

# RP-HPLC Method Development and Validation for Estimation of Naftidrofuryl Oxalate Using Box-Behnken Design

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## ABSTRACT

A simple, rapid, precise, sensitive, economical, robust and QbD based RP -HPLC method has been developed for Naftidrofuryl oxalate and validated as per ICH guidelines. The response surface methodology employed with a 3-factor, 3- level Box-Behnken statistical design and used to facilitate method development and optimization. The response surface methodology and multiple response optimizations utilizing a polynomial equation were used to select suitable mobile phase combination. The independent variables studied were the Organic phase ( $X_1$ ), Aqueous phase ( $X_2$ ) and ( $X_3$ ) and the responses was Retention time ( $Y_1$ ), Resolution ( $Y_2$ ) and Tailing factor ( $Y_3$ ). Chromatographic separation was achieved on Zodiac C18 column (100mm×4.6, 3 $\mu$ m), using optimized mobile phase Acetonitrile and pH-7 Tetrabutyl-ammonium buffer solution (90:10), at 282nm. The flow rate and injection volume were 1.0 mL-1 min and 20  $\mu$ L, respectively. Analysis of variance (ANOVA) confirmed that the three factors were significant. The method was validated for linearity, system suitability, recovery (accuracy), precision, robustness, ruggedness, limit of quantitation (LOD) and detection (LOQ) as per the ICH guidelines. The precision, ruggedness and robustness values were also within the prescribed limits. Calibrations curves were linear ( $r^2=0.997$ ) at the concentration range of 10 to 50 $\mu$ g/mL. LOD and LOQ values were 12.914 $\mu$ g/mL and 39.132 $\mu$ g/mL respectively. The proposed method was successfully developed by applying QBD based concept and proposed method can be used for routine analysis of Naftidrofuryl oxalate in quality control laboratories.

## Introduction

Naftidrofuryl, chemically it is a (RS)-2-(diethylamino) ethyl-3-(1-naphthyl)- 2-(tetrahydro furan-2-ylmethyl) propanoate and it is available in the form of oxalic acid. It is also known as nafronyl, INN, NF, NAFTI or as the oxalate salt nafronyl oxalate [1]. The chemical formula of Naftidrofuryl oxalate is (C<sub>26</sub>H<sub>35</sub>N<sub>07</sub>) with molecular weight is 473.56 as shown in Figure 1 [2]. Naftidrofuryl oxalate shows smooth muscle relaxation, increases cerebral blood flow as well as peripheral blood flow, cerebral adenosine triphosphate concentrations and glucose utilization properties attracts its

pharmacological applications in the treatment of senile brain diseases a vasodilator. Therefore, it is widely used in the treatment of peripheral and cerebral vascular disorders [3,4].

The British Pharmacopoeia (BP) describes a potentiometric non-aqueous titration and high-performance liquid chromatography (HPLC) methods for the assay of NF in bulk form and capsules, respectively [5,6]. The literature survey reveals that few analytical methods have been reported for the determination of Naftidrofuryl oxalate in biological fluids and/or

pharmaceutical preparations by using high-performance liquid chromatography [7], high-performance liquid chromatography with fluorescence detection [8,9], RP-HPLC fluorimetry [10], phosphorimetry [11], phosphorescence [12,13], Phosphorimetric determination [14], ion-selective membrane electrodes method [15], spectrophotometric analysis [16], Spectrophotometric, spectrofluorimetric and voltammetric analysis [17] and Stability Indicating Methods [18] were reported till date. Naftidrofuryl

oxalate molecule, is composed with the ester linkage associates naphthalene propionic acid and diethylaminoethyl moieties and easily hydrolysis in aqueous solutions by either acid- or base-catalyzed reaction to form 3-(1-naphthyl)-2-tetrahydrofurfuryl propionic acid, (Naftidrofurylacid, NFA) and diethylaminoethanol. Figure 2 shows the degradation reaction pathway of Naftidrofuryl oxalate. NFA is the metabolite of naftidrofuryl oxalate has been found in human plasma [4,19].

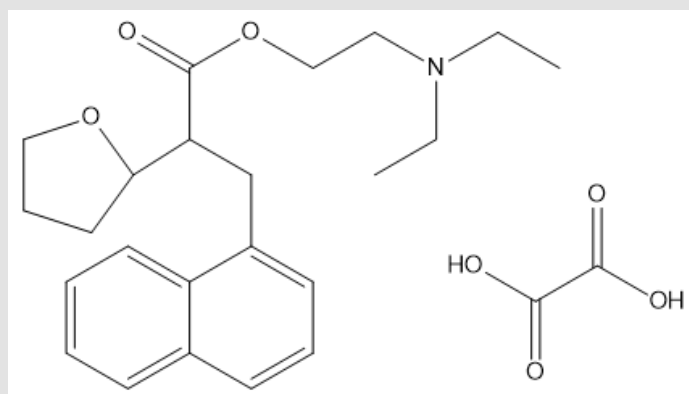


Figure 1: Structure of Naftidrofuryl Oxalate.

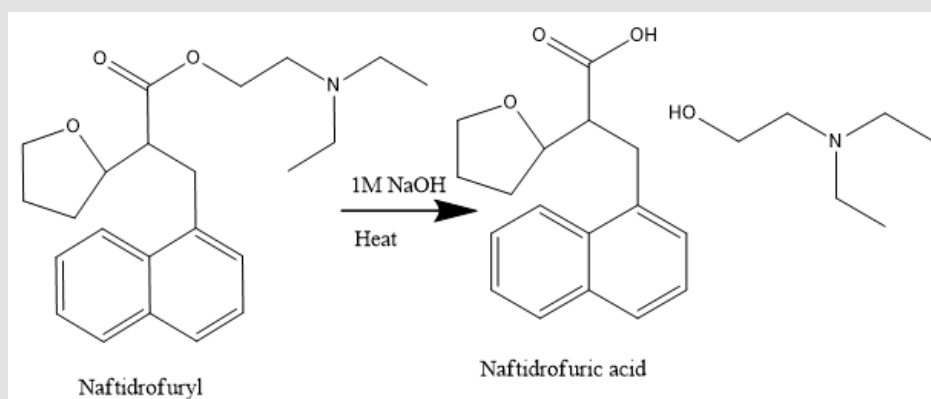


Figure 2: Hydrolysis reaction of Naftidrofuryl.

It is desirable to develop a simple, precise, robust and fast procedure that could be applied in quality control laboratories for the selective determination of Naftidrofuryl oxalate in the pharmaceutical product. The developed method can be utilized for the determination of drug content in its pharmaceutical dosage form is also demonstrated. For this we select QbD based RP-HPLC method development. In a olden days, one factor at a time (OFAT) and keeping the others fixed as a traditional approach used for optimization of HPLC methods, results in achievement of narrow robust, precise and rugged behavior of the method for instrumental variables used in method development phase. This approach may cause high risk in method failure and always requires revalidation protocol after method transfer or alternative method development used to ensure the consequences; thereby it has been increasing

the cost of the method, regulatory burden, recall of drugs and batch failure cost [20,21].

Nowadays, QbD is used as a systematic, scientifically based, holistic and proactive approach and powerful tool for chromatographic method development. In this approach we can understand process parameters that influence chromatographic separation by determination of critical ones, their positive or negative effect on the selected responses, and the multidimensional interaction between them. In addition, the change in variable of QbD facilitates the purposeful variables changes will gives desired response and suggests the optimal solution with variables value that best gives the maximum, minimum or target response, while at the same time it finds the spot with the minimum error transmitted

to the responses. So, this should represent robust process conditions that not affected by slight variations in factor settings. It also suggests a mathematical model that relates the response and the experimental variables, thus allowing response prediction with minimum error transmitted to the response [22-26].

Application of DoE principles facilitates understanding of multiple method parameters and variables that tend to affect CMAs, while unravelling the prevalence of (any) interactions and reducing intricacies. For the successful execution of DoE study, the knowledge of response variables or CMAs, CMVs, their ranges, and best fitting of the mathematical model(s) is mandatory. DoE-based Response Surface Methodology (RSM) is helpful in systematic development of analytical methods involving significant nonlinearity between CMV-CMA relationship(s) using diverse experimental designs like Factorial design, Central Composite design, Box Behnken design, Optimal design, etc. The experimental designs help in mapping the responses on the basis of the studied objective(s), CMAs being explored, at high (coded as +1), medium (coded as 0), or low (coded as -1) levels of CMVs. It tends to unearth the mechanistic understanding of CMVs and CMAs relationship, and associated interactions among them. Various 3D and 2D-plots like response surface plots, contour plots, perturbation charts, linear correlation plots, outlier plot and Box-Cox plot are some of the key pictorial/graphical tools of the experimental designs useful for the purpose [27,31].

The literature survey reveals that the BBD was one of the design strategies employed for selection and optimization of mobile phase during chromatographic analysis. The BBD is different from all screening designs as it contains combinations or midpoints of edges of the process space and at the center, i.e., variables at their highest or lowest levels, so that the designs will avoid all factors which affects method. These designs are useful in avoiding experiments performed under extreme conditions as these may give unsatisfactory results [32-34]. In the RSM design, Box-Behnken statistical design is an independent, rotatable or nearly rotatable, quadratic design, requires fewer experimental runs and less time and thus provides a far more effective and cost-effective technique than the conventional processes of formulating and optimization of dosage forms [35].

Literature survey revealed that there is no previously reported analytical QbD based RP- HPLC method for NF. Thus, the main goal of this work was to establish a precise, accurate and sensitive QbD based RP-HPLC method for NF using RSM and Multiple responses optimization utilizing quadratic polynomial equation. Screening of the critical factors was achieved using BBD, the independent variables for the present study was Organic phase ( $X_1$ ), Aqueous phase ( $X_2$ ) and ( $X_3$ ) and the responses was Retention time ( $Y_1$ ),

Resolution ( $Y_2$ ) and Tailing factor ( $Y_3$ ). The developed method was validated for linearity, system suitability, recovery (accuracy), precision, robustness, ruggedness, limits of quantitation (LOD) and detection (LOQ) as per the ICH guidelines [36,37].

## Experimental

### Materials

Naftidrofuryl oxalate was obtained as a gift sample from Zim Lab, Nagpur, India. All the chemicals such as Methanol and Acetonitrile (HPLC grade), Ammonium acetate, Tetra butyl ammonium hydroxide and Concentrated Nitric acid (AR grade), procured from Merk life science private limited Mumbai and Final limited Ahemdabad.

**Instrumentation and Chromatographic Conditions:** HPLC analysis was carried out using Shimadzu HPLC series 1100. Separation was carried out on Zodiac column (C18 100mm×4.6 I.D., 3µm particle size column), Detection was achieved using SPD-10UV detector and LC 10 ADVP-pumps connected to a hp computer. The mobile phase was prepared by mixing Tetrabutyl- ammonium buffer and Acetonitrile (adjusted to pH7 with concentrated Nitric acid) in a ratio of 10:90 v/v, then filtered through 0.45µm membrane filter (Millipore, Milford, MA, USA). The injection volume was 20µL with a flow rate of 1.0mL/min and UV detection at 282nm. The wavelength of maximum absorbance was detected by UV-Visible spectrometer (double beam), Shimadzu UV-1700 model and wavelength scanning range was 200-400nm was exercised using UV probe software. For applying quality by design Design Expert® 11.0 – trial version software was used.

**Preparation of Standard Solutions:** A stock solution of 1000 µg/mL was prepared by dissolving 10mg of Naftidrofuryl oxalate standard in 10mL of a mixture Tetrabutyl- ammonium buffer and Acetonitrile (adjusted to pH7 with Concentrated Nitric acid) in a ratio of 10:90 v/v. Standard solutions were prepared by further dilution of stock solution with the same solvent to get 30 µg/mL.

### Experimental Design

**Scouting Step:** This step included some trials of the mobile phase that gives an acceptable well resolved chromatographic peak of analyte. At the beginning, different mobile phases containing Tetrabutyl ammonium buffer and Acetonitrile as the aqueous/ organic part of the mobile phase were tried. In addition, change in ratio of Tetrabutyl- ammonium buffer and Acetonitrile were tested. Finally, the variables that may affect the selected CQA were selected.

**Screening Design:** In the present study, 3-factor, 3-level Box-Behnken statistical design was used to evaluate the effect of selected responses, to characterize the drug contents and to optimize the developed method. BBD is efficacious for exploration of quadratic

response surfaces, mathematical screening and thus helping to optimize the process by using a small number of experimental runs. The dependent and independent variables selected are given

Table 1 along with their low, medium and high levels, which were selected based on the results from preliminary experimentation. The observed responses are given in Table 2.

**Table 1:** Variables in Box Behnken Design.

Variable in Design	Levels used, actual (Coded)		
	Low (-1)	Medium (0)	High (+1)
X <sub>1</sub> = Organic phase (%)	85	90	95
X <sub>2</sub> =Aqueous phase (%)	5	10	15
X <sub>3</sub> =flow rate (ml/min)	0.8	1	1.2

**Table 2:** The observed responses in Box-Behnken Design for Naftidrofuryl Oxalate.

Experiment (Run)	Independent variables			Dependent variables		
	X <sub>1</sub> (%)	X <sub>2</sub> (%)	X <sub>3</sub> (ml/min)	Y <sub>1</sub> (min)	Y <sub>2</sub> (RS)	Y <sub>3</sub> (TF)
BB 1	90	10	1	2.614	5.751	1.117
BB 2	85	10	1.2	2.165	7.068	1.523
BB 3	95	15	1	2.645	6.307	1.619
BB 4	90	15	0.8	3.529	7.978	1.527
BB 5	90	5	0.8	3.279	7.37	1.412
BB 6	90	5	1.2	2.238	5.509	1.277
BB 7	90	10	1	2.607	5.596	1.157
BB 8	95	10	1.2	2.181	6.114	1.653
BB 9	85	10	0.8	2.252	7.628	1.46
BB 10	90	10	1	2.558	5.049	1.174
BB 11	85	5	1	2.182	7.522	1.528
BB 12	85	15	1	2.595	8.752	1.45
BB 13	95	10	0.8	3.643	5.508	1.489
BB 14	90	15	1.2	2.165	7.457	1.243
BB 15	90	10	1	2.559	5.529	1.131
BB 16	90	10	1	2.607	5.046	1.129
BB 17	95	5	1	2.861	5.928	1.523

Note: \*Variables X=Organic phase (X<sub>1</sub>), Aqueous phase(X<sub>2</sub>) and Flow rate (X<sub>3</sub>). Responses Y= Retention Time (Y<sub>1</sub>), Resolution (Y<sub>2</sub>) and Tailing factor (Y<sub>3</sub>).

BBD a also used to optimize and evaluate the main effects, interaction effects and quadratic effects of the independent factors on the dependent factors as 3-factor, 3-level Box- Behnken statistical design suitable for exploring quadratic response surfaces and constructing second order polynomial model with Design Expert® 11.0 – Trial Version software. The nonlinear computer-generated quadratic model is given as

$$Y_0 = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_2 + b_{22}X_2 + b_{33}X_2$$

where Y<sub>0</sub> is the dependent variable; b<sub>0</sub> is an intercept; b<sub>1</sub>-b<sub>33</sub> are regression coefficients computed from the observed experimental values of Y; and X<sub>1</sub>-X<sub>3</sub> are the coded levels of independent variables. The terms X<sub>1</sub>, X<sub>2</sub> and X<sub>i</sub>(i=1,2or3) represent the interaction and quadratic terms, respectively

**Optimization Data Analysis:** The responses Retention Time (Y<sub>1</sub>), Resolution (Y<sub>2</sub>) and Tailing factor (Y<sub>3</sub>) of were treated by Design-Expert software design such as BBD which consist of three components include linear, quadratic and special cubic models. The best fitting mathematical model such as ANOVA method and Good fit evaluation were selected based on the comparisons of several statistical parameters including the coefficient of variation (C.V), the multiple correlation coefficient (R<sup>2</sup>), adjusted multiple correlation coefficient (adjusted R<sup>2</sup>); and the predicted residual sum of square (PRESS), proved by Design-Expert software. Among them, PRESS indicates how well the model fits the data, and for the chosen model it should be small relative to the other models under consideration. Also various 3-D response surface graphs and counter plots were provided by the Design- Expert software.

By intensive grid search performed over the whole experimental region, the optimum checkpoint mobile phase composition was selected to validate the chosen experimental domain and polynomial equations. The optimized checkpoint mobile phase composition was prepared and evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with that of the predicted values. Also, linear regression plots between actual and predicted values of the responses were produced using MS- Excel version 2019.

**Validation:** The method was validated in accordance with the International Conference on Harmonization (ICH) requirements [37], which involved linearity, system suitability, recovery (accuracy), precision, robustness, ruggedness, limits of quantitation (LOD) and detection (LOQ).

**Linearity, LOD, and LOQ:** The linearity of the method was assessed at five concentrations within the appropriate range. To construct the calibration curves the peak areas were plotted against concentrations. For an analyte in a sample, LOD is the lowest concentration which can be detected, not quantified. On the other hand, LOQ is the lowest concentration was calculated.

**System Suitability:** The system suitability parameters area, retention time, tailing factor, theoretical plates and resolution were calculated by injecting standard Naftidrofuryl oxalate solution.

**Accuracy:** The accuracy of the proposed method was indicated by % recovery of the five different concentrations of Naftidrofuryl oxalate.

**Robustness:** In addition to identifying the design space that represents a robustness zone, the insensitivity of the proposed method to small changes in the optimized conditions such as deliberate changes in the method were done i.e., Change in flow rate ( $1.0 \pm 0.2$  mL/min), change in mobile phase concentration (organic portion of  $90\% \pm 5\%$ ), change in column and change in pH of Buffer solution ( $7.0 \pm 0.2$  pH) in mobile phase.

**Ruggedness:** The studies were carried out for two different parameters i.e., Days (Intraday and Interday) and Analyst to Analyst variation. Single concentration level was selected and analyzed as described before three times within the same day (intra-day precision) by different analysts, and on successive three days (inter-day precision). The % RSD was calculated as a measure for

method precision.

## Results and Discussion

### Scouting Step

An isocratic mobile phase consisting of Tetrabutyl- ammonium buffer 20% and acetonitrile 80% was applied at the beginning. This mobile phase gave strong tailing and no well resolved peak shape. When % of Tetrabutyl- ammonium buffer was increased and % of acetonitrile reduced, tailing increased strongly. Then we reduced % of tetrabutyl-ammonium buffer and % of acetonitrile increased in order to have better resolution. In this case, good peaks shape was obtained. In this step, three factors were chosen: the % aqueous part of the mobile phase, % of organic phase and flow rate.

### Screening with BBD

The significance of model so obtained can be evaluated by two ways i.e. ANOVA method and Good fit evaluation. ANOVA is a statistical method based on F-test to estimate the significance of model. It involves subdividing total variation into variation due to Residual error, Main effects and Interactions.

### ANOVA Technique

The ANOVA (one-way Analysis of Variance) is used to ensure about the significant differences between the means of three or more independent groups. The results of ANOVA of Variance for Retention time ( $Y_1$ ), Resolution ( $Y_2$ ) and Tailing factor ( $Y_3$ ) shown in Table 3. The Model F-value of Retention time ( $Y_1$ ), Resolution ( $Y_2$ ) and Tailing factor ( $Y_3$ ) was found to be 16.69, 10.72 and 7.11 respectively and it implies that the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms such as -A, C, AC in Retention time( $Y_1$ ), - A, B,  $A^2$ ,  $B^2$ ,  $C^2$  in Resolution ( $Y_2$ ) and  $-A^2$ ,  $B^2$ ,  $C^2$  in Tailing factor ( $Y_3$ ) are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms. The Lack of Fit F-value of 66.01 and 32.46 in Retention time ( $Y_1$ ) and Tailing factor ( $Y_3$ ) respectively implies that the Lack of Fit is significant. Whereas in the Resolution ( $Y_2$ ), Lack of Fit F-value of 3.02 implies the Lack of Fit is not significant relative to the pure error. There is a 15.69% chance that a Lack of Fit F-value this large could occur due to noise. The average values obtained from the software are given in Table 4.

**Table 3:** ANOVA results of the Box Behnken design for response surface quadratic model.

Source	Retention time( $Y_1$ )		Resolution ( $Y_2$ )		Tailing factor ( $Y_3$ )	
	F-value	p-value	F-value	p-value	F-value	p-value
Model	16.69	0.0001	10.72	0.003	7.11	0.009
A-% org phase	18.19	0.002	31.8	0.001	1.67	0.238
B-% Aq phase	0.558	0.472	10.9	0.0131	0.156	0.704
C-flowrate	62.34	< 0.0001	3.43	0.106	0.588	0.468



AB	3.16	0.106	0.91	0.372	0.966	0.358
AC	15.08	0.003	1.71	0.232	0.326	0.586
BC	0.832	0.383	2.26	0.177	0.709	0.428
A <sup>2</sup>			8.06	0.025	41.38	0.0004
B <sup>2</sup>			26.37	0.001	6.62	0.037
C <sup>2</sup>			6.84	0.035	6.77	0.035
Lack of Fit	66.01	0.0006	3.02	0.157	32.46	0.003
Result	Significant		Not significant		Significant	

**Table 4:** Table for average values obtained from the software.

Source	Retention time(Y <sub>1</sub> )	Resolution (Y <sub>2</sub> )	Tailing factor (Y <sub>3</sub> )
Std. Dev.	0.18	0.45	0.089
Adj R-Squared	0.8548	0.8454	0.7745
Pred R-Squared	0.5462	0.217	-0.522
Adeq Precision	14.752	9.811	7.14

### Main Effects (Lack of Fit)

The Lack of Fit is one of the components of partition of the sum of squares in an ANOVA which can tell that that purpose model is fit or not. The results of Lack of Fit Test and Model Summary Statistics of Retention time (Y<sub>1</sub>), Resolution (Y<sub>2</sub>) and Tailing factor (Y<sub>3</sub>) showed in Table 5.

**Table 5:** Model Summary Statistics for Lack of Fit Test.

Retention time(Y <sub>1</sub> )						
Source	Std. Dev.	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	PRESS	Remark
Linear	0.265	0.736	0.675	0.451	1.9	-
2FI	0.177	0.909	0.855	0.546	1.57	-
Quadratic	0.148	0.956	0.899	0.308	2.39	Suggested
Cubic	0.028	0.999	0.996			-
Resolution (Y <sub>2</sub> )						
Source	Std. Dev.	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	PRESS	Remark
Linear	0.937	0.446	0.318	0.135	17.81	-
2FI	1.02	0.493	0.189	-0.38	28.39	-
Quadratic	0.446	0.932	0.845	0.217	16.11	Suggested
Cubic	0.327	0.979	0.917			-
Tailing factor (Y <sub>3</sub> )						
Source	Std. Dev.	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	PRESS	Remark
Linear	0.203	0.034	-0.189	-0.537	0.854	-
2FI	0.228	0.062	-0.501	-1.802	1.56	-
Quadratic	0.089	0.901	0.775	-0.522	0.846	Suggested
Cubic	0.023	0.996	0.984			-

**Table 6:** Table for final Equation in Terms of Actual Factors.

Final Equation in Terms of Actual Factors			
Factors	Retention time(Y <sub>1</sub> )	Resolution (Y <sub>2</sub> )	Tailing factor (Y <sub>3</sub> )
Model	-37.206	262.33	97.119
A (% org phase)	0.46	-4.827	-2.032

B (% Aq phase)	0.656	-0.358	-0.206
C (flowrate)	29.273	-59.459	-7.63
AB	-0.006	-0.009	0.002
AC	-0.344	0.292	0.025
BC	-0.081	0.335	-0.037
A <sup>2</sup>		0.025	0.011
B <sup>2</sup>		0.045	0.004
C <sup>2</sup>		14.207	2.805
Model	2FI	Quadratic	Quadratic

Contour Plots and Response Surface Analysis

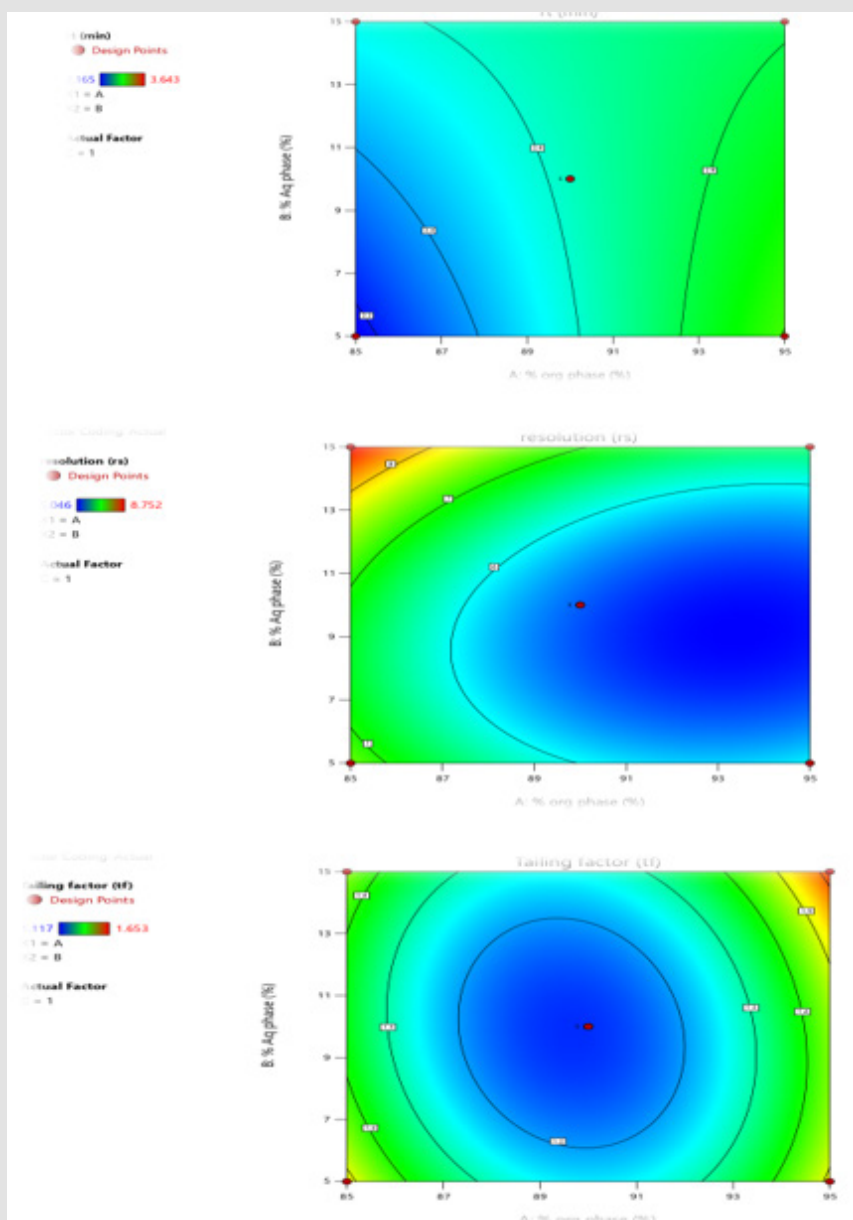
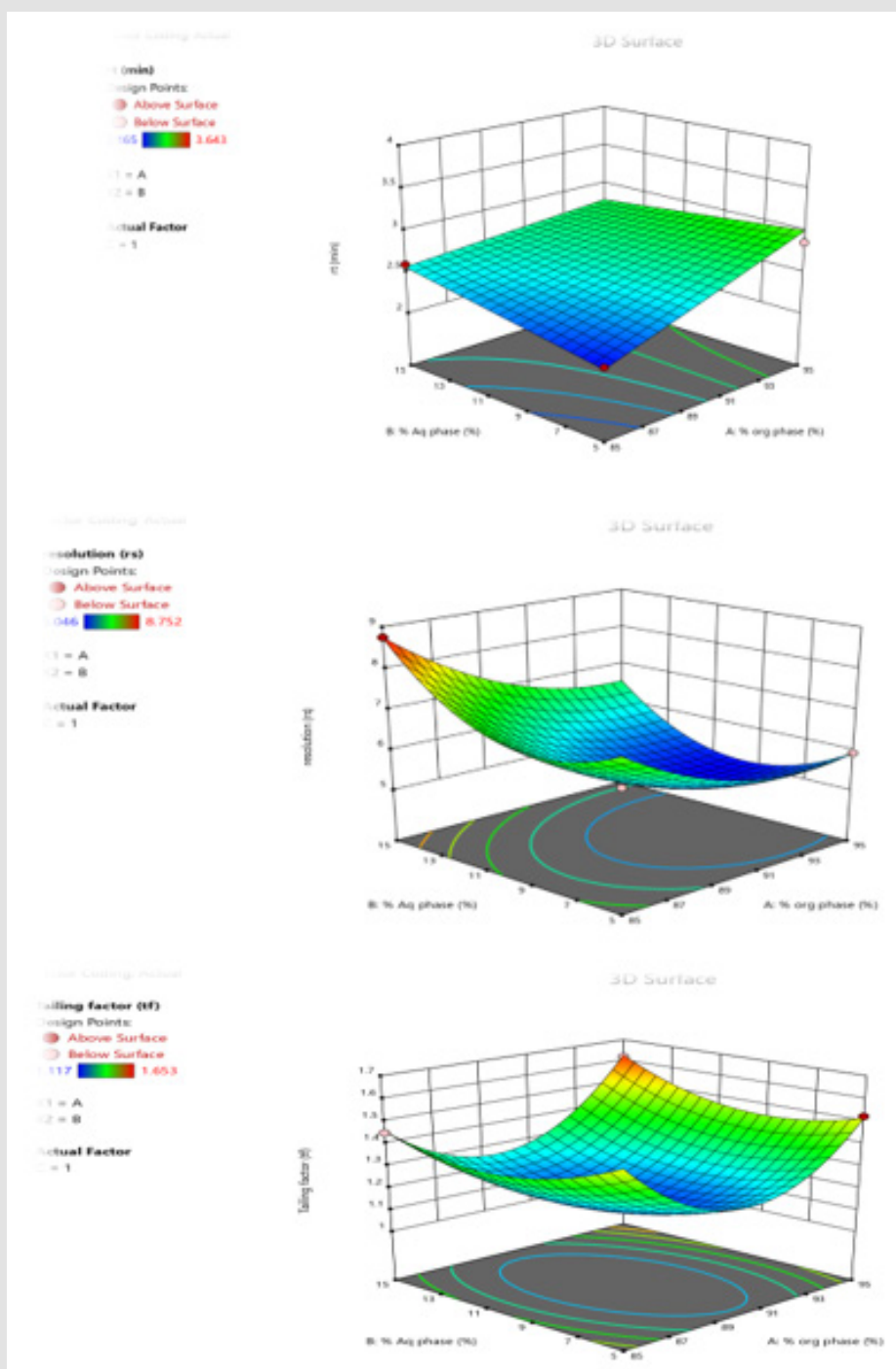


Figure 3:  
 a. Counter Plot for Response Retention Time.  
 b. Counter Plot for Response Resolution.  
 c. Counter Plot for Response Tailing factor.

The graphical optimization was another tool used to specify the design space or sweet spot where the desired CQAs meet. The goal of graphical optimization was to maximize both responses after specifying their lowest acceptable limits. 3D plot showed the interaction effect of the critical factors. Two-dimensional contour

plot of Retention time ( $Y_1$ ), Resolution ( $Y_2$ ) and Tailing factor ( $Y_3$ ) shown in Figures 3a-3c and three-dimensional response surface plot are presented in Figures 4a-4c which is very useful to study the interaction effects of the factors on the responses. These types of plots show the effects of two factors on the response at a time.



**Figure 4:**  
 a. Surface Response Curve for Response Retention Time.  
 b. Surface Response Curve for Response Resolution.  
 c. Surface Response Curve for Response Tailing factor.



## Validation

### Linearity, LOD, and LOQ

Linearity study was performed on Naftidrofuryl oxalate under the optimized chromatographic conditions; a good linearity was obtained between the peak areas and the concentration ranging

from 10 to 50 µg/mL. The observations are shown in Table 7, from the data, a plot concentration (µg/mL) Vs Area Under Curve (AUC) was constructed for the Naftidrofuryl oxalate (Figure 5). The study of graphical plots represents correlation coefficient of Naftidrofuryl oxalate was found to be 0.997.

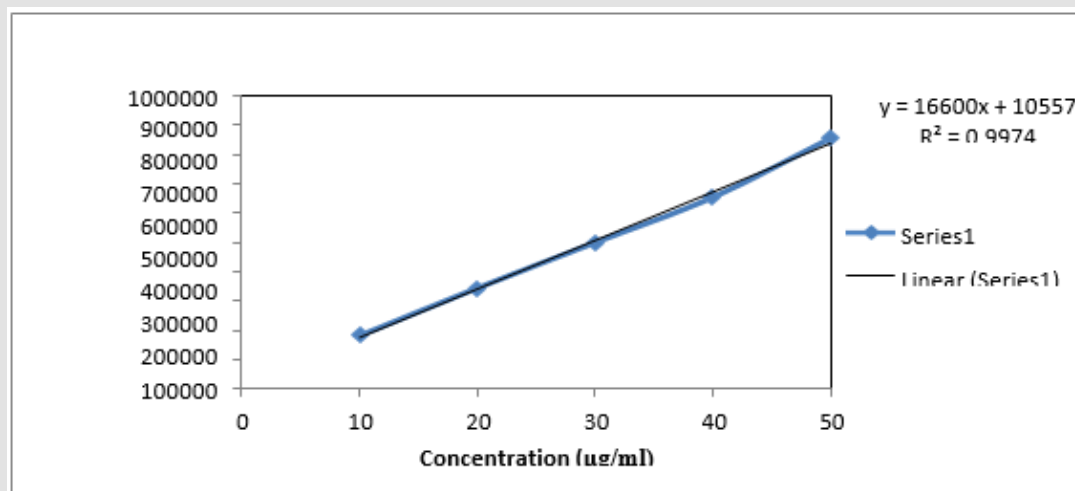


Figure 5: Plot of Linearity Curve.

Table 7: Regression data and System suitability data.

Sr.No	Parameters	Results
1	Linearity range (µg/mL)	10 to 50µg/mL
2	Coefficient of determination (R2)	0.997
5	LOD	12.91357
6	LOQ	39.13203
7	Mean	543601.8
8	±S.D.	3243.27
9	% RSD	0.597
10	Theoretical plate/ column	21,462.38
11	Retention time	2.772
12	Tailing factor	1.273
13	Resolution	6.244

### System Suitability

The study of chromatogram reveals that the Naftidrofuryl oxalate was well resolved hence, resolution of the same needs to be studied. The system suitability was performed by preparing the 30 µg/mL solution of standard Naftidrofuryl oxalate performed under optimized condition. From the observation of results, all the obtained results are in under the acceptance limit. Hence, according to acceptance criteria the system is suitable for analysis. The results for system suitability parameters are given in Table 7 and standard

chromatogram is given in Figure 6. The correlation coefficient was found to be 0.997 for the drug.

### Accuracy

Accuracy of proposed method was as certain on the basis of recovery studies performed by standard addition method. Percent Recovery was performed by preparing the 30 µg/mL solution of Standard Naftidrofuryl Oxalate. The ±S. D and % RSD was calculated and the results for % Recovery is given in Table 8.

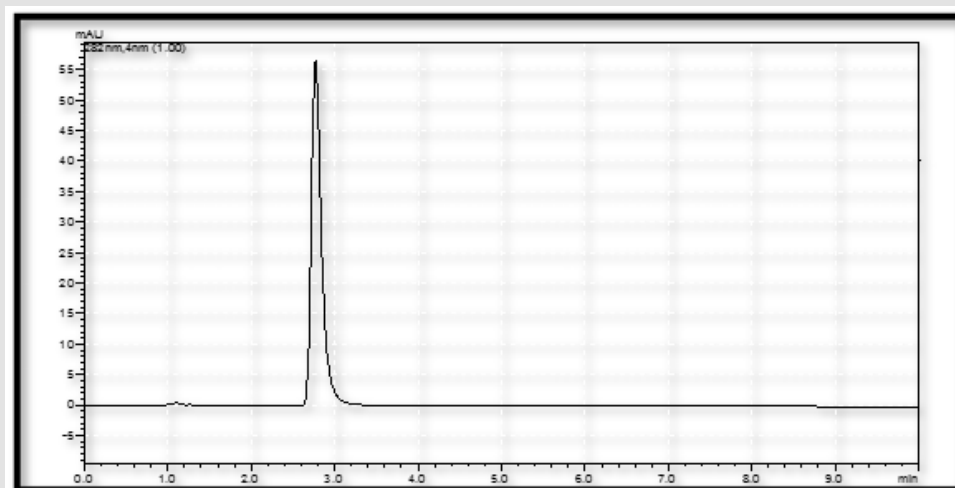


Figure 6: Chromatogram of Std NF.

Table 8: Observation and Results of Recovery Study.

Accuracy Level	Total Amount Recovered (mg)	% Recovery
80%	7.9993	99.87
	8.0539	100.3
90%	8.9992	99.88
	9.0126	100.03
100%	10.0594	100.29
	10.0675	100.47
110%	11.1864	101.51
	11.0045	99.95
120%	12.1828	101.36
	12.1287	100.99
±SD		0.581
%RSD		0.58

### Robustness

In these study deliberate changes in the method were done i.e. Change in flow rate ( $1.0 \pm 0.2$  mL/min), change in mobile phase concentration (organic portion of  $90\% \pm 5\%$ ), Change in column

(Agilent ZORBAX and ZODIAC) and change in pH of Buffer solution ( $7.0 \pm 0.2$  pH) in mobile phase. The robustness results Mean, SD and %RSD of series of measurement were found to be within limit as shown in Table 9.

Table 9: Table for summary data of Robustness results.

Parameters	Change in flow rate (mL/Min)		Change in mobile phase composition		Change in column		Change in pH of buffer in mobile phase	
	±SD	%RSD	±SD	%RSD	±SD	%RSD	±SD	%RSD
Rt (mL/Min)	0.026	0.968	0.022	0.824	0.03	1.087	0.038	1.41
Area (mV)	563.307	0.103	2886.184	0.529	277.48	0.05	1032.169	0.187
HETP	97.4	0.45	120.221	0.558	4.51	0.02	96.525	0.45
Tailing factor	0.011	0.863	0.015	1.168	0.012	1.029	0.009	0.762

## Ruggedness

The studies were carried out for two different parameters i.e., Days (Intraday and Interday) and Analyst to Analyst variation. In intraday and inter-day variations, results of estimation by proposed methods were found to be varying. In inter-day study % RSD and % estimation was found to be increased after one day storage suggest

**Table 10:** Results of Ruggedness study for the proposed method.

Conc. ( $\mu\text{g/mL}$ )	Recovery%		
	Intra-day precision (1h,3h and 5h)	Inter-day precision (1 <sup>st</sup> , 2 <sup>nd</sup> and 3rd Day)	Analyst to analyst ruggedness (n=3)
30 $\mu\text{g/mL}$	100	100	100.695
	100	102.38	100.68
	99.99	102.94	100.66
$\pm\text{SD}$	0.002	1.563	0.016
%RSD	0.002	1.536	0.016

## Conclusion

A validated QbD based RP-HPLC method has been developed for Naftidrofuryl oxalate utilizing "Analytical Quality by Design" (AQbD)- DOE approach. Multivariate regression analysis was successfully applied to study the main effects of three factors on the of retention time, resolution and tailing factor. BBD was employed for optimization of chromatographic conditions and used to analyse the analytical target profile by studying the interaction and quadratic effects on the factors for three selected responses. The models used for screening and optimization steps were found to be significant and confirmed method predictability. The developed method was validated for linearity, accuracy, robustness of test method, ruggedness, intermediate precision, recovery study, LOD and LOQ. The method is simple, robust, accurate and can be successfully applied to the analysis of Naftidrofuryl oxalate.

## Conflict of Interest

No conflict of interest with any institution/organization.

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that the NF is unstable in solution form while in intraday study, solution was found to be stable up to 3 h. The result of estimation for Naftidrofuryl oxalate by different analysts was very much reproducible. This indicates the ruggedness of the method in the hands of different analysts. The results of Ruggedness study for the proposed method is given in Table 10.

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