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Analytical QbD Approach to HPLC Method Development and Validation for Naltrexone

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Abstract: A Quality-by-Design approach to method development can potentially leads to a more robust/rugged method due to emphasis on risk assessment and management than traditional or conventional approach. This study applied a QbD approach to HPLC method development and validation of Naltrexone. An efficient experimental design based on central composite design of 2 key component of HPLC method i.e. mobile phase and Detection Wavelength at 3 different levels. The responses to be evaluated are retention time, theoretical plates and tailing factor. The chromatographic condition were optimized, i.e.; column C18, mobile phase used were Acetonitrile: Methanol: Water, 44:30:26 V/V/V at 1 ml/min flow rate. The retention time was found to be 6.96 min. The described method was linear at 287 nm detection wavelength with 10-50 µg/ml for Naltrexone. The precision ruggedness and robustness values were also within the prescribed limits (<1% for system precision and <2% for other parameters). The LOD & LOQ were found to be 1.69 µg/ml & 5.12 µg/ml for Naltrexone. The %Recovery was found to be 98.36%. A better understanding of the factors that influence chromatographic separation with greater confidence in the ability of the developed HPLC method to meet their intended purposes is done. The QbD approach to analytical method development was used for better understanding of method variables with different levels. The proposed method can be used for routine analysis of Naltrexone in quality control laboratories.

Key Words **- Analytical QbD, HPLC, Naltrexone (NAL), Central Composite Design (CCD)**

I. INTRODUCTION

Quality by design is define as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [1]."

Analytical QbD

Analytical sciences are considered an integral part of pharmaceutical development. Analytical method and product development go hand in hand during the entire life cycle of any pharmaceutical product. The traditional approach of analytical method development is quite tedious owing to high degree of variability involved at each stage of method development. In order to eliminate the hiccups encountered during method development, the systematic QbD-based approach has slowly been permeating into the mind-set of analytical scientists. Accordingly, efforts have been made to extend QbD approach to analytical method development, popularly termed as "Analytical QbD (AQbD)." AQbD is a science and risk-based paradigm for analytical method development, endeavouring for understanding the predefined objectives to control the Critical Method Variables (CMVs) affecting the Critical Method Attributes (CMAs) to achieve enhanced method performance, high robustness, ruggedness and flexibility for continual improvement. AQbD helps 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.[4] The major objective of AQbD has been to identify failure modes and establish robust 'Method Operable Design Region (MODR)', which is also called as 'Design Space', within meaningful system suitability criteria and continuous life cycle management. Among

analytical researchers, to date there is no or negligible experience or exposure with the AQbD approach for analytical methods. A key advantage of the QbD approach is the flexibility to perform a qualification against the specific Analytical Target Profile defined for the intended use of the method [2-8].

Naltrexone is a pure opiate antagonist and has little or no agonist activity. Naltrexone is thought to act as a competitive antagonist at mc, κ, and δ receptors in the CNS, with the highest affinity for the μ receptor. Naltrexone competitively binds to such receptors and may block the effects of endogenous opioids. This leads to the antagonization of most of the subjective and objective effects of opiates, including respiratory depression, miosis, euphoria, and drug craving.

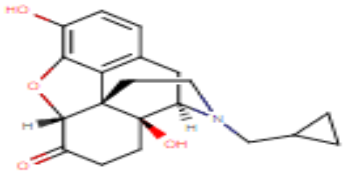

Figure 1: Structure of Naltrexone

The literature survey reveals that various UV Spectrometric [9], Spectrofluorimetric [10-11], HPLC [12-15] methods were available. So it was thought to develop and validate the Analytical QbD HPLC method for Naltrexone in pharmaceutical dosage form.

II. MATERIALS AND METHOD

Instrumentation

The Shimadzu P series integrated HPLC was equipped with a quaternary gradient unit, a LC-20 AD solvent delivery unit, DGU-20AR degassing unit, detector, a CTO-10ASVP column oven, SPD-M40 PDA detector and a SIL-20AC programmable auto sampler controlled by LAB SOLUTION software. The FTIR Spectrophotometer (8400-S Shimadzu) and Electronic analytical balance (ME204) were used.

Materials

The Marketed Formulation used for assay was obtained NODICT, Naltrexone Tablet 50mg (IP). All Chemicals and Reagents used were of Analytical Grade and HPLC Grade obtained from Ranchem Ltd., India.

Chromatographic conditions

The Shimpack ODS C18 column 25 cm (4.6 mm x 250mm, 5 um) was used as a stationary phase. The optimized mobile phase used were Acetonitrile: Methanol: Water, 44:30:26 V/V/V**.** The flow rate was set at 1 ml/min and column was kept at ambient temperature. The eluent was detected by PDA detector at 287 nm. A satisfactory separation and peak symmetry for the drug were obtained with the above chromatographic condition. The HPLC method for Naltrexone was optimized for various parameters: mobile phase and pH as two variables at three different levels using central composite design. The responses to be evaluated were retention time, theoretical plates and tailing factor.

Preparation of reference standard solution

Accurately weighed and transferred about 10mg of Naltrexone (NAL) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Naltrexone (NAL) will be 100 µg/ml. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Naltrexone (NAL) 10 µg/ml. The optimum wavelength was selection using PDA detector where gives maximum absorbance.

Selection of detection wavelength

10 µg/ml Naltrexone was scanned from 200–400 nm, and 287 nm was selected as detection wavelength.

HPLC method development by QbD approach

HPLC method development by Analytical QbD was as follows.
HPLC method development using QbD approach was done by following stages [16, 17]

Stage 1: Quality Target Product Profile (QTPP)

Selection of Quality Target Product Profile is a potential method for identifying variables which directly effect on the quality. Generally, in liquid chromatographic method there are many QTPP-variables is available in terms of system suitability test. Examination of potential variables was performed in this defined phase. Here in the HPLC method which was developed for the analysis of antimigraine the quality target product profile chosen was Retention Time, Peak Asymmetry, respectively.

Stage 2: Determine Critical Quality Attributes (CQAs)

Definition of CQAs states that "It is characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality." In the chromatographic method CQAs were considered as method parameter which are directly affected to the quality target product profile. Here in HPLC method variables selected were Mobile Phase Ratio, pH of Buffer. These was a better understanding of the specific levels of control require for critical method parameter to maintain the allowable response range, that is, the critical method attributes.

Stage 3: Develop a Design Space and Design of Experiment

After defining the method variable, formal experimental designs such as statistical design of experiments were applied to select method understanding for obtaining in-depth understanding and perform optimization. Here the DOE based on systemic scouting of two key components of the HPLC method one was mobile phase and second was pH is present. It forms a chromatographic database that will assist with method understanding, optimization, and selection. The DOE helps to eliminate the need for performing a large number of runs achieves desirable results from a limited number of experiments. Since multivariable interaction of variables and process parameter have been studied, and increased understand of method variability, thus there is greater understanding of the method. There is a better understanding of the specific levels of control required for critical method parameter to maintain the allowable response range that is a critical method attribute. The experimentally measured responses were then modelled to determine the design space.

Table 1: Coded values for Independent Variables

Factor		Low (-1)	Middle (0)	High (+1)
A Mobile Phase	30	25	30	35
B Detection Wavelength	282	277	282	287

Table 2: Different batches with their respective composition

No. Exp.	Batch code	A. Aqua phase	B. Wavelength
1	NAL1	25	277
2	NAL 2	35	287
3	NAL 3	30	282
4	NAL 4	30	275
5	NAL 5	35	277
6	NAL 6	25	282
7	NAL 7	30	289
8	NAL 8	25	287
9	NAL 9	35	282
10	NAL 10	30	282
11	NAL 11	30	282

These method conditions were evaluated using the three-tiered approach. At the first level, the conditions were evaluated for peaks symmetry, retention time and peaks tailing. This resulted in different chromatographic conditions for API. The best suited experimental conditions shall be optimized using design expert software.

Stage 4: Risk assessment
Stage 5: Implement a Control Strategy
Stage 6: Manage Analysis Lifecycle, including Continual Improvement

Method Validation
The method was validated in accordance with ICH guidelines Q2R1 for evaluation of various parameters linearity, specificity, accuracy, precision, LOD, LOQ and robustness [18-26].

Linearity and Range
The 20-100 µg/ml Tolfenamic acid was prepared by pipette out 2, 4, 6, 8 and 10 ml of 100 µg/ml Naltrexone (NAL). The straight-line equations and correlation coefficients for Naltrexone (NAL) was determined. Dilute the solution were filtered through 0.45 µm membrane filters.

Specificity:
A blank (mobile phase), placebo, standard solution of Naltrexone spiked with API and test solution were injected and % interference was checked.

Accuracy:
Accuracy of method was ascertained by performing recovery at 3 levels i.e. 80%, 100% and 120%. Recovery between 98-102% justifies the accuracy of the method.

Precision:
Repeatability was determined by analysing solution containing mixture of Naltrexone (NAL) having concentration of 60ppm respectively. Peak area of same concentration was measured six times and % RSD was calculated. Take 40, 60 and 80ppm of NAL respectively and sample were analyze at different time intervals in a day for 0 hr, 3 hr and 6 hr (Morning, Afternoon and Evening) and %RSD was calculated

LOD and LOQ
The LOD was estimated from the set of 6 calibration curves. The LOD and LOQ was calculated as, LOD = 3.3 * (SD / Slope) and LOQ = 10 * (SD / Slope); Where, SD = Standard deviation of the Y – intercepts of the 3 calibration curves. Slope = Mean slope of the 3 calibration curves

Robustness
Small and purposeful variations in change in Change in Flow rate (0.9 ml/min ± 0.1), Change in Wavelength (287 nm ± 5) and Change in mobile phase ratio were used to test the method's robustness. The effect was investigated by measuring the peak area of solutions and calculating the percent RSD.

Assay of marketed dosage formulation
The tablet marketed formulation contained 50 mg of NAL. Weighed and finely powdered twenty tablets. Weighed accurately a quantity of tablet powder equivalents to about 10 mg of drugs were transferred into 100 ml glass volumetric flask, dissolved the drugs in the minimum amount of solvent and diluted to 100 ml with mobile phase. The solution was filtered through 0.22µ nylon filter paper and appropriate volume of filtrate was diluted to obtained final concentration of 40 µg/ml of NAL. Final solution was analyzed using optimized chromatographic condition.

RESULT AND DISCUSSION

Optimization of Chromatographic condition

Optimization of HPLC Method:
The C 18 (250 mm x 4.6 mm), 5 µm was selected as stationary phase. The various mobile phase trials for optimization of HPLC method were used. Based on review of literature, several mobile phases were selected based on solubility of drug in the solvents. Various solvents and mixtures of solvents was tried using methanol, acetonitrile and HPLC grade water and their combinations. And the best result was obtained by using mixture Acetonitrile: Methanol: Water, 44:30:26 V/V/V shown desirable system suitability parameters like good peak

shape and tailing factor less than 1.5 as well as theoretical plate more than 2000. Flow rate was 1 ml/min monitor with PDA detector at 287 nm detection wavelength.

Selection of quality target product profile:

The QTPP plays an important role for identifying the variables that affect the QTPP parameters. The retention time, theoretical plates, and peak asymmetry were identified as QTPP for proposed HPLC method.

Determine critical quality attributes:

The CQAs are the method parameters that are directly affect the QTPP. The mobile phase composition and pH of buffer were two critical method parameters required to be controlled to maintain the acceptable response range of QTPP.

Factorial design:

Optimization of various parameters for analysis of Naltrexone using HPLC (by central composite design).

Design summary for optimization:

After defining the QTPP and CQAs, the central composite experimental design was applied to optimization and selection of two key components: mobile phase and pH of HPLC method. The various interaction effects and quadratic effects of the mobile phase composition and pH of buffer solution on the retention time, theoretical plates, and peak asymmetry was studied using central composite statistical screening design.

A 2-factor, mobile phase composition and pH of buffer solution at 3 different levels, design was used with Design Expert® (Version 13.0, Stat-Ease Inc., and M M), the best suited response for second-order polynomial exploring quadratic response surfaces.

$$Y=\beta0+\beta1A+\beta2B+\beta12AB+\beta11A2+\beta22B2B+\beta22B2A+\beta11A2$$

where A and B are independent variables coded for levels, Y is the measured response associated with each combination of factor level, β0 is an intercept, and β1 to β22 are regression coefficients derived from experimental runs of the observed experimental values of Y. Interaction and quadratic terms respectively represent the terms AB, A2, and B2.

Since multivariable interaction of variables and process parameter have been studied, the factors were selected based on preliminary analysis. As independent variables, mobile phase composition and pH of buffer were chosen and shown in given Table. The dependent variables were retention time, peak area, and peak asymmetry as dependent variables for proposed independent variables.

Table 3: Chromatographic Data Obtained from Central Composite Design

Sr. No.	Mobile phase ratio (ml) (V/V/V)	Wavelength	Peak Area (PA)	Retention Time (RT) (Min.)	Tailing Factor (TF)	Resolution
1	Acetonitrile: Methanol: Water 20:45:35	277	216883	12.994	1.032	10.829
2	Acetonitrile: Methanol: Water 20:45:35	282	235796	12.993	1.031	10.850
3	Acetonitrile: Methanol: Water 20:45:35	287	213633	12.993	1.032	10.855
4	Acetonitrile: Methanol: Water 30:40:30	277	183255	7.03	1.598	13.434
5	Acetonitrile: Methanol: Water 30:40:30	282	201202	7.03	1.598	13.418
6	Acetonitrile: Methanol: Water 30:40:30	287	178375	7.04	1.599	13.122
7	Acetonitrile: Methanol: Water 40:35:25	277	168585	5.714	1.586	6.778
8	Acetonitrile: Methanol: Water 40:35:25	282	167109	5.714	1.585	6.775

9	Acetonitrile: Methanol: Water 40:35:25	287	166034	5.714	1.583	6.777
10	Acetonitrile: Methanol: Water 30:40:30	282	211200	7.02	1.578	13.325
11	Acetonitrile: Methanol: Water 30:40:30	282	206051	7.04	1.567	13.452

Design space

The response surface study type, Central Composite Design and quadric design model with 10 runs were used. The proposed CCD experimental design was applied and the evaluation of mobile phase composition and pH of buffer was done against the three responses; retention time, theoretical plates and peak asymmetry and the result was summarized.

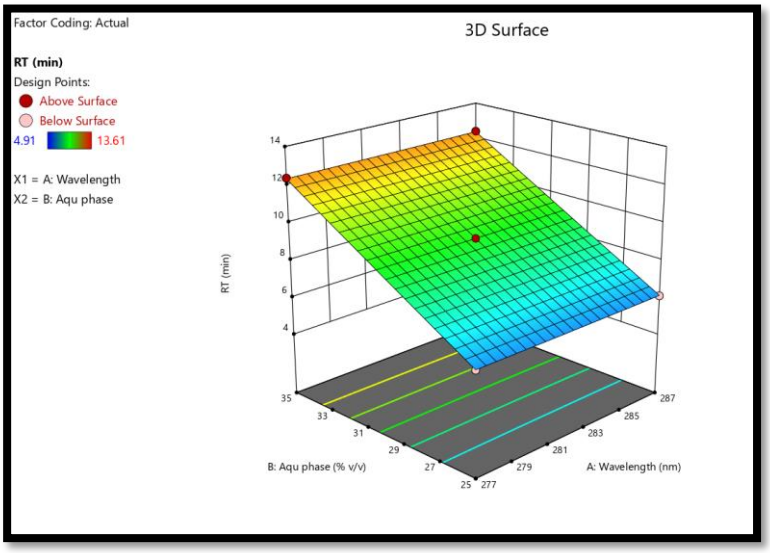

Figure 2: 3D surface plot for Effect of Combination of Factors on R1 Retention time of Naltrexone by using CCD

From Figure 2 and coded equation for Retention time was RT=+9.22-0.0007A+3.10B+0.0100AB+0.0119A²+0.0244B², it was concluded that as β1 negative coefficient (-0.0007) suggests that as the amount of water in the mobile phase (A) decreases and β2 negative coefficient (+3.10) suggests that as detection wavelength (B) increases, the value of retention time was increased.

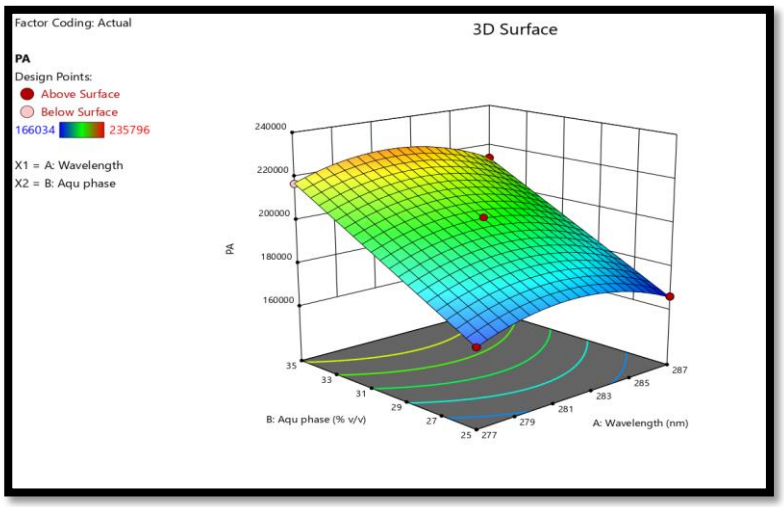

Figure 3: 3D surface plot for Effect of Combination of Factors on R2 Peak Area Naltrexone by using CCD

Form Figure 3 and coded equation for the Peak Area PA=+2.017E+05-1587.80A+24129.39B-174.75AB-10406.00A²-87.25B², it was concluded that it was concluded that as β1 negative coefficient (-1587.80) suggests that as the amount of water in the mobile phase (A) decreases and β2 positive coefficient (+24129.39) suggests that as detection wavelength (B) increases, the value of theoretical plates was increased.

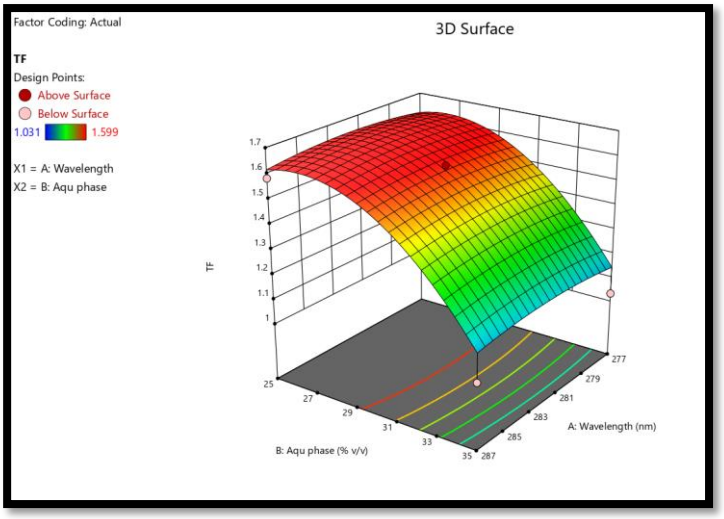

Figure 4: 3D surface plot for Effect of Combination of Factors on R3 Tailing Factor of Naltrexone by using CCD

From Figure 4 and coded equation for Tailing Factor, TF=+1.58-0.0002A-0.2361B+0.0008AB-0.0260A²-0.1713B², it was concluded that as β1 negative coefficient (-0.0002) suggests that as the amount of water in the mobile phase (A) decreases but not significantly as value is too little for the same and β2 negative coefficient -0.2361) suggests that as detection wavelength (B) decreases, the value of peak asymmetry was increased.

Optimized condition obtained

It was obtained by studying all responses in different experimental conditions using Design expert 13.0 software and optimized HPLC conditions and predicted responses are shown in Table 4 and 3D surface plot of Desirability for Obtaining Optimized Formulation shown in Figure 5.

Table 4: Suggested chromatographic conditions form Design Expert

Drug Name	Aqu. Phase	Wavelength	Retention time	Peak Area	Tailing Factor	Desirability
NAL	26.20	287	6.89	17343	1.599	0.831

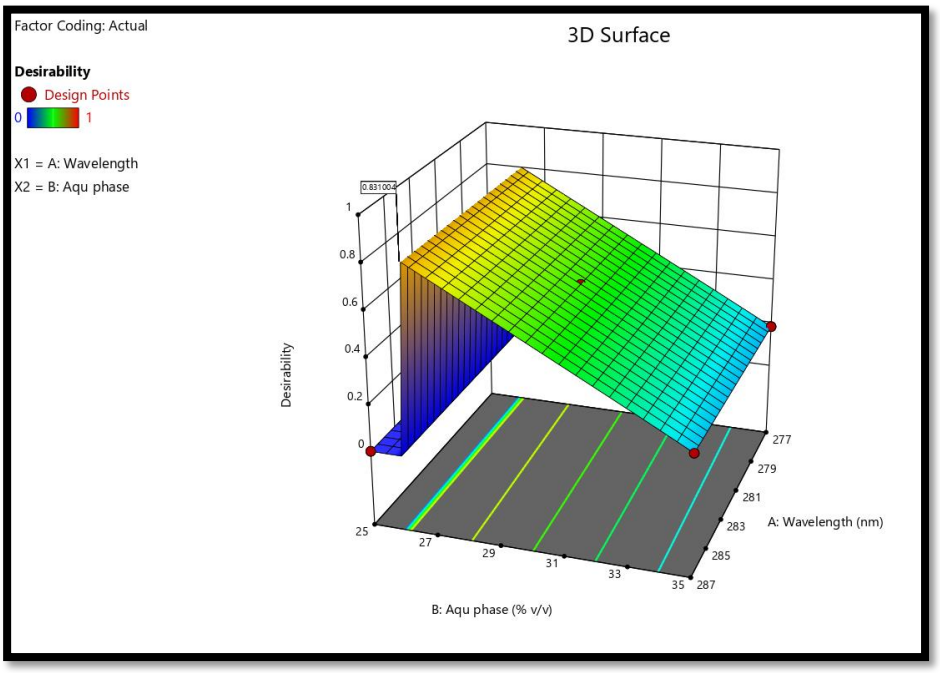

Figure 5: 3D surface plot of Desirability for Obtaining Optimized Formulation

The observed value for responses was calculated by running the HPLC chromatogram for given set of mobile phase and pH of buffer and then compared with the predicted values to evaluate for % prediction error.

METHOD VALIDATION

Linearity

Linear responses were obtained in concentration range of 20-100 µg/ml for Naltrexone (NAL). The data for linearity has shown in given table No. 5 for Naltrexone (NAL). The calibration curve for Naltrexone (NAL) was given in fig.

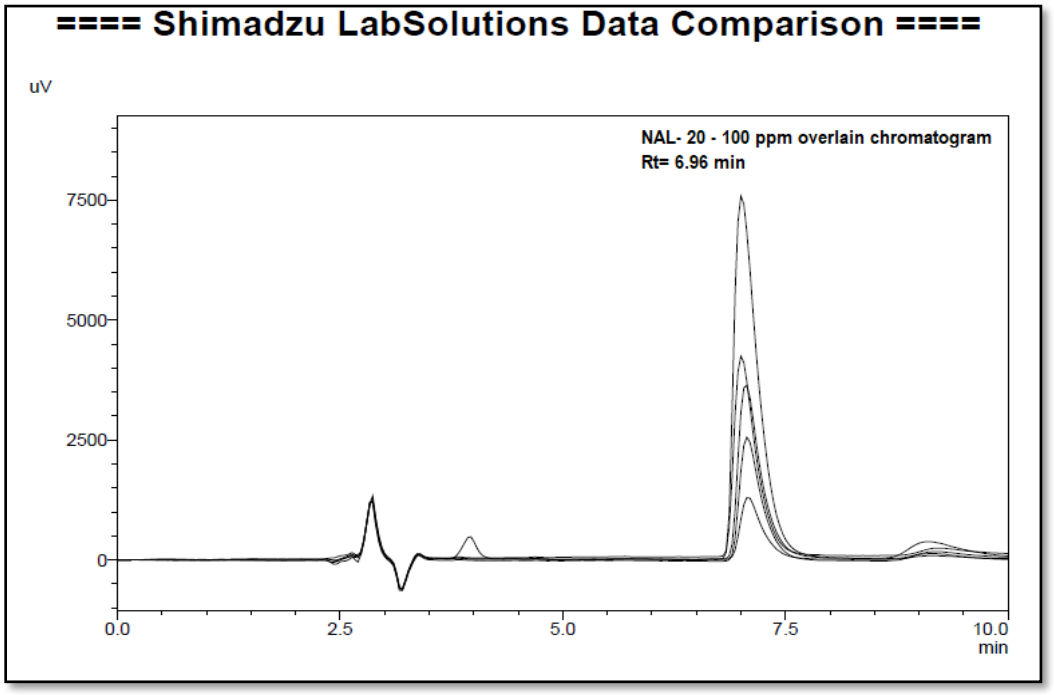

Fig.: Overlay HPLC chromatogram of Naltrexone (NAL) over the concentration range 20 -100 µg/ml using optimized chromatographic condition

Table 5: Linearity Data of Naltrexone

Naltrexone (NAL)		
Conc.	**Peak Area ± SD**	**% RSD (n=3)**
20	23139.17 ± 366.26	1.58
40	46539.67 ± 805.70	1.73
60	72495 ± 912.88	1.26
80	102912 ± 1930.51	1.88
100	131110 ± 829.23	0.63

Accuracy:

Accuracy of method was carried out at three levels (80 %, 100 % and 120 %). Percentage recovery for Naltrexone (NAL) hydrate was found to be in range of 97.23 – 102.60 % are shown in Tables 6.

Table 6: Accuracy data

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	%Recovery ± SD
Naltrexone (NAL)						
0	20	0	20	21310	20.39 ± 0.2799	101.96 ± 1.399
80	20	16	36	41790	35.43 ± 0.38	98.42 ± 1.07
100	20	20	40	47120	39.35 ± 0.30	98.37 ± 0.74
120	20	24	44	52237	43.11 ± 0.57	97.97 ± 1.28

Precision:

The repeatability study was shown in Table 7 and Intraday and Interday precision data were shown in Table 8. The precision RSD was found to be less than 2 revels that the proposed method is acceptable as per ICH guidelines.

Table 7: Repeatability Data of Naltrexone

Sr. No	Conc.(µg/ml)	Area
1	60 µg/ml	71724
2	60 µg/ml	72724
3	60 µg/ml	72242
4	60 µg/ml	71921
5	60 µg/ml	71785
6	60 µg/ml	72874
Average	72212	
SD	491.21	
% RSD (n = 3)	0.68	

Table 8: Intraday and Interday precision of method

Conc.	Intraday precision		Interday precision	
	Peak Area (Mean ± SD)^n	**%RSD** (n = 3)	**Peak Area (Mean ± SD)^n**	**%RSD** (n = 3)
40	47120.67 ± 403.22	0.86	47292.00 ± 504.27	1.07
60	72230.00 ± 500.11	0.69	72426.67 ± 995.94	1.38
80	105080.67 ± 1346.26	1.28	104082.33 ± 1954.39	1.88

LOD and LOQ:
LOD & LOQ of Naltrexone was found 1.69 µg/ml and 5.12 µg/ml respectively.

Robustness:
Small and purposeful variations in instrumental settings such as mobile phase composition, Detection Wavelength, and Flow rate to test the method's robustness by injecting triple injections of standard solutions of 20µg/ml of NAL. The effect was investigated by measuring the peak area of solutions and calculating the percent RSD.

Table 10: Robustness study

Drug Conc.	Effect of Change in Flowrate					
	0.9 ml/min		1 ml/min		1.1 ml/min	
	Peak ± SD	%RSD (n=3)	Peak ± SD	%RSD (n=3)	Peak ± SD	%RSD (n=3)
NAL (20 µg/ml)	217216.33 ± 1527.53	0.70	211809.00 ± 2939.79	1.39	184921.67 ± 2081.67	1.13
	Effect of Change in Detection Wavelength					
	277 nm		282 nm		287 nm	
	218883.00 ± 2645.75	1.21	233462.67 ± 4041.45	1.73	212463.00 ± 1605.21	0.76
	Effect of Change in Volume of Aqueous: Organic Ratio					
	25:75		30:70		35:65	
	184353.33 ± 1014.40	0.55	203769.00 ± 3337.62	1.64	212299.67 ± 1527.53	0.72

The % RSD for peak area were found to be less than 2 indicate the robust method.

Assay of Formulation:
Marketed Dosage of Naltrexone (NAL) containing 50 mg when analysed using the developed method, showed 98.36% assay for Naltrexone (NAL). The assay result indicated the method's ability to measure accurately and specifically in presence of excipients presents in tablet powder.

CONCLUSION
A Quality-by-Design approach to HPLC method development has been described. The method goals are clarified based on the process understanding. The experimental design describes the scouting of the key HPLC method components including mobile phase and Detection Wavelength. Their interrelationships are studied and optimized. QbD principles were applied to HPLC method development for Naltrexon using Design Expert Software by CCD. And a multivariant analysis of several critical method parameters like combination of 2 factor at 3 different level was done used to determine the best performing chemistry system and the final Design space.
Here a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Moreover, this approach provides an in-depth knowledge and enables the creation of a chromatographic database that can be utilized to provide alternative method conditions at a future time should changes to the method be required. Furthermore, the method development is not considered finished until a thorough risk assessment and all the necessary robustness and ruggedness studies are carried out. All the validated parameters were found within acceptance criteria.
The validated method is specific, linear, precise, accurate, robust and rugged for determination based on knowledge of method obtained through the method development and the results of risk assessment along with robustness and ruggedness studies, detailed analytical method performance control strategy can be defined to manage the risk.
QbD Approach to method development has helped to better understand the method variables, leading less chance of failure during method validation and transfer. The automated QbD method Development Approach using Design Expert software has provided a better performing more robust method in less time compared to manual method development.

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