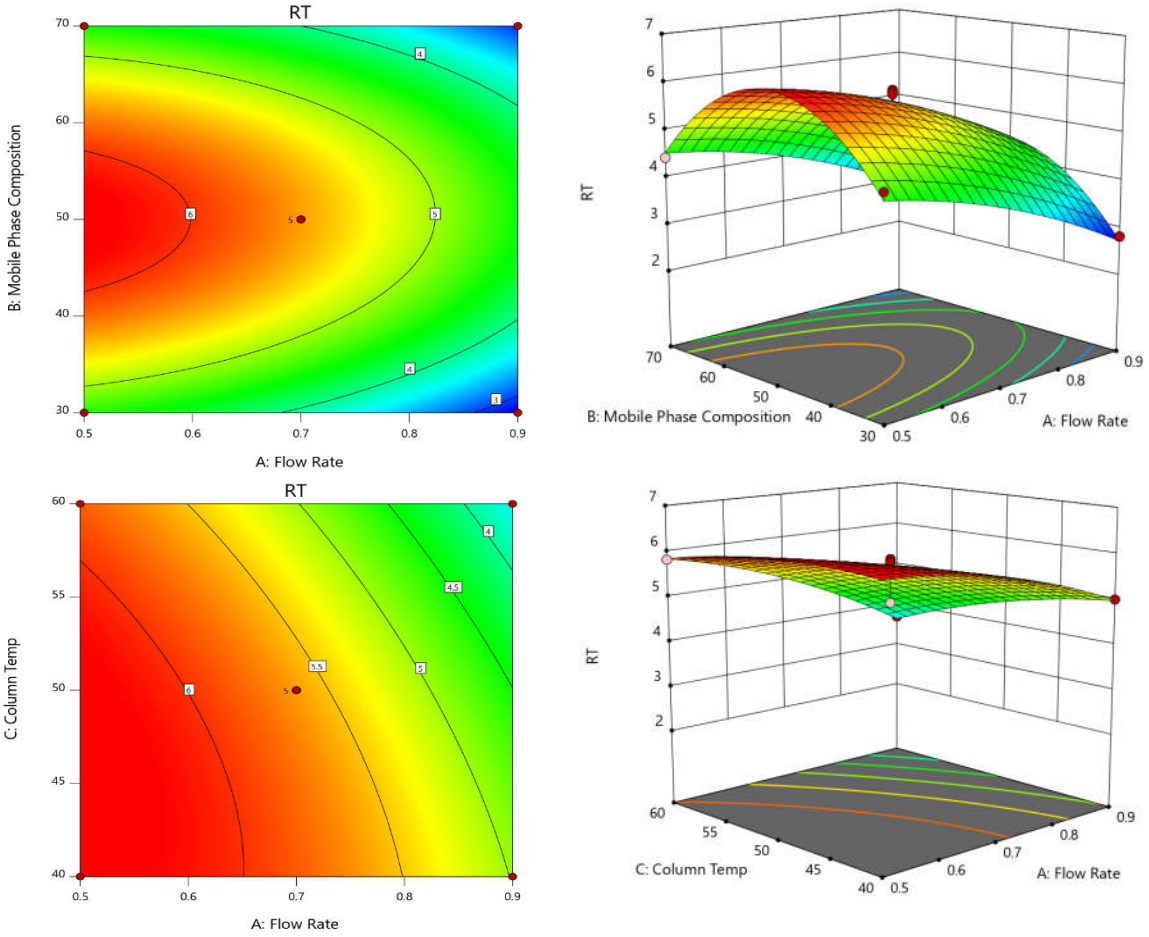
3.3. Analytical Method Optimization using Quality by Design Approach

In the present research work, three independent factors viz. (A) flow rate, (B) mobile phase composition and (C) column temperature were selected based on the preliminary trial runs. Various trial was taken and the responses like response 1 (Retention Time), response 2 (Tailing Factor) and response 3 (Number of Theoretical Plates). The data of optimization and its analysis was carried out by selecting the quadratic polynomial model for analysing the both main and interaction effects. Table 5 describe the all the design batches with independent factors and their respective responses. By examining the model equation created

in accordance with the quadratic equation for each response, the coefficient of analysis was calculated.

3.3.1. Interaction of Independent Factors with Retention Time as Response

The interactive effect of independent factors viz. flow rate, mobile phase composition and column temperature on the responses like retention time, tailing factor and number of theoretical plates[63]. The 2D contour plots and 3D response surface plots for the retention time, tailing factor, number of theoretical plates and three Critical Analytical Attributes (CAA) are shown in Fig. (4).



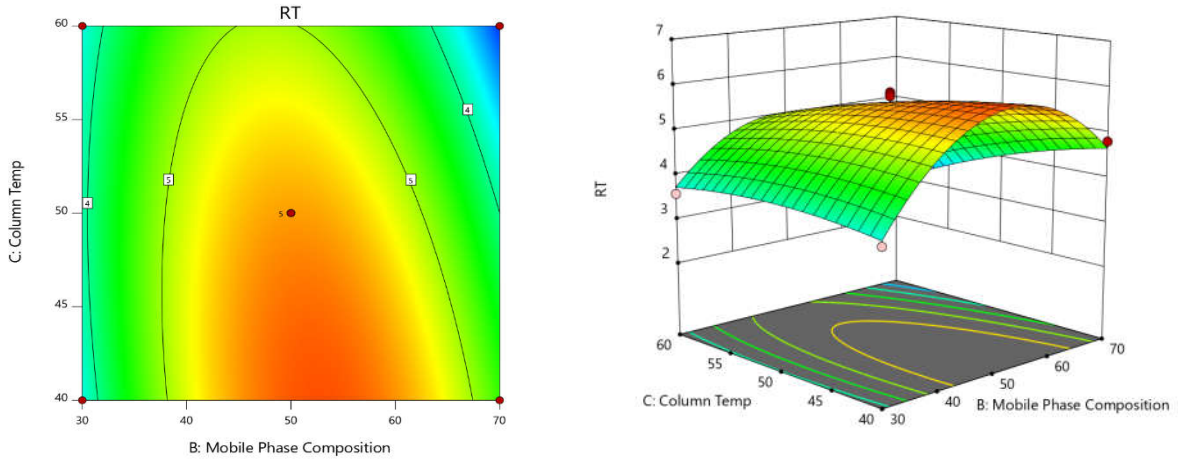


Fig. (4). 2D contour plots and 3D response surface plots showing the effect of flow rate, mobile phase composition and column temperature on Retention Time as Response

The Model F-value of 19.74 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant[64]. In this case A, C, BC, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.30 implies the Lack of Fit is not significant relative to the pure error. There is 82.23% chance that a Lack of Fit F-value this large could occur due to noise.

The Predicted R^2 of 0.8392 is in reasonable agreement with the Adjusted R^2 of 0.9134; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13.238 indicates an adequate signal. This model can be used to navigate the design space[65].

From the above plot of it was clear that the actual values are closer the predicted values that showed the quadratic model of design was fitted and the results were obtained was précised with the accuracy (Fig. (5)).

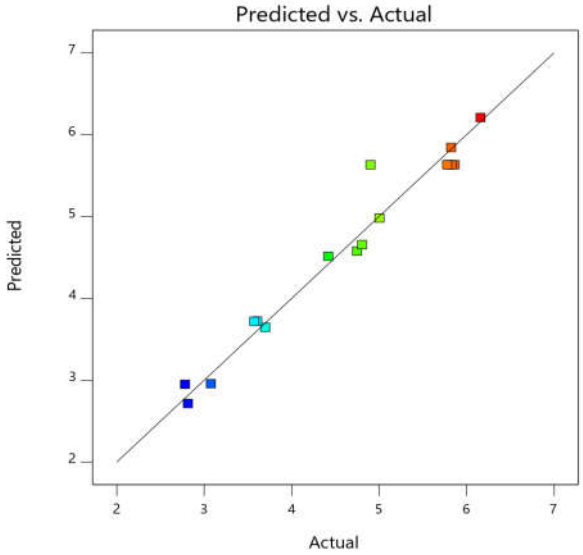
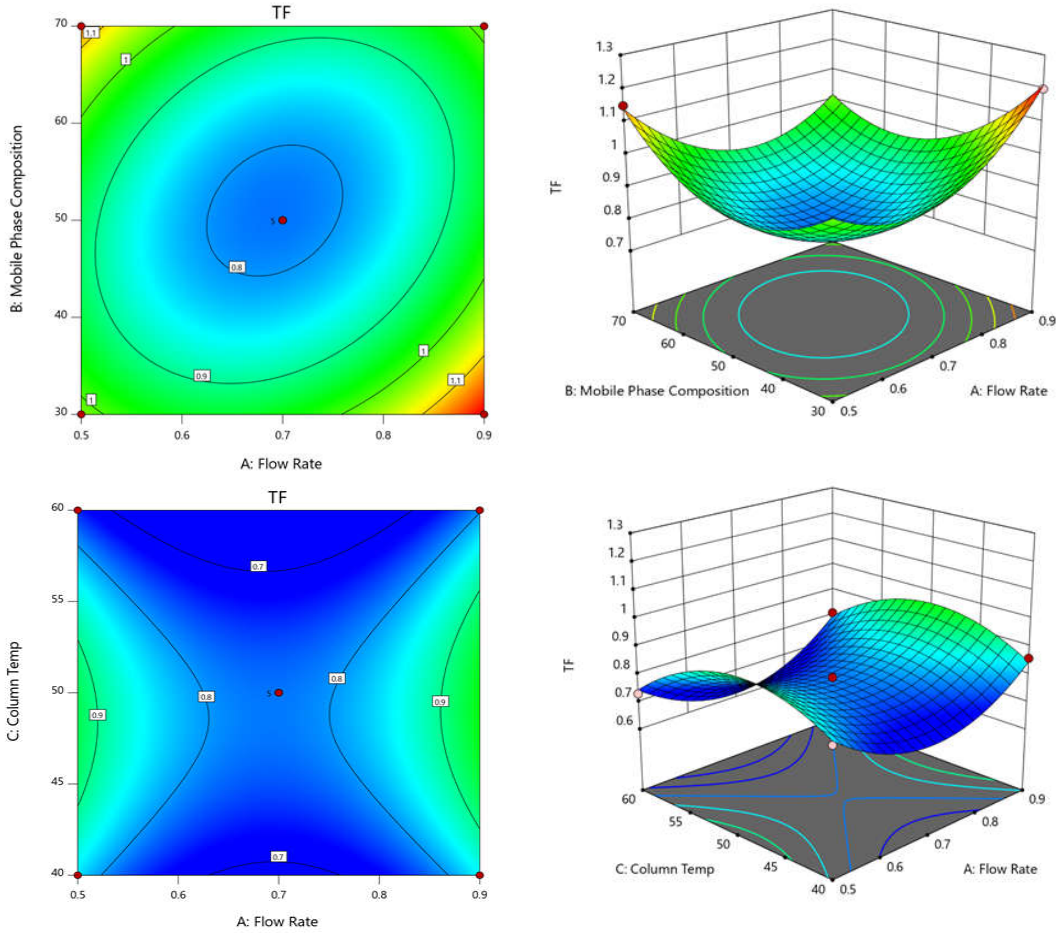


Fig. (5). Predicted verses Actual values for Retention Time as Response

3.3.2. Interaction of Independent Factors with tailing factor as Response

The effect of flow rate, mobile phase composition and column temperature on the tailing factor was also studies with 2D contour plots and 3D response surface plots[66]. The Fig. (6) illustrate the all the interactive effects on the tailing factor.



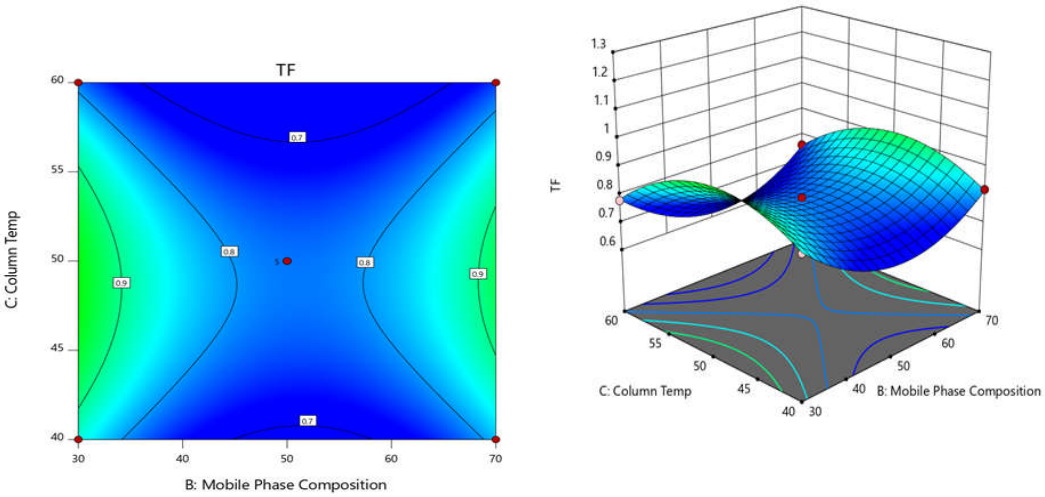


Fig. (6). 2D contour plots and 3D response surface plots showing the effect of flow rate, mobile phase composition and column temperature on Tailing Factor as Response

The model is significant, according to the model's F-value of 39.22. This large of an F-value is 0.01% likely to be the result of noise. Model terms are considered significant when P-values are less than 0.0500[67, 68]. In this instance, important model terms are C, A2, B2, and C2. Values higher than 0.1000 suggest that, there is no significance between the model terms. Model reduction can help your model if it has a large number of inconsequential model terms (not including those needed to maintain hierarchy). The 0.62 Lack of Fit F-value indicates that the Lack of Fit is not statistically significant in comparison to the pure error.

The Adjusted R2 of 0.9556 and the Predicted R2 of 0.8805 are reasonably in agreement, meaning that the difference is less than 0.2. Adeq Precision calculates the ratio of signal to noise. It is preferred to have a ratio higher than 4. Your signal strength ratio of 17.307 is sufficient. You can navigate the design area with the help of this model. A good predictive model will have a close relationship between the actual and predicted values(Fig. (7)). This means that the points on the graph will be close to the line.

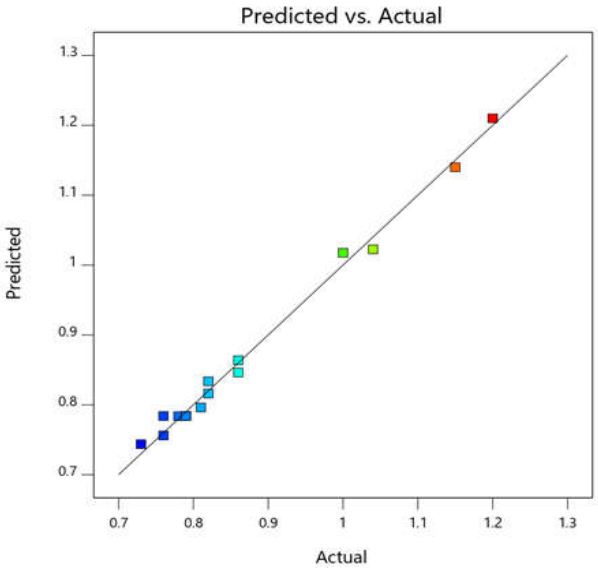
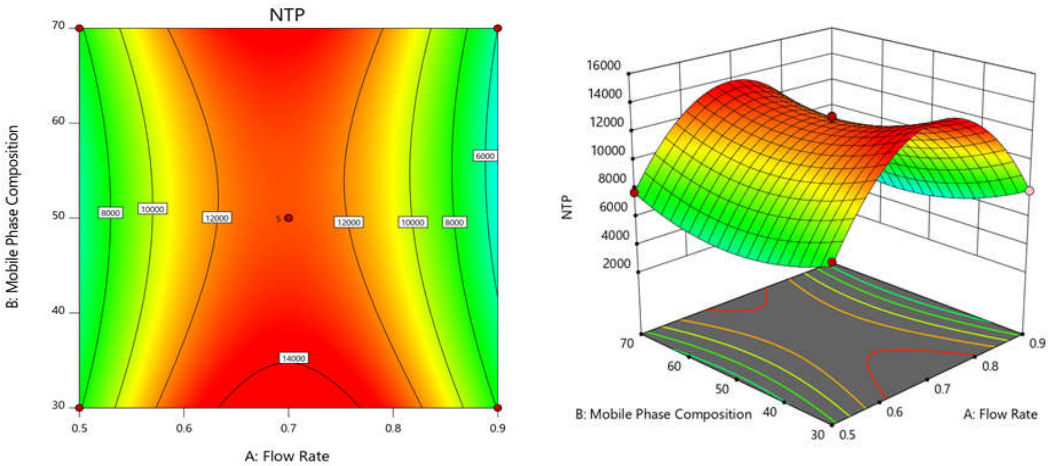


Fig. (7). Predicted verses Actual values for Tailing Factor as Response

3.3.3. Interaction of Independent Factors with Number of Theoretical Plate as Response

The model is significant, according to the model's F-value of 39.22. This large of an F-value is 0.01% likely to be the result of noise. Model terms are considered significant when P-values are less than 0.0500. In this instance, important model terms are C, A2, B2, and C2. Values higher than 0.1000 suggest that there is no significance between the model terms. Model reduction can help your model if it has a large number of inconsequential model terms (not including those needed to maintain hierarchy). The 0.62 Lack of Fit F-value indicates that the Lack of Fit is not statistically significant in comparison to the pure error(Fig. (8))[69-71].



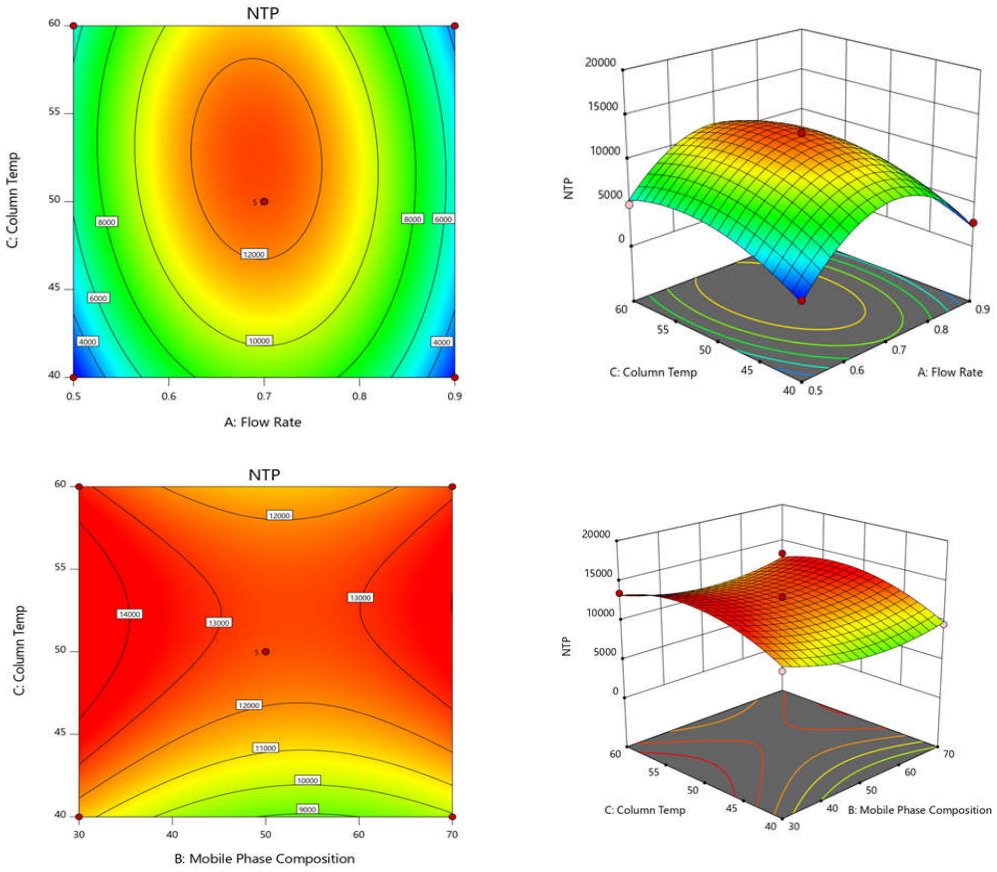


Fig. (8). 2D contour plots & 3D response surface plots showing effect of flow rate, mobile phase composition and column temperature on Number of Theoretical plate as Response

The Adjusted R2 of 0.9556 and the Predicted R2 of 0.8805 are reasonably in agreement, meaning that the difference is less than 0.2. Adeq Precision calculates the ratio of signal to noise. It is preferred to have a ratio higher than 4. Your signal strength ratio of 17.307 is sufficient. You can navigate the design area with the help of this model (Fig. (9)). Closeness of the point to the line results in the predicted values is matching with actual values. Clustering of data points in specific regions of the overlay plot indicates regions where certain combinations like Fig. 10 (A), 10 (B) and 10 (C) are frequently used or tested. Patterns or trends in the distribution of data points provide insights into interactions or dependencies between the two factors.

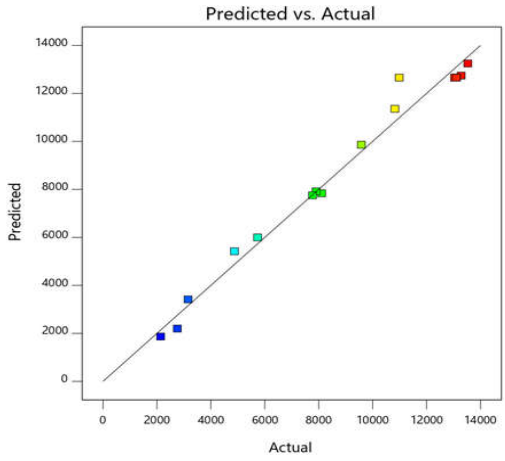


Fig. (9). Predicted verses Actual values for Tailing Factor as Response

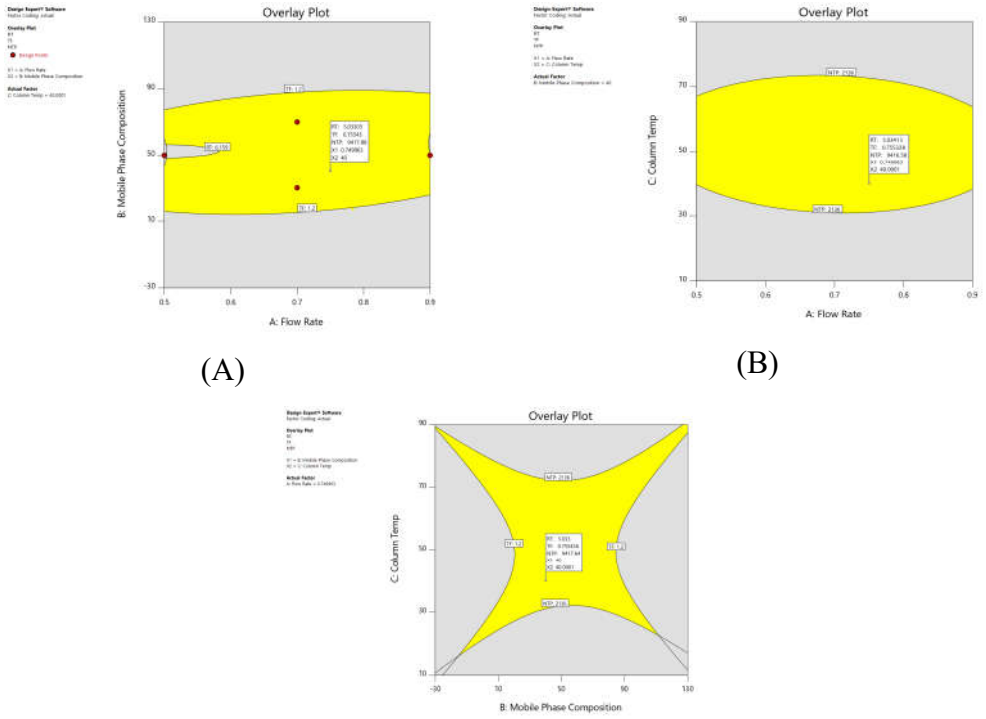


Fig. (10). Represents the overlay plot of (A) Flow rate & Mobile Phase Composition (B) Flow rate & Column Temperature (C) Mobile Phase Composition & Column Temperature

3.4. Analytical Method Validation

3.4.1. Linearity

The standard calibration curve for Doxycycline Hyclate was developed in the concentration ranging from the 5 to 30 µg/ml and showed good linearity with coefficient of correlation (r^2) 0.9939. The linear regression equation was found to be y=65098x-46560.

From the results obtained it was cleared that, all the responses are within the acceptance limit criteria [72, 73].

3.4.2. Repeatability

The repeatability parameter was tested by calculating the percent relative standard deviation (% RSD) and % Recovery factor. From the results it was showed that, the developed method gives good repeatability with % RSD, found to be 0.00247% which was found to be less than 2%. This shown that the developed method was repeatable.

3.4.3. Inter day Precision

The inter-day precision was a crucial validation criterion, and the Doxycycline Hyclate test was conducted at three different concentration levels: 8, 10, and 12 µg/ml. Three days of sample solution analysis were used to evaluate the inter-day precision. In Table 9, the results were summarized. The calculated % RSD values are 0.521% for the 80% level, 0.349% for the 100% level and 0.051% for the 120% level. A low % RSD (less than 2%) indicates that the method demonstrates high precision across the different days, meaning it is reliable for repeated measurements. The lower the % RSD, the more precise the method.

3.4.4. Accuracy

Accuracy is also important parameter to validate the developed method. The accuracy data was determined by the % recovery method. The accuracy data showed good percent recovery from the range of 99.99% to 100.01% given in Table 10. The accuracy data also showed the % RSD value not more than 2% which indicates the high degree of accuracy of developed Doxycycline Hyclate method[74, 75]. These results confirm that the method is both accurate and precise for the tested concentration.

3.4.5. Limit of Quantitation and Limit of Detection

The method showed LOQ and LOD as 0.0846 µg/ml and 0.0279 µg/ml respectively which indicate the very high sensitivity of method develop for the quantification of Doxycycline Hyclate.

3.4.6. Robustness

The robustness of the analytical method was determined by the slight change in the HPLC parameters viz. wavelength, flow rate and mobile phase composition. The results were illustrated in Table 11. The % RSD of peak area of was analyzed and which was observed within the acceptable limit of less than 2 % for all the parameters. Slight change was observed in the HPLC parameters which indicate the developed analytical method of Doxycycline Hyclate was optimized and robust for the estimation of Doxycycline Hyclate in different formulations[73].

3.4.7. Forced Degradation Samples of Doxycycline Hyclate

The chromatograms of the Doxycycline Hyclate sample, which were subjected to different forced degradation conditions, revealed distinct peaks for the active ingredients and degradation products at varying retention durations[76]. On the other hand, under some circumstances, the actives showed a decline in the height and area of the peak rather than distinct peaks of the degradation products. After being located and compared to the standard solution's peak, the peaks of the degradation products were discovered to be clearly separated from the actives' peaks. The results of the degradation investigations showed that the Doxycycline Hyclate was more stable in peroxide, water, thermal and photolytic stress condition. It was found that while Doxycycline Hyclate deteriorated more in an alkali environment(Fig. (11)).

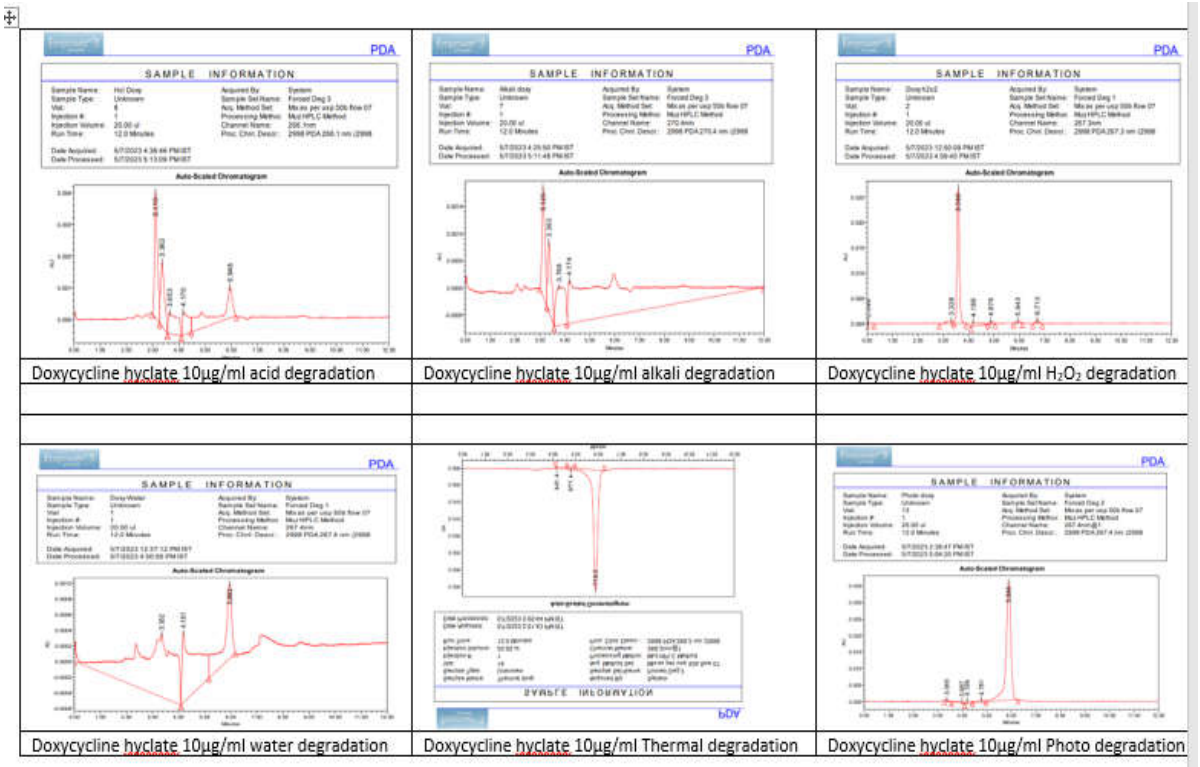


Fig. (11). Forced Degradation studies of Doxycycline hyclate

3.4.8. Evaluation of Doxycycline Hyclate in Nanostructured Lipid Carriers (NLC) Formulation

The developed RP-HPLC method demonstrated robustness, accuracy, and reproducibility for the quantification of Doxycycline Hyclate in both bulk drug and NLC formulations. The peak of doxycycline hyclate was observe at 5.601 minute in bulk and 5.603 minute in NLC formulation.consistent retention time of 5.6 minutes highlighted the method's reliability. This validated analytical approach is well-suited for routine quality control of Doxycycline Hyclate in pharmaceutical preparations, ensuring the integrity and effectiveness of the NLC delivery system. (Fig. (12)), and (Fig. (13)).

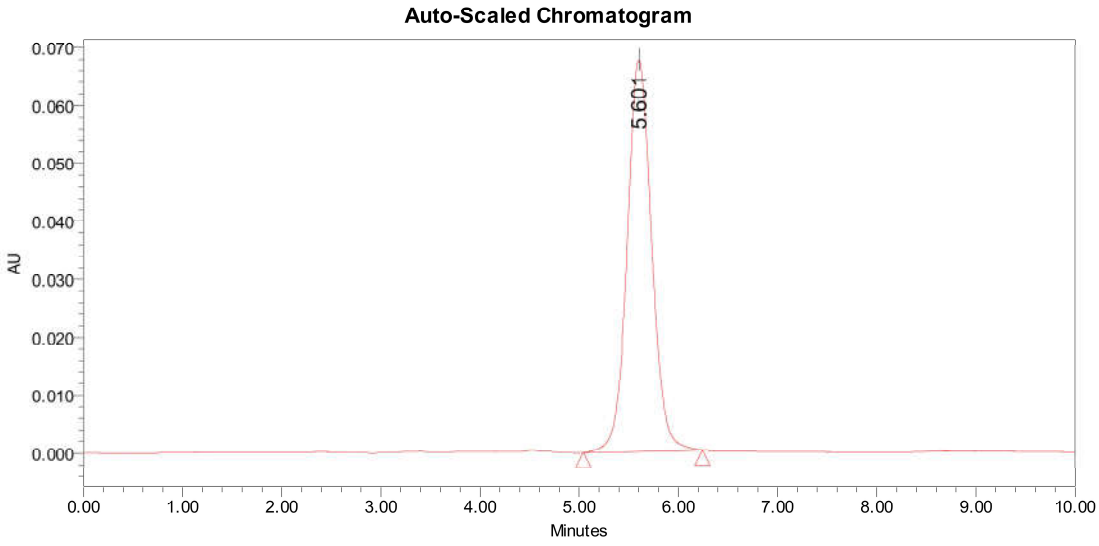


Fig. (12). HPLC Peak of Doxycycline Hyclate in bulk.

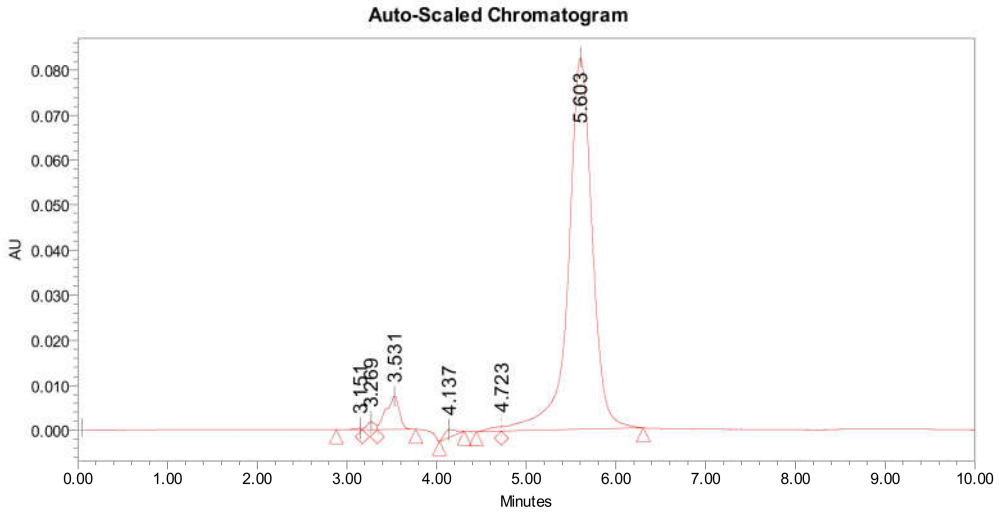


Fig. (13). HPLC Peak of Doxycycline Hyclate in NLC Formulation.

CONCLUSION

In this research project, we successfully developed and validated an RP-HPLC method for the estimation of Doxycycline Hyclate by employing the principles of Quality by Design (QbD). The systematic and science-based approach facilitated a robust and reliable method with enhanced precision and accuracy. The QbD approach allowed us to comprehensively evaluate critical method parameters, including flow rate, mobile phase composition, and column temperature. By systematically varying these factors and assessing their impact on the responses (retention time, tailing factor, and number of theoretical plates), we successfully optimized the method for the estimation of Doxycycline Hyclate. Robustness testing revealed that the optimized method was tolerant to minor variations in method parameters, confirming its reliability under different operational conditions. The force degradation studies showed that, the Doxycycline Hyclate was more sensitive to an alkaline environment. This developed method is also used to estimate doxycycline hyclate from its novel formulation. This method is well-suited for routine pharmaceutical quality control, offering accurate and precise quantification of Doxycycline Hyclate in various sample matrices. The successful application of QbD principles in method development and optimization enhances the method's quality and underscores its potential for use in pharmaceutical research and manufacturing. This research project contributes to the field of analytical chemistry by providing a comprehensive method that aligns with modern quality assurance principles and regulatory expectations.

ETHICAL STATEMENT:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of supporting data:

No additional data and materials are associated with this article to support the findings of the article.

Competing interests:

Not applicable.

Funding:

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Author's contributions:

All authors contributed to the study conception and design. All authors also read and approved the final manuscript.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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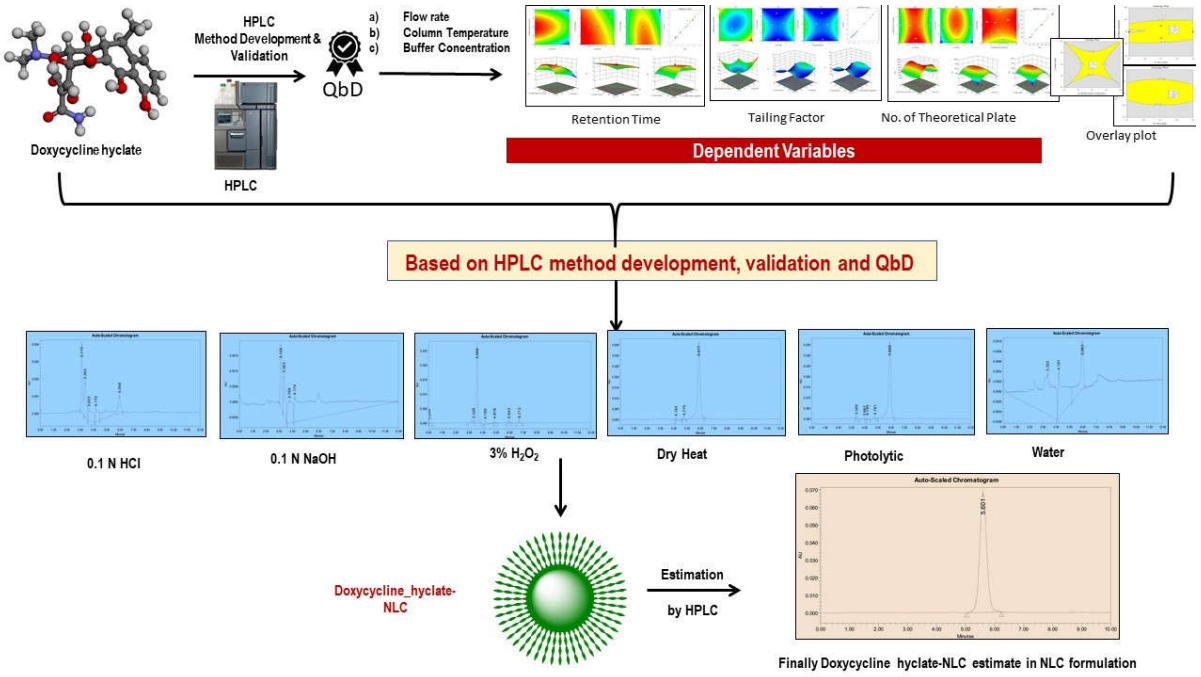
Doxycycline hyclate
HPLC Method Development & Validation
HPLC
QbD
a) Flow rate
b) Column Temperature
c) Buffer Concentration
Retention Time
Tailing Factor
No. of Theoretical Plate
Overlay plot
Dependent Variables
Based on HPLC method development, validation and QbD
0.1 N HCl
0.1 N NaOH
3% H_2O_2
Dry Heat
Photolytic
Water
Doxycycline_hyclate-NLC
Estimation by HPLC
Finally Doxycycline hyclate-NLC estimate in NLC formulation