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# Application of Factorial Design for Optimization of Spectrophotometric Determination of Cefdinir Using MBTH

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## ABSTRACT

The target of the present study is to use experimental design in screening and optimizing experimental variables to develop a spectrophotometric method for determination of Cefdinir (CFN) antibiotic. The proposed method is based on the oxidative coupling reaction of CFN with 3-methylbenzothiazolinone-2- hydrazone (MBTH) in presence of $FeCl_3$ in acidic medium forming a green colored chromogen measured at $\lambda_{max}$ of 660 nm. A $2^{5-2}$ fractional factorial design was utilized to screen the effect of $FeCl_3$, HCl and MBTH concentrations, in addition to reaction time and nature of diluting solvent on the absorbance of the formed chromogen. One way ANOVA and Pareto ranking analyses have shown that all variables were statistically significant. Full factorial design was then utilized to evaluate the effects of the variables on the selected response. With the help of a response surface and contour plots the optimum value of each variable was determined and used for further experiments. These optimum values were 5mg/mL $FeCl_3$, 0.06% HCl, 4mg/mL MBTH, reaction time of 10 min and methanol as diluting solvent. Beer's law is obeyed in the range of 0.5-6.0 µg/mL. The proposed method was successfully applied for the determination of CFN in bulk powder and commercial dosage forms without interference from the commonly encountered excipients and additives. The mean recoveries of the analyte in pharmaceutical preparations were in agreement with those obtained from a comparison method, as revealed by statistical analysis of the obtained results using the Student's *t*-test and the variance ratio *F*-test.

**Keywords:** Cefdinir; MBTH; experimental design; factorial design; spectrophotometry.

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## INTRODUCTION

Cefdinir (CFN), [(-)-(6R, 7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3- vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid] is a semisynthetic, broad-spectrum, third-generation cephalosporin antibiotic (Figure. 1). The bactericidal activity of CFN results from disrupting the synthesis of the peptidoglycan layer of bacterial cell wall. It is used for treatment of upper and lower respiratory tract infections and urinary tract infections [1]**.** CFN is the subject of a monograph in each of United States Pharmacopoeia, USP[2] and Japanese Pharmacopoeia, JP[3]. Both USP and JP recommend high performance liquid chromatography (HPLC) method for assay of the raw material, capsules and oral powder for suspension.

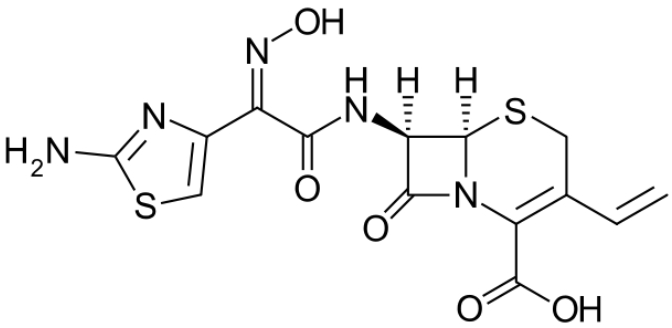


**Figure 1.Structural formula of Cefdinir (CFN).**

The literature revealed some methods for determination of CFN in pharmaceutical preparations including spectrophotometry[4-7], spectrofluorimetry[8], high performance liquid chromatography [9-12] and electrochemical methods [13, 14].

The aim of this work was to establish a relatively simple, sensitive and validated visible spectrophotometric method for the determination of CFN in pure form and in pharmaceutical dosage forms, since most of the reported methods use sophisticated equipments[9-13] or suffer from drawbacks of poor stability of the formed colored product [6]or being time consuming[7].

MBTH undergoes oxidative-coupling reaction with aromatic amines or phenols in the presence of an oxidant under acidic conditions to form intense colored oxidative coupling products. Consecutively, MBTH have been used as chromogenic reagent for spectrophotometric determination of many pharmaceutical amines [15-20] based on the reaction between the drug and MBTH-Fe (III). In this work an attempt was made in this direction and succeeded in developing a method depends on this reaction. The method can be extended for the routine assay of CFN formulations.

The univariate approach optimizes conditions one-by one by varying levels of one condition while levels of other conditions are held at constant levels, which is time and reagent consuming. Moreover, it is unable to consider interaction effect between conditions and hence the maximum efficiency of analytical methods might not be obtained. On the other hand, chemometrics, as a group of multivariate approaches, is more powerful than the univariate approach. The strategy of chemometrics is that to obtain the highest efficiency of analytical methods in the shortest way. Hence, chemometrics reduces consumption of reagents and sample, besides it saves time and minimizes effort. Chemometrics gains its strategy throughout the following ways: (i) examining the effect of conditions and their interactions on the efficiency of analytical methods, (ii) optimizing conditions with considering their interactions, (iii) developing more than one analytical aspect at the same time, (iv) reducing a large amount of data that can be easily interpreted and (v) testing the ruggedness[21,22]. Among the most common effective chemometric optimization approaches are the experimental design-based methods. The remarkable applications of experimental design include factor screening, response surface examination, system optimization and system robustness. Factorial design, which is the dominant factor screening method, allows to select which factors are significant and at what levels[21, 22.]

The aim of the present work was to utilize the experimental design approach for screening and optimizing the experimental variables affect the oxidative coupling reaction to develop a spectrophotometric method for determining the content of CFN in bulk and pharmaceutical formulations.

## MATERIALS AND METHOD

### Apparatus

Spectrophotometric analyses were carried out on a Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer with two matched 1 cm path-length quartz cells.

### Software

Experimental design and data analysis were performed by Design-Expert® trial version 9.0 (Stat-Ease Inc., Minneapolis, MN, USA). The rest of calculations for the analysis of spectral data were performed using Microsoft Excel 2010 software (Microsoft, USA).

### Materials and reagents

CFN pure sample was kindly supplied by Kahira pharm. Co. Cairo, Egypt. 3-Methylbenzothiazolinone-2-hydrazone hydrochloric hydrate (MBTH) 97 % purity and

dimethylformamide (DMF) were obtained from Merck (Darmstadt, Germany). Anhydrous ferric chloride ($FeCl_3$) 96 % purity was obtained from Fine-Chem Limited, Mumbai, India. Distilled water was used throughout the study.

**Standard solutions**

Standard solution of CFN (500 µg/ mL) was prepared in DMF. The standard solution was kept in the refrigerator and was found to be stable for 2 days. MBTH (0.2%) was prepared by dissolving 200 mg of MBTH in 100 mL distilled water and Ferric chloride (1%) was prepared by dissolving 500 mg of ferric chloride anhydrous in 50 mL of 0.06% HCl. Both MBTH and $FeCl_3$ solutions must be freshly prepared.

**Formulations**

Two commercial pharmaceutical formulations containing CFN were purchased from local pharmacy and subjected to the analytical procedure. Cefdin® capsules (300 mg CFN per capsule) and Cefdin® suspension (125 mg CFN per 5.0 mL) as products of Smithkline Beecham Egypt LLC for NOVARTIS PHARMA S.A.E.-Egypt, with batch number of N100931 and N100432, respectively.

**General procedure**

***Construction of Calibration Curves***

Accurately measured aliquots equivalent to 5-60 µg CFN were quantitatively transferred from the stock solution of the drug into a series of 10 mL volumetric flasks using adjustable volume micropipettes. To each flask 2 mL MBTH and 0.5 mL $FeCl_3$ were added successively, kept aside for 15 minutes for complete color development. The solutions were made up to the mark with methanol. The absorbance of the green colored solution was measured at 660 nm against the corresponding reagent blanks for each concentration containing the same volume of DMF in sample flasks, in addition to the other ingredients and the calibration curve was constructed.

***Analysis of the pharmaceutical preparations***

For Cefdin® capsules and suspension, a quantity of the powder equivalent to 50 mg of CFN was transferred to a 100 mL volumetric flask. The content was mixed with DMF (50 mL), sonicated for 30 min. to dissolve the drug as completely as possible and the volume was adjusted up to the mark with DMF. The contents were mixed well and filtered rejecting the first portion of the filtrate.

These solutions were used without any further dilution and analyzed as described under the construction of calibration curve. The content of the drug was determined by triplicate

measurement of three independently prepared solutions. The nominal contents of the pharmaceutical preparations were calculated either from the previously plotted calibration graph or using the corresponding regression equation.

## RESULTS AND DISCUSSION

The proposed method utilized the oxidative coupling reaction for determination of CFN, where the drug reacts with MBTH in the presence of $FeCl_3$ in acidic medium to give a green colored product peaking at 660 nm, where MBTH is oxidized by ferric chloride in acidic medium, forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes an electrophilic attack on the most nucleophilic site of the drug to form the colored product.

**Stability of chromogen**

The reaction between CFN and MBTH is completed within 10 minutes. The absorbance of the chromogen remains stable for at least 12 hours; which may be attributed to using an organic solvent like DMF. This allowed the convenience measurement of large number of samples and allowed the method to be followed for the intra-day studies.

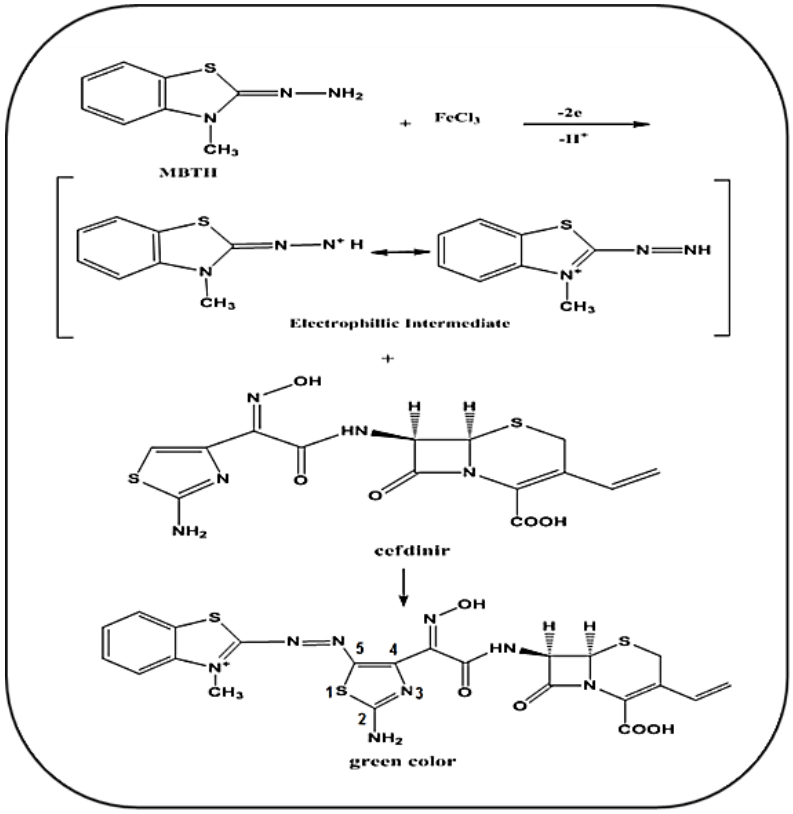


**Figure 2. Scheme for the proposal mechanism of the reaction.**

CFN solubility profile[23, 24] reveals that, it dissolves only in DMF, DMSO and 0.1 M phosphate buffer (pH 7); the latter affects the pH of the reaction, and decrease the stability of the produced color. While DMF and DMSO react with MBTH under the reaction conditions. So, DMF was found to be solvent of choice due to high stability of the reaction product color. Consequently, the blanks should contain the same volume of DMF present in the sample flasks, and the stock solution was used directly without any further dilution, where the absorbance decreases dramatically in case of diluting the stock solution.

Although the structure of the colored product has not been established experimentally, the formation of oxidative coupling product may be postulated as shown in Figure. 2 by analogy with the coupling reaction between ceftazidime and MBTH [15].

**Optimization of variables using experimental design**

When more than one variable is potentially important, it is difficult to obtain optimal conditions throughout the commonly used step-by-step optimization procedure. In these cases, multivariate analysis has been extensively applied to determine the best conditions for the analytical methods employed[25-27]. Experimental design as multivariate analysis tool, offers an efficient route to identify the conditions yielding the best results.

- ***Screening design: [Two level ($2^{5-2}$) fractional factorial designs]***

Screening designs are normally used when a large number of factors are likely to affect a particular response**.** A $2^{5-2}$ fractional factorial design was utilized to evaluate the main effect of independent variables on the selected response (absorbance). The primary purpose was to identify significant main effects with the least number of runs as possible. For the reaction between CFN and MBTH in acidic medium, five variables were involved in the experimental design: concentrations of $FeCl_3$, HCl and MBTH, reaction time and nature of diluting solvent. In this planning, the five variables were studied at two levels: low (-1) and high (+1). Eight experiments were necessary for this design, which were realized in triplicate and randomized to eliminate any environmental variation. The range and levels of variables utilized in the screening design are given in Table 1.

**Table 1. Factors and levels**

| Factors | | Factor levels (-) | (+) |
|---|---|---|---|
| $x_1$: | $FeCl_3$ Conc. (mg/mL) | 4 | 8 |
| $x_2$: | HCl (%) | 0.05 | 0.3 |
| $x_3$: | MBTH Conc. (mg/mL) | 4 | 8 |
| $x_4$: | Reaction time (min.) | 0 | 20 |
| $x_5$: | Nature of diluting solvent | water | methanol |

The corresponding fractional experimental design and the experimental results are shown in Table 2. The highest and lowest values of each variable were defined based on the review and preliminary experiments.

**Table 2. A fractional $2^{5-2}$ factorial design matrix and results of experiments**

| Run Order | Variables | | | | | Absorbance values | |
|---|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$* | Experimental | Predicted |
| 1 | -1 | -1 | 1 | 1 | { -1 } | 0.144 | 0.144 |
| 2 | 1 | -1 | 1 | -1 | { 1 } | 0.688 | 0.687 |
| 3 | -1 | -1 | -1 | 1 | { 1 } | 0.728 | 0.728 |
| 4 | -1 | 1 | 1 | -1 | { -1 } | 0.015 | 0.016 |
| 5 | 1 | 1 | -1 | 1 | { -1 } | 0.563 | 0.562 |
| 6 | 1 | -1 | -1 | -1 | { -1 } | 0.366 | 0.366 |
| 7 | -1 | 1 | -1 | -1 | { 1 } | 0.462 | 0.462 |
| 8 | 1 | 1 | 1 | 1 | { 1 } | 0.852 | 0.852 |

*****As categorical factor.

The linear regression model was obtained based on the statistical analysis of the absorbance data Eq. (1):

$$y = b_0 + b_1 \boldsymbol{X_1} + b_2 \boldsymbol{X_2} + b_3 \boldsymbol{X_3} + b_4 \boldsymbol{X_4} + b_5 \boldsymbol{X_5} \quad \ldots\ldots \quad \boldsymbol{(1)}$$

Where y is the absorbance, $b_0$ is the intercept, $b_i$ ($b_1$, $b_2$, $b_3$, …, $b_5$) represent the regression coefficient and $\boldsymbol{X_i}$ ($\boldsymbol{X_1}$, $\boldsymbol{X_2}$, $\boldsymbol{X_3}$, ……, $\boldsymbol{X_5}$) represent the independent variable. The calculated coefficients and their associated P-values are presented in Table 3.

**Table 3.Estimated regression coefficient for selected response**

| Factor | Coefficient | Standard Error | p-value |
|---|---|---|---|
| Intercept | 0.74 | 1.212E-003 | < 0.0001 |
| $FeCl_3$ Conc. | 0.17 | 1.212E-003 | < 0.0001 |
| HCl % | -0.018 | 1.212E-003 | 0.0046 |
| MBTH Conc. | -0.075 | 1.212E-003 | 0.0003 |
| Reaction time | 0.12 | 1.212E-003 | 0.0001 |
| Dil. Solvent | 0.24 | 1.212E-003 | < 0.0001 |

The data were analyzed by ANOVA method and the results are presented in Table 4. The qualities of the fitted mathematical model was ascertained by correlation coefficient $R^2$ of 1, adjusted $R^2$ of 0.9999 and predicted $R^2$ of 0.9996 all were within acceptable limits of ($R^2 \geq 0.80$), indicating good matching between the experimental results and the predicted values obtained using the polynomial model as shown in Figure. 3. The "Adequate Precision" measures the signal (response) to noise (deviation) ratio and a value greater than 4 is desirable. For this model, it was 355.214 indicating an adequate signal.

**Table 4. Analysis of variance (ANOVA) for the selected response (absorbance)**

| Source | SS | DF | MS | F- value | p-value |
|---|---|---|---|---|---|
| Model | 0.85 | 5 | 0.17 | 14443.03 | < 0.0001 |
| $FeCl_3$ Conc. | 0.23 | 1 | 0.23 | 19932.87 | |
| HCl % | 2.552E-003 | 1 | 2.552E-003 | 217.12 | |
| MBTH Conc. | 0.045 | 1 | 0.045 | 3828.56 | |
| Reaction time | 0.11 | 1 | 0.11 | 9338.91 | |
| Dil. Solvent | 0.46 | 1 | 0.46 | 38897.70 | |
| Residual | 2.351E-005 | 2 | 1.175E-005 | | |
| Cor Total | 0.85 | 7 | 0.17 | | |

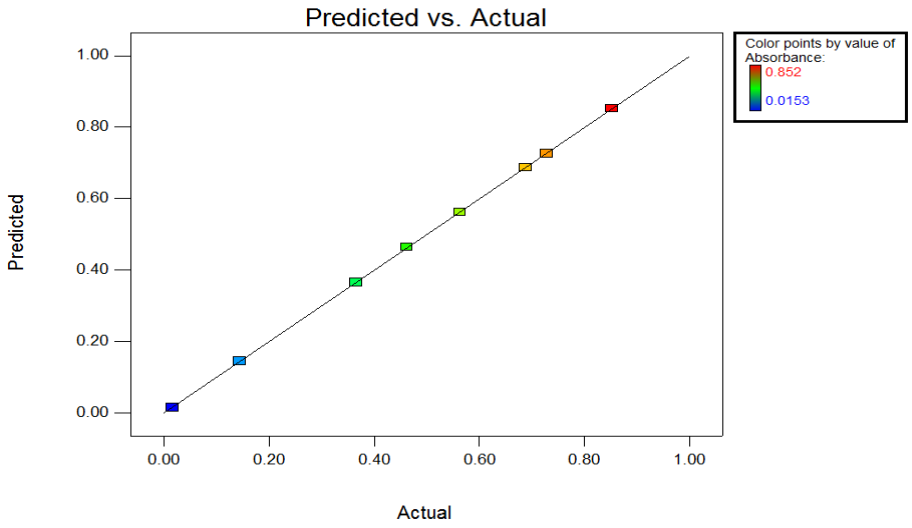


**Figure 3. Plot of actual experimental results *vs.* predicted values obtained using the polynomial model.**

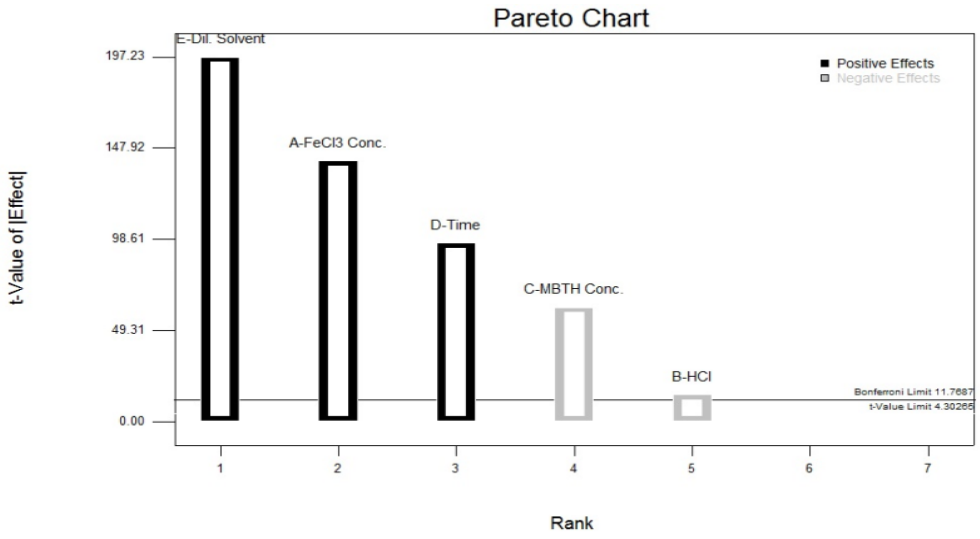


**Figure 4: Pareto chart for visualizing the effects of the chemical variables on the absorbance measurements using a $2^{5-2}$ factorial design.**

The results of fractional factorial study were plotted by Pareto chart (Figure. 4) which showed the effects of each variable corresponding to a 95% confidence level response. The importance of each variable depends on its sign and value. Positive signs indicate that the absorbance signal is increased with an increase of the value of the respective variable within the studied range, while negative signs indicate that the absorbance signal is favored with a decrease of the variable. If the resulting graph goes over the statistical *t*-line (*i.e.*with p-value less than 0.05), the variation of the response caused by changing the variable is higher than the experimental error. Therefore, the variable is considered to be significant[28, 29]. From which, it was revealed that all the studied variables are significant. The type of diluting solvent had the largest influence on absorbance with a positive sign which means that solvent in +1 level (methanol) is the optimum diluting solvent under these conditions. Consequently, variables still need to be studied was reduced to four instead of five.

- ***Optimization design: [Four level full factorial designs]***

Based on the results obtained, the next step was carried out in order to evaluate the effect of the remaining variables on the absorbance. Two full factorial designs were carried out in four levels. The first for $FeCl_3$ and HCl concentrations, ranging from 2 to 8 mg/mL and 0.01 to 0.1 %, respectively (using 4 mg/mL MBTH and waiting time of 20 min).While the second for time and MBTH concentration, ranging from 0 to 20 min and 2 to 8 mg/mL, respectively (using 8 mg/mL $FeCl_3$ and 0.05% HCl).Tables 5 & 6 show the optimization matrices created from the full factorial design.

**Table 5. Matrix of full factorial design for $FeCl_3$ and HCl concentrations with the measured response values**

| **Run Order** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $FeCl_3$ Conc. | 6 | 2 | 8 | 4 | 4 | 6 | 8 | 8 | 8 | 4 | 2 | 6 | 2 | 6 | 2 | 4 |
| HCl Conc. | 0.1 | 0.01 | 0.1 | 0.07 | 0.05 | 0.01 | 0.01 | 0.07 | 0.05 | 0.01 | 0.1 | 0.05 | 0.05 | 0.07 | 0.07 | 0.1 |
| Absorbance | 0.559 | 0.506 | 0.462 | 0.786 | 0.777 | 0.655 | 0.561 | 0.634 | 0.628 | 0.562 | 0.463 | 0.778 | 0.589 | 0.764 | 0.581 | 0.563 |

**Table 6. Matrix of full factorial design for MBTH concentration and reaction time with the measured response values**

| **Run Order** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reaction time | 20 | 20 | 5 | 10 | 5 | 10 | 0 | 0 | 20 | 10 | 0 | 20 | 5 | 0 | 5 | 10 |
| MBTH Conc. | 4 | 2 | 2 | 2 | 8 | 8 | 8 | 4 | 8 | 4 | 2 | 6 | 4 | 6 | 6 | 6 |
| Absorbance | 0.77 | 0.729 | 0.503 | 0.721 | 0.174 | 0.309 | 0.092 | 0.204 | 0.301 | 0.777 | 0.151 | 0.695 | 0.563 | 0.139 | 0.347 | 0.689 |

The software recommended mathematical transformations as pre-treatment of the data. For this purpose the Box Cox plot was used as tool to determine the most appropriate power transformation that can be applied to response data. In this, case inverse (with lambda = −1) was recommended for $FeCl_3$ & HCl model and Log (with lambda = 0) was recommended for reaction time & MBTH model as shown in Figure. 5.

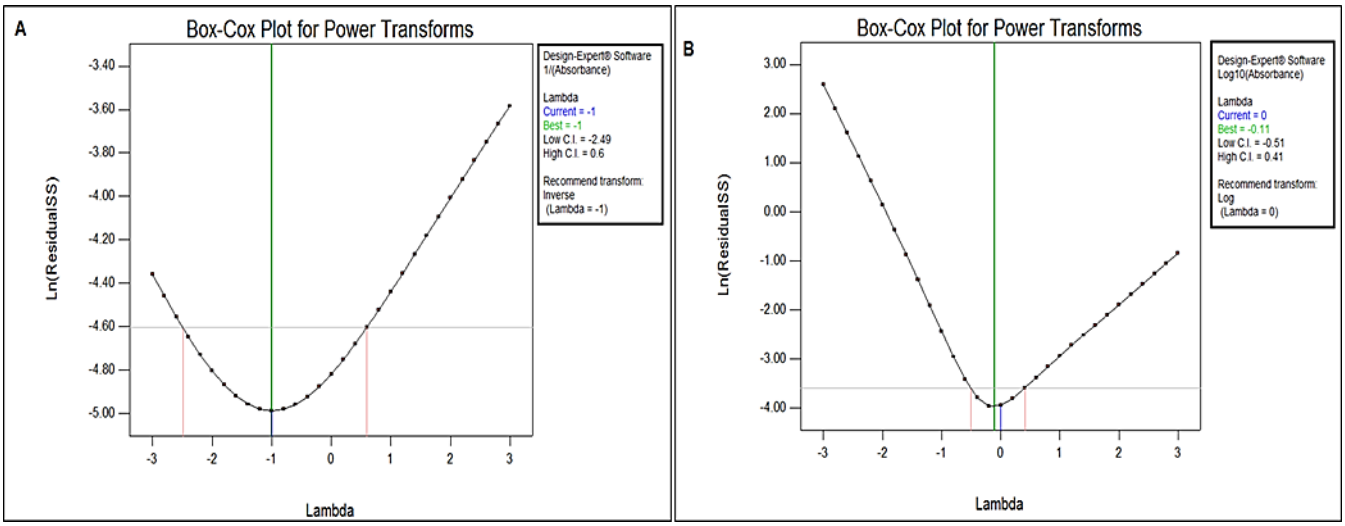


**Figure 5. Box-Cox transformation for absorbance in A) $FeCl_3$ & HCl Conc. Model B) Reaction time & MBTH Conc. model.**

The quadratic regression models are given by Eq. (2 &3):

$$1/A = 1.23 - 0.060\,x_1 + 0.094\,x_2 + 0.060\,x_1x_2 + 0.37\,{x_1}^2 + 0.43\,{x_2}^2 \quad \ldots\ldots \textbf{(2)}$$

Where *A* represents the response corresponding to the absorbance value. The factors $x_1$ and $x_2$ are $FeCl_3$ and HCl concentrations, respectively.

$$Log_{10}(A) = -0.11 + 0.31\,x_1 - 0.18\,x_2 - 0.013\,x_1x_2 - 0.32{x_1}^2 - 0.20\,{x_2}^2 \ldots\ldots \textbf{(3)}$$

Where *A* represents the response corresponding to the absorbance value. The factors $x_1$ and $x_2$ are time and MBTH concentration, respectively. The qualities of both optimization matrices were examined by ANOVA (Table 7).

**Table 7. Statistical parameters of the full factorial designs obtained from ANOVA**

| Model | SS | MS | P- value | $R^2$ | Adj. $R^2$ | Pred. $R^2$ | Adeq. Precision |
|---|---|---|---|---|---|---|---|
| $FeCl_3$ & HCl | 1.20 | 0.24 | < 0.0001 | 0.9601 | 0.9401 | 0.8650 | 20.495 |
| Reaction time & MBTH | 0.94 | 0.19 | < 0.0001 | 0.9470 | 0.9205 | 0.8196 | 18.389 |

Before adopting the model on the basis of the ANOVA test, the adequacy of the model should be checked. One can use the analysis of the residuals as a primary diagnostic tool for this purpose. The residual plots of absorbance in the model equations for the optimization matrices are

displayed in Figure.6. In the normal probability plots of residuals, the points on this plot lied reasonably close to a straight line, implying that the errors were normally distributed with mean zero and constant but unknown variance as the underlying assumption of the analysis. These figures confirm the models adequacy.

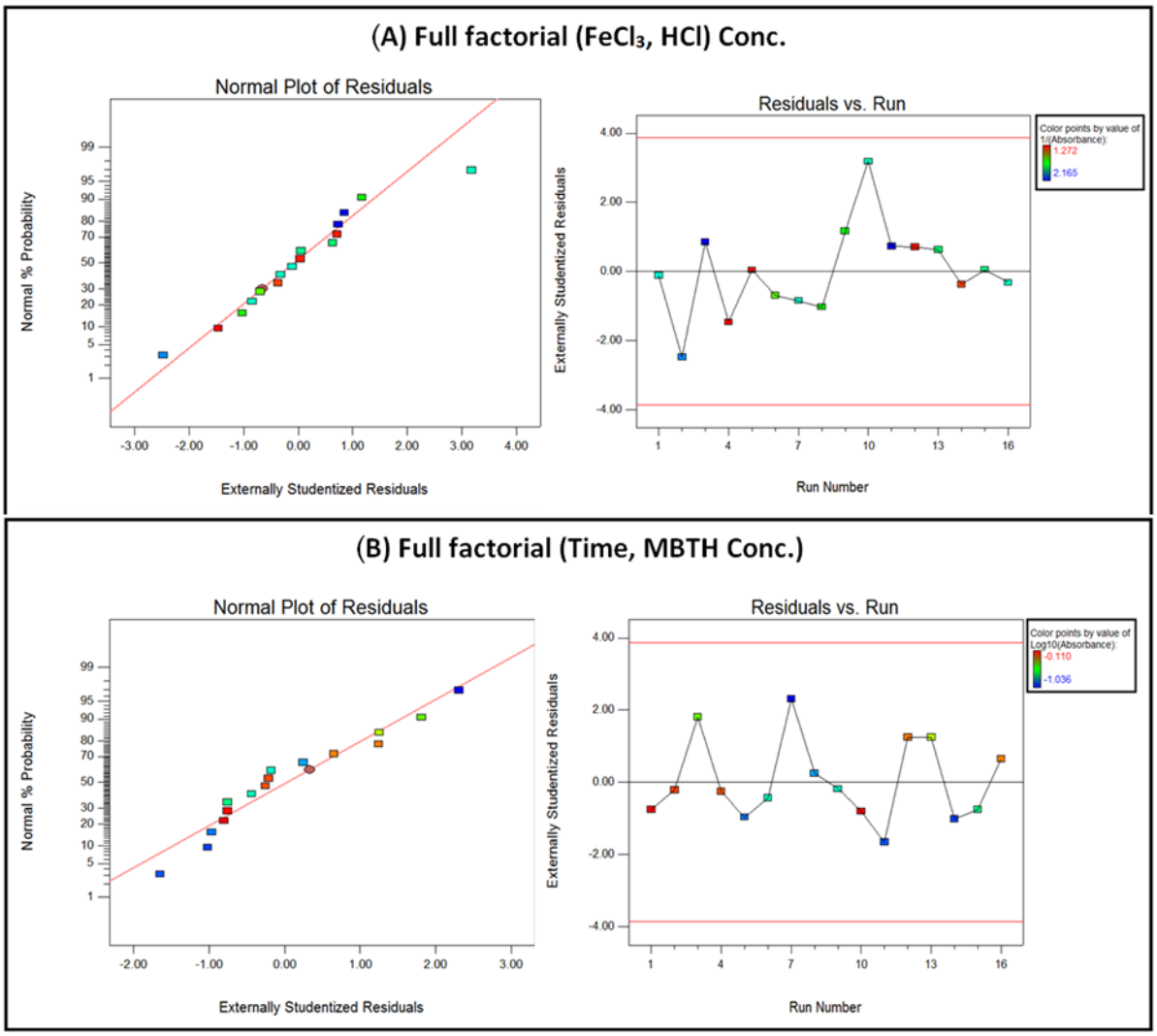


**Figure 6. Residual plots of absorbance for (A) $FeCl_3$ & HCl Conc. design (B) Reaction time & MBTH Conc. design.**

The results obtained from the full factorial designs showed that, there was an interaction between the $FeCl_3$ and HCl concentrations. While for time and MBTH concentration, no interaction was observed between them as shown in Figure. 7. Response surface and contour plots were also analyzed to visualize the effects of the variables on absorbance (Figure.8 & 9).The contour plots show non-linear effects of these factors on selected response. By analyzing the

response surface plots, it is possible to identify that 5 mg/ mL $FeCl_3$, 0.06% HCl, 4 mg/mL MBTH and time of 15 min give the maximum absorbance response.

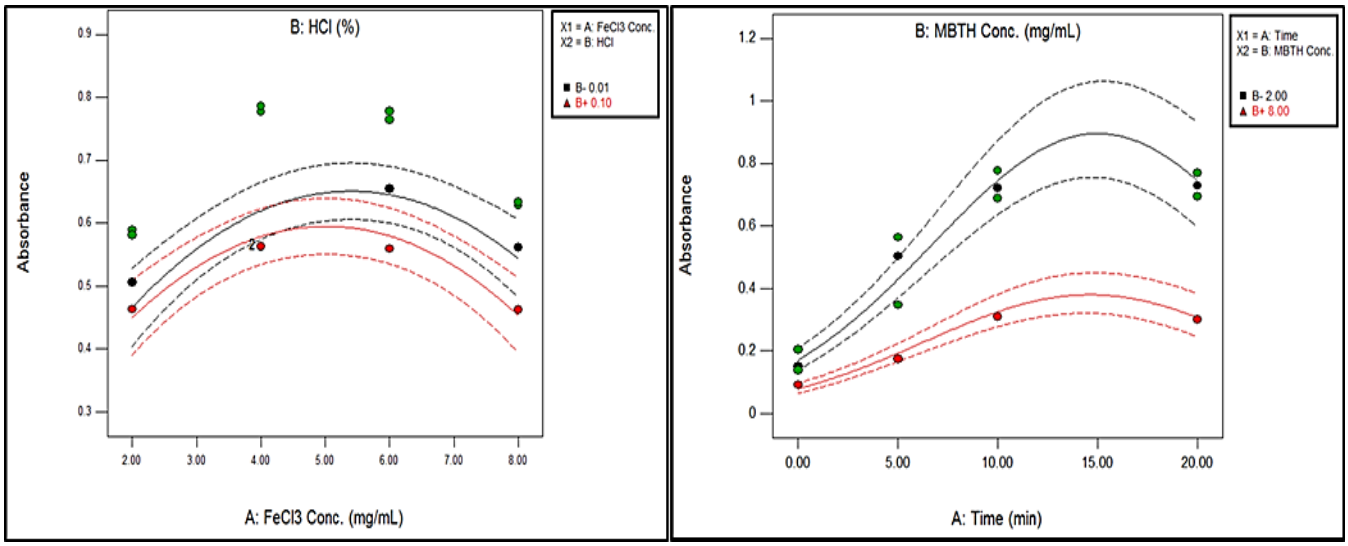


**Figure 7.Interaction plots for absorbance in optimization matrices.**

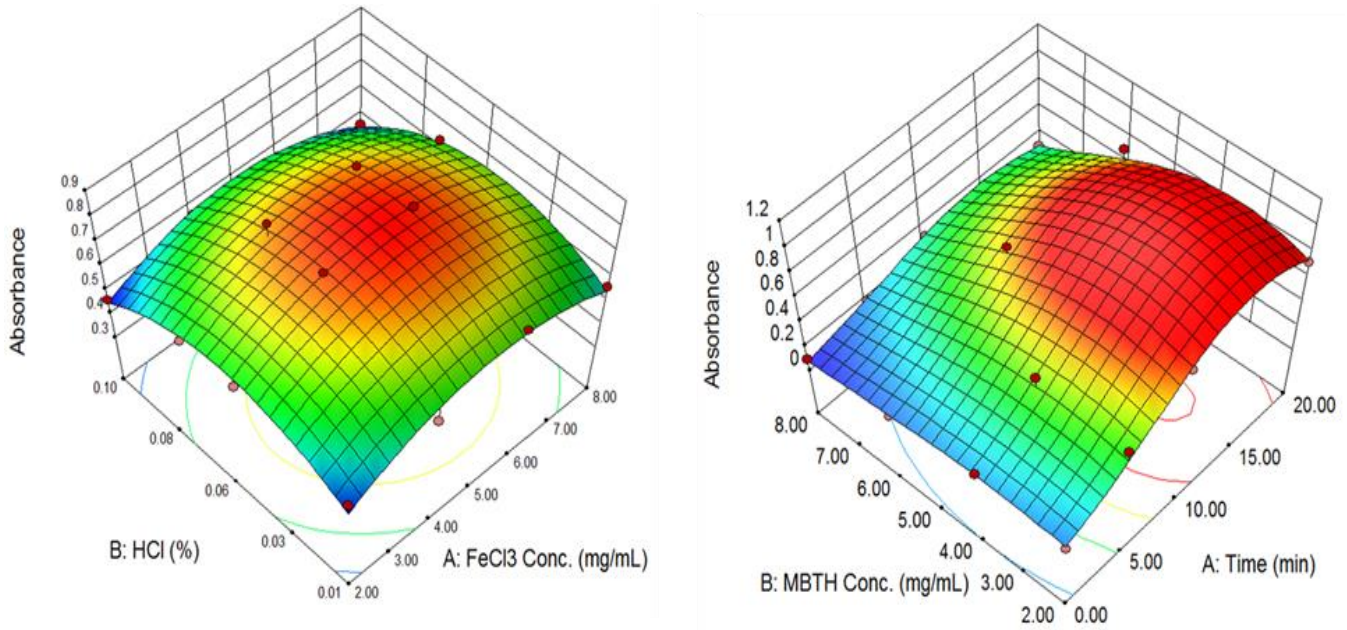


**Figure 8. Response surface plots of quadratic models for absorbance in optimization matrices.**

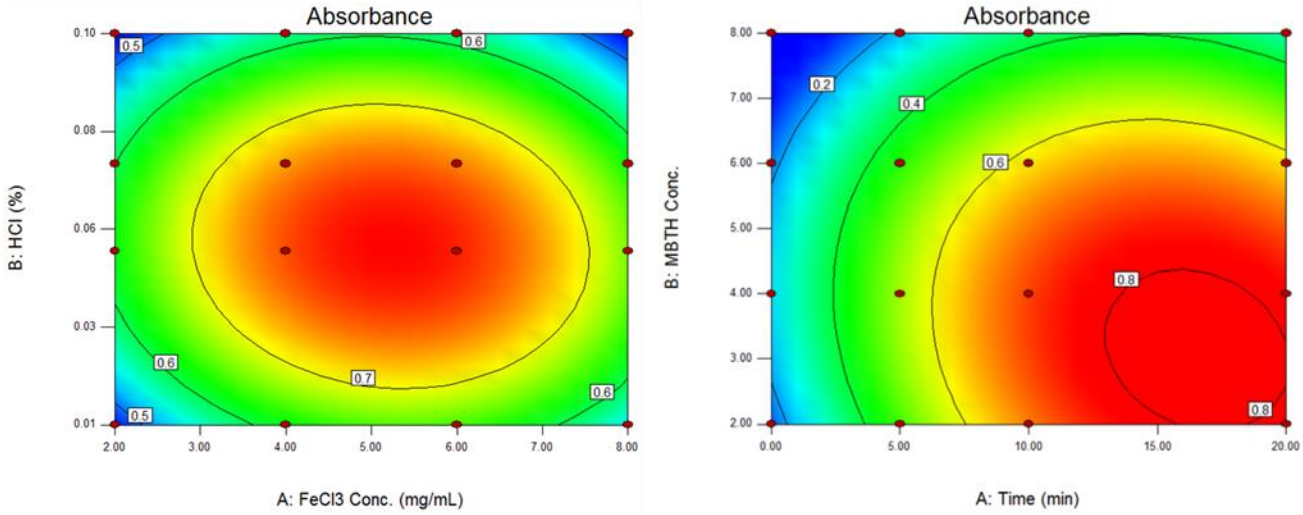


**Figure 9. Contour plots of absorbance for optimization matrices.**

Also the order of addition was investigated and it was found that, reversing the order of addition of reagents, i.e. $FeCl_3$ first, and then MBTH, results in a significant decrease in absorbance which may be attributed to the oxidation of CFN prior to the addition of MBTH.

**Validation of the method**

The developed method was validated according to ICH guidelines [30]. The following parameters were considered: linearity, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision.

***Linearity***

The method is linear over the range of 0.5-6.0 µg/mL for CFN (Figure. 10) and the linear regression equation was obtained. The regression data in Table 8 showed a linear dependence of the absorbance values on drug concentration over the selected range. The linearity of calibration graphs and adherence to Beer's law were validated by the high value of the correlation coefficient.

***Specificity***

Specificity of the method was proved as no interference was encountered from neither capsule nor suspension excipients like starch, sucrose, citric acid, glucose, sodium benzoate, gum acacia, and magnesium stearate.

***Limit of quantification (LOQ) and limit of detection (LOD)***

The (LOQ) was determined according to ICH recommendations[30] to establish the lowest concentration that can be measured, below which the calibration graph is non linear ($LOQ = 10\ \sigma/S$) where S is the slope and σ is the standard deviation of the intercept of regression line of

the calibration curve. The (LOD) was determined by evaluating the lowest concentration of the analyte that can be detected (LOD = 3.3 σ/S). The results of LOQ and LOD of CFN obtained by the proposed method were given in Table 8. The proposed method is sensitive as integrated by the high molar absorptivity value of the drug.

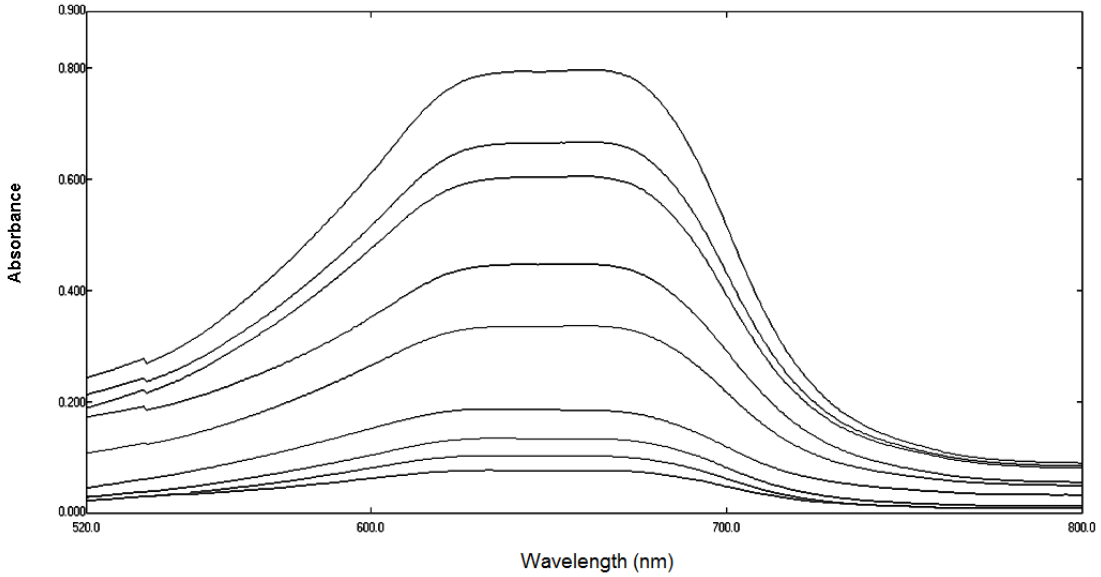


**Figure 10. Absorption spectra of CFN (0.5-6.0 µg/mL) in pure state using the proposed method.**

**Table 8: Analytical data for determination of CFN adopting the proposed method**

| Parameters | Values |
|---|---|
| Concentration range (µg/mL) | 0.5- 6.0 |
| Correlation coefficient (r) | 0.9999 |
| Slope | 0.1255 |
| Intercept | 0.0406 |
| % RSD[a] | 0.64 |
| % Er[b] | 0.21 |
| $S_{y/x}$[c] | $2.57 \times 10^{-3}$ |
| $S_a$[d] | $1.43 \times 10^{-3}$ |
| $S_b$[e] | $4.45 \times 10^{-4}$ |
| LOD (µg/mL)[f] | 0.04 |
| LOQ (µg/mL)[g] | 0.11 |
| ΔЄ (Mole$^{-1}$. L. cm $^{-1}$)[h] | $6.20 \times 10^4$ |

[a] % RSD, relative standard deviation ($\%\ RSD = SD \times 100/\bar{x}$ where SD is the standard deviation and $\bar{x}$is the mean recovery); [b] % Er, percent error ($\%Er = RSD/\sqrt{n}$ where n is the number of values); [c]$S_{y/x}$, standard deviation of the residuals; [d]$S_a$, standard deviation of the intercept; [e] $S_b$, standard deviation of the slope; [f] LOD, limit of detection; [g] LOQ, Limit of quantification; [h] ΔЄ, molar absorptivity.

**Accuracy and Precision**

Accuracy was revealed by calculating % the recovery of pure samples analyzed by the proposed methods (Table 9). Inter-day and intra-day accuracy and precision of the proposed method were also determined (Table 10) by analysis of CFN in concentrations of 1, 3 and 5 µg/mL, each three times a day for three consecutive days. The inter-day and intra-day accuracy were proved by the low values of % Er. The relative standard deviations (% RSD) for the results did not exceed 1.5%, proving the high reproducibility of the results and the precision of the method**.** This good level of precision was suitable for quality control analysis of CFN in pharmaceutical dosage forms.

**Table 9. Application of the proposed method and comparison method for the determination of CFN in pure state**

| Proposed method | | | | Comparison method [7] | |
|---|---|---|---|---|---|
| **Amount taken (µg/mL)** | **Amount found* (µg/mL)** | **% Found** | **ΔЄ (Mole$^{-1}$. L. cm $^{-1}$)** | **Amount taken (µg/mL)** | **% Found** |
| 0.50 | 0.497 | 99.44 | $8.15 \times 10^4$ | 4.00 | 100.59 |
| 0.70 | 0.696 | 99.49 | $7.23 \times 10^4$ | 8.00 | 100.10 |
| 0.90 | 0.896 | 99.51 | $6.72 \times 10^4$ | 12.00 | 101.89 |
| 1.00 | 0.999 | 99.92 | $6.56 \times 10^4$ | 16.00 | 99.37 |
| 2.00 | 2.019 | 100.96 | $5.81 \times 10^4$ | | |
| 3.00 | 3.023 | 100.77 | $5.54 \times 10^4$ | | |
| 4.00 | 3.963 | 99.08 | $5.32 \times 10^4$ | | |
| 5.00 | 4.983 | 99.67 | $5.27 \times 10^4$ | | |
| 6.00 | 6.019 | 100.32 | $5.25 \times 10^4$ | | |
| | % R ± SD = 99.91 ± 0.64 | | | % R ± SD = 100.48 ± 1.06 | |
| | | | *t*- value = 1.67 (2.20) | | |
| | | | *F*- value = 2.72 (4.07) | | |

In each of proposed and comparison method, three samples were repeated.

*Each result is the mean recovery of three separate determinations.

% R, the mean of % recovery.

Figures between brackets are the tabulated *t* and *F*- values at ($p$= 0.05).

**Table 10. Evaluation of the accuracy and precision data of the proposed method for the determination of CFN in Cefdin® capsules and oral powder for suspension**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Cefdin® capsules** | | | | | | |
| **Amount added (µg/mL)** | **1.00** | | **3.00** | | **5.00** | |
| | **Amount found (µg/mL)** | **% Found** | **Amount found (µg/mL)** | **% Found** | **Amount found (µg/mL)** | **% Found** |
| • ***Intra-day*** | 1.02 | 101.51 | 3.05 | 101.83 | 5.01 | 100.14 |
| | 0.99 | 99.12 | 2.98 | 99.18 | 4.93 | 98.55 |
| | 1.00 | 100.00 | 3.03 | 101.04 | 4.98 | 99.67 |
| $\overline{X} \pm SD$ | 100.19 ± 1.22 | | 100.68 ± 1.36 | | 99.45 ± 0.82 | |
| % RSD | 1.22 | | 1.35 | | 0.82 | |
| % Er | 0.70 | | 0.78 | | 0.47 | |
| • ***Inter-day*** | 1.01 | 100.72 | 2.97 | 98.91 | 4.98 | 99.51 |
| | 0.98 | 98.33 | 2.99 | 99.71 | 4.99 | 99.82 |
| | 0.99 | 99.12 | 3.02 | 100.50 | 5.02 | 100.30 |
| $\overline{X} \pm SD$ | 99.39 ± 1.22 | | 99.71 ± 0.80 | | 99.88 ± 0.40 | |
| % RSD | 1.23 | | 0.80 | | 0.40 | |
| % Er | 0.71 | | 0.46 | | 0.23 | |
| **Cefdin® (Oral powder for suspension)** | | | | | | |
| • ***Intra-day*** | 1.00 | 99.92 | 3.04 | 101.30 | 5.01 | 100.14 |
| | 0.99 | 99.12 | 2.97 | 98.91 | 4.94 | 98.71 |
| | 1.01 | 100.72 | 3.02 | 100.77 | 4.98 | 99.67 |
| $\overline{X} \pm SD$ | 99.92 ± 0.80 | | 100.33 ± 1.26 | | 99.51 ± 0.73 | |
| % RSD | 0.80 | | 1.25 | | 0.73 | |
| % Er | 0.46 | | 0.72 | | 0.42 | |
| • ***Inter-day*** | 0.99 | 99.12 | 3.05 | 101.57 | 5.00 | 99.98 |
| | 0.99 | 99.12 | 2.98 | 99.18 | 4.94 | 98.71 |
| | 1.01 | 100.72 | 2.98 | 99.44 | 4.98 | 99.67 |
| $\overline{X} \pm SD$ | 99.65 ± 0.92 | | 100.06 ± 1.31 | | 99.45 ± 0.66 | |
| % RSD | 0.92 | | 1.31 | | 0.67 | |
| % Er | 0.53 | | 0.76 | | 0.39 | |

Each result is the mean recovery of three separate determinations, three samples were repeated.

Batch No. of N100931 and N100432 for capsules and suspension, respectively.

**Assay of dosage forms**

The proposed method was applied for determination of CFN in capsules and oral powder for suspension. The absorbance values of standard solution of CFN (0.5 mg/mL) relative to the absorbance values of the test solution was used for the determination of the drug in dosage forms and the concentrations percent recoveries were calculated according to the corresponding regression equation. The excipients of capsule and suspension did not interfere with the assay. The results of dosage forms are given in Table 11.

**Table 11. Assay results for the determination of CFN in different dosage forms by the proposed method and comparison method**

| **Cefdin® capsules** | | | |
|---|---|---|---|
| **Proposed method** | | | **Comparison method** [7] |
| **Amount taken (µg/mL)** | **Amount found * (µg/mL)** | **% Found** | **% Found** |
| 1.00 | 1.00 | 100.00 | 97.66 |
| 3.00 | 3.01 | 100.24 | 97.66 |
| 5.00 | 4.99 | 99.82 | 99.22 |
| % R ± SD = 100.02 ± 0.21 | | | % R ± SD = 99.18 ± 0.9 |
| *t*- value = -2.42 (2.78) | | | |
| *F*- value = 18.81 (19) | | | |
| **Cefdin® oral powder for suspension** | | | |
| 1.00 | 1.01 | 100.72 | 97.66 |
| 3.00 | 3.00 | 100.00 | 100.26 |
| 5.00 | 5.00 | 100.00 | 100.78 |
| 100.24 ± 0.41 | | | 99.57 ± 1.67 |
| *t*- value = -1.85 (2.78) | | | |
| *F*- value = 16.35 (19) | | | |

*Each result is the mean recovery of three separate determinations.

Figures between brackets are the tabulated *t* and *F*-values at ($p$=0.05).

Batch No. of N100931 and N100432l for capsules and suspension, respectively.

## CONCLUSION

The present study described a simple and sensitive visible spectrophotometric method for determination of CFN in pure form and pharmaceutical dosage forms. An experimental design approach was applied for optimization of the colored reaction conditions. A two-level fractional factorial design was used to provide data regarding influencing factors that had significant effects

on the selected response (absorbance). One-way ANOVA and Pareto ranking analyses showed that all factors were statistically significant. Full factorial designs were then utilized to evaluate the main, interaction and quadratic effects of the factors on the selected response Y. With the help of a response surface plot and contour plot, the optimum values of the selected factors were identified. The predicted values of the response matched the experimental values reasonably well, as integrated by high values of correlation coefficients ($R^2 \geq 0.80$). The proposed method has advantages of simplicity, low-cost and high stability of the colored product. Furthermore the sensitivity of the proposed method was confirmed by high molar absorptivity values of CFN. These advantages allowed the application of the proposed method to the analysis of CFN in pharmaceutical dosage forms and in quality control laboratories.

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