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# RP-HPLC DEVELOPMENT & VALIDATION OF CITICOLINE IN BULK DRUG & ITS PHARMACEUTICAL DOSAGE FORM BY QUALITY BY DESIGN (QbD)

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## 1. ABSTRACT

The work describes the Reverse phase high performance liquid chromatographic method for the estimation of citicoline in bulk as well as in tablet dosage form. The estimation was carried out on C18 using the mobile mixture of water and methanol (70:30) as a mobile phase. All analyte were detected by measuring absorbance at 271 nm with flow rate of 1.0 ml/min. the total run time of the study was 4 min. The method was validated for accuracy, precision, linearity, specificity, as per ICH guidelines. The calibration curves were found linear over the concentration range of 1.0-15.00 µg/ml for Citicoline. From the validation Study it was found that the method is specific, rapid, accurate & precise.

## 2. INTRODUCTION

Analytical method validation is the process of documenting / proving that an analytical method provides analytical data acceptable for the intended use.

There are many factors to consider when developing methods. The initially collected information about the analyst's physiochemical properties (these include appearance, boiling point, density, volatility, water solubility and flammability etc.)

Quality by Design (QbD) is a strategic process for development and manufacturing. It is meant to ensure that the intended performance of a final drug product is as expected both in terms of purity and efficacy.

## 3. OBJECTIVES

1. During the literature survey I found that Citicoline Sodium is a well-established molecule. There is various research work done but the study of validation of Citicoline and its formulation Quality by Design (QbD) not yet done.
2. Hence in this research an attempt will be made to study the method validation of Citicoline and its formulations by Quality by Design (QbD).
3. To increase process capability and reduce product variability and defects by enhancing product and process design
4. Understanding and control. To increase product development and manufacturing efficiencies.

   To enhance root cause analysis and post approval change management.

## 4. OBJECTIVES

### 4.1. PRELIMINARY CHARACTERIZATION OF DRUG

#### 4.1.1 Color, odour and appearance

Citicoline sodiumwas evaluated for parameters like color; odour& appearance are shown in result**.**

**4.1.2. Melting point**forCiticoline sodiumwas determined by open capillary method and compared with literature values given in results.

#### 4.1.3. Citicoline sodiumfactor calculations

Molecular weight of Citicoline sodium: 510.31.

Molecular weight of Citicoline: 488.32.

$$Factor = \frac{Molecular weight of \text{Citicoline}}{Molecular weight of \text{Citicoline sodium}}$$

$$Factor = \frac{488.32}{510.31}$$

Factor = 0.957

#### 4.1.4. Determination of solubility

The solubility was determined in Water at a concentration of 3mg/mL as follows and are given in results**.**

**4.2 Selection of analytical wavelength**

**4.2.1. Selection of solvent**

**4.2.2. Preparation of standard stock solutions**

**4.2.3. Selection of analytical wavelength**

**4.3 Method Development by RP – HPLC**

**4.3.1. Preparation of standard stock solution for Chromatographic development**

**4.3.2 Optimization of HPLC method.**

**4.4. Optimization of Developed RP-HPLC Method with Design Space and Control Strategy determination by optimization study**

All the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert version 7.0.0; State-Ease Inc., Minneapolis, MN, USA).

**4.4.1 Application of design of experiments for method optimization**

**4.4.2. Design of experiments (DOE-1)**

**Preparation of standard solutions to inject in DOE runs**

**Translation of coded levels in actual values**

| Level of Variable | Range of Factors | | |
|---|---|---|---|
| | Methanol (%v/v) | Flow Rate (mL/min) | Column oven temperature (°C) |
| Low Level (-1) | 60 | 0.8 | 37 |
| Medium Level (0) | 70 | 1.0 | 40 |
| High Level (1) | 80 | 1.2 | 43 |

**4.4.3. Preparation of System suitability test (Citicoline standard solution)**

**Acceptance criteria**

1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

**4.4.4. Analysis of marketed Test sample**

Marketed test sample Having Name Citiros 500 mg tablets are selected for analysis and for doing validation.

**Average weight of test sample (Citiros 500 mg)**

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula.

Average weight (mg) = Weight of 20 tablets (mg) / 20

**Formula for % Assay calculation**

$$\%\ Assay = \frac{CiticolineSplarea}{CiticolineStd\ avgarea} X \frac{CiticolineSTDwt\ (mg)}{50} X \frac{0.4}{20} X \frac{50}{Tabletsampleweight\ (mg)} X$$

$$\frac{20}{0.2} X \frac{Avgwtofsample\ (mg)}{LabelclaimofCiticoline} XFactorX\ 100$$

## 4.5. VALIDATION OF RP-HPLC METHOD

The developed method for estimation of Citicoline was validated as per ICH guidelines for following parameters.

1. FILTRATION STUDY
2. STABILITY OF ANALYTICAL SOLUTION
3. SPECIFICITY
4. LINEARITY AND RANGE.

**Linearity levels prepared as follows**

| Sr. No. | Level (%) | mL of stock solution | Diluted to with water (mL) | Citicoline Concentration (µg/mL) |
|---|---|---|---|---|
| 1 | 10 | 0.50 | 20 | 1.00 |
| 2 | 50 | 2.50 | 20 | 5.00 |
| 3 | 100 | 5.00 | 20 | 10.00 |
| 4 | 125 | 6.25 | 20 | 12.50 |
| 5 | 150 | 7.50 | 20 | 15.00 |

5. Limit of Detection (LOD) and Limit of Quantitation (LOQ).
6. ACCURACY (% RECOVERY)

**Accuracy levels details**

Refer Following table for each sample

| Level (%) | Citicoline Sodium API (mg) | Placebo (mg) | Diluted to (mL) | Volume taken (mL) | Diluted to (mL) | Citicoline Concentartion (µg/mL) |
|---|---|---|---|---|---|---|
| 50 | 26.3 | 22.6 | 50 | 0.2 | 20 | 5.03 |
| | 26.2 | 22.7 | 50 | 0.2 | 20 | 5.01 |
| | 26.4 | 22.8 | 50 | 0.2 | 20 | 5.05 |
| 100 | 52.3 | 22.5 | 50 | 0.2 | 20 | 10.01 |
| | 52.3 | 22.4 | 50 | 0.2 | 20 | 10.01 |
| | 52.4 | 22.6 | 50 | 0.2 | 20 | 10.03 |
| 150 | 78.5 | 22.7 | 50 | 0.2 | 20 | 15.02 |
| | 78.4 | 22.5 | 50 | 0.2 | 20 | 15.01 |
| | 78.6 | 22.5 | 50 | 0.2 | 20 | 15.04 |

**Acceptance criteria**

1. % Recovery for each sample and Mean recovery and overall recovery should be in the range of 98-102%.

2. The Relative Standard Deviation should not be more than 2.0%.

**7. PRECISION**

I. Repeatability

Precision (Repeatability) Sample details are as follows

| Sample No. | Test powder material (mg) | Diluted to (mL) | Volume taken (mL) | Diluted to (mL) |
|---|---|---|---|---|
| 1 | 74.9 | 50 | 0.2 | 20 |
| 2 | 75.1 | 50 | 0.2 | 20 |
| 3 | 74.8 | 50 | 0.2 | 20 |
| 4 | 74.9 | 50 | 0.2 | 20 |
| 5 | 75.0 | 50 | 0.2 | 20 |
| 6 | 74.7 | 50 | 0.2 | 20 |

**Acceptance criteria**

% Assay: 90-110% for each sample and mean assay value

% RSD for % assay value of 6 samples: NMT 2%.

**II. Intermediate precision**

| Sample No. | Test powder material (mg) | Diluted to (mL) | Volume taken (mL) | Diluted to (mL) |
|---|---|---|---|---|
| 1 | 74.8 | 50 | 0.2 | 20 |
| 2 | 74.7 | 50 | 0.2 | 20 |
| 3 | 75.1 | 50 | 0.2 | 20 |
| 4 | 74.8 | 50 | 0.2 | 20 |
| 5 | 74.9 | 50 | 0.2 | 20 |
| 6 | 74.8 | 50 | 0.2 | 20 |

**Acceptance criteria**

% Assay: 90-110% for each sample and mean assay value

% RSD for % assay of 6 samples of Intermediate precision**:** NMT 2

% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

**8) ROBUSTNESS**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

## 5. RESULTS

### 5.1. PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF DRUG

#### 5.1.1. Color, odour and appearance

| Sr. No | Name | Colour, odour and appearance of drug |
|---|---|---|
| 1 | Citicoline Sodium | White, odourless and slightly amorphous powder |

#### 5.1.2. Melting point determination

| Sr. No. | Name | Melting point std. value (°C) | Melting point observed (°C) |
|---|---|---|---|
| 1 | Citicoline Sodium | 259-268 °C | 261-268 °C |

#### 5.1.3. Solubility study

| Sr. No. | Name of Solvent | Observation | Conclusion | Summary |
|---|---|---|---|---|
| 1 | Water | No Drug Particles seen after sonication | Drug was found soluble in water. | Water used as a diluent for preparing stock solution. |

### 6.2 Optimization of HPLC method

**Developed Chromatographic Condition**

| Parameter | Description |
|---|---|
| Mode | Isocratic |
| Column Name | BDS hypersil C8, 250 mm X 4.6mm ID, 5 µm |
| Detector | UV Detector |
| Injection Volume | 20 µl |
| Wavelength | 271 nm |
| Column Oven temp | 40ºC |
| Mobile Phase | Methanol: Water (70:30%V/V) |
| Flow Rate | 1.0 ml/min |

### 6.3. Chromatograms of DOE run

**Chromatography as follows**

| Parameter | Description |
|---|---|
| Mode | Isocratic |
| Column Name | BDS hypersil C8, 250 mm X 4.6mm ID, 5 µm |
| Detector | UV Detector |
| Injection Volume | 20 µl |
| Wavelength | 271 nm |
| Column Oven temp | 40ºC |
| Mobile Phase | Methanol: Water (70:30%V/V) |
| Flow Rate | 1.0 ml/min |

### 6.4. Results for the Retention time of DOE

1. **Fit Summary:** After entering the data in Design-Expert software, fit summary applied to the data after which the "quadratic vs 2FI" was suggested by the software.

**Fit summary table for R.T. of DOE**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob> F | |
|---|---|---|---|---|---|---|
| Mean vs Total | 347.04565 | 1 | 347.04565 | | | |
| Linear vs Mean | 18.84543 | 3 | 6.28181 | 66.414 | < 0.0001 | |
| 2FI vs Linear | 0.27213 | 3 | 0.09071 | 0.947 | 0.4542 | |
| Quadratic vs 2FI | 0.94209 | 3 | 0.31403 | 142.695 | < 0.0001 | Suggested |
| Cubic vs Quadratic | 0.01533 | 3 | 0.00511 | 255.417 | < 0.0001 | Aliased |
| Residual | 0.00008 | 4 | 0.00002 | | | |
| Total | 367.12070 | 17 | 21.59534 | | | |

## 2. ANOVA for retention time of DOE

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the retention time of DOE are as following Table.

**ANOVA table for a retention time of DOE**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob> F | |
|---|---|---|---|---|---|---|
| Model | 20.04736 | 6 | 3.34123 | 1206.96 | < 0.0001 | significant |
| A-METHANOL | 2.95366 | 1 | 2.95366 | 1066.96 | < 0.0001 | |
| B-FR | 2.97237 | 1 | 2.97237 | 1073.72 | < 0.0001 | |
| C-COT | 0.03001 | 1 | 0.03001 | 10.84 | 0.0081 | |
| AB | 0.26010 | 1 | 0.26010 | 93.96 | < 0.0001 | |
| A^2 | 0.56454 | 1 | 0.56454 | 203.93 | < 0.0001 | |
| B^2 | 0.32668 | 1 | 0.32668 | 118.01 | < 0.0001 | |
| Residual | 0.02768 | 10 | 0.00277 | | | |
| Lack of Fit | 0.02760 | 6 | 0.00460 | 230.02 | < 0.0001 | significant |
| Pure Error | 0.00008 | 4 | 0.00002 | | | |
| Cor Total | 20.07505 | 16 | | | | |

The Model F-value of 1206.96 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, $A^2$, $B^2$ are significant model terms.

## 3. Fit Statistics for R.T. of DOE

| | | | |
|---|---|---|---|
| Std. Dev. | 0.053 | R-Squared | 0.999 |
| Mean | 4.518 | Adj R-Squared | 0.998 |
| C.V. % | 1.164 | Pred R-Squared | 0.993 |
| PRESS | 0.139 | Adeq Precision | 128.028 |

The "Pred R-Squared" of 0.993is in reasonable agreement with the "Adj R-Squared" of 0.998.

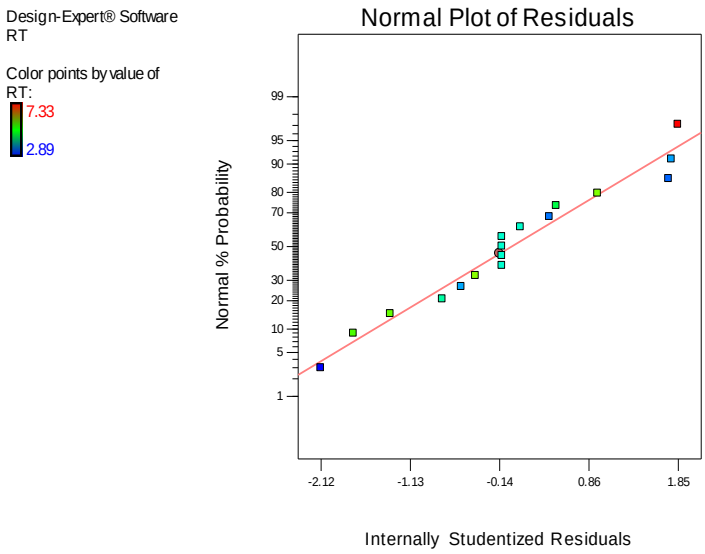
"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 128.028indicates an adequate signal. This model can be used to navigate the design space.

## 4. Final Equation in Terms of coded Factors for R.T. of DOE

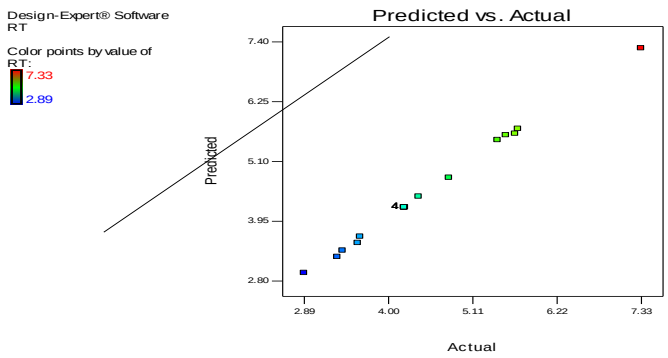
| RT | = |
|---|---|
| 7.2753 | |
| -1.9763 | * A |
| -1.9826 | * B |
| -0.0613 | * C |
| 0.2550 | * A * B |
| 0.3657 | * A^2 |
| 0.2782 | * B^2 |

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor.

## 5. Graphical Presentation: Diagnostics of R.T. for DOE




**Normal % Probability for DOE of R.T.**




**Predicted Vs Actual for DOE of R.T.**

### 6.5. Results for the asymmetry of DOE

**1. Fit Summary:** After entering the data in Design-Expert software, fit summary applied to the data after which the "quadratic vs 2FI" was suggested by the software.

**Fit summary table for asymmetry of DOE.**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob> F | |
|---|---|---|---|---|---|---|
| Mean vs Total | 30.57882 | 1 | 30.57882 | | | Suggested |
| Linear vs Mean | 0.00677 | 3 | 0.00226 | 0.53 | 0.6708 | |
| 2FI vs Linear | 0.01243 | 3 | 0.00414 | 0.96 | 0.4491 | |
| Quadratic vs 2FI | 0.03642 | 3 | 0.01214 | 12.58 | 0.0033 | Suggested |
| Cubic vs Quadratic | 0.00668 | 3 | 0.00223 | 111.25 | 0.0003 | Aliased |
| Residual | 0.00008 | 4 | 0.00002 | | | |
| Total | 30.64120 | 17 | 1.80242 | | | |

**2. ANOVA for Asymmetry of DOE**

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the asymmetric factor of DOEare as following Table.

**ANOVA table for asymmetry of DOE as such**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob> F | |
|---|---|---|---|---|---|---|
| Model | 0.05562 | 9 | 0.00618 | 6.40 | 0.0115 | significant |
| A-METHANOL | 0.00071 | 1 | 0.00071 | 0.74 | 0.4183 | |
| B-FR | 0.00132 | 1 | 0.00132 | 1.36 | 0.2812 | |
| C-COT | 0.00025 | 1 | 0.00025 | 0.26 | 0.6264 | |
| **AB** | 0.00903 | 1 | 0.00903 | 9.35 | **0.0184** | |
| AC | 0.00250 | 1 | 0.00250 | 2.59 | 0.1515 | |
| BC | 0.00090 | 1 | 0.00090 | 0.93 | 0.3663 | |
| **A^2** | 0.00584 | 1 | 0.00584 | 6.05 | **0.0434** | |
| B^2 | 0.00125 | 1 | 0.00125 | 1.30 | 0.2920 | |
| **C^2** | 0.02678 | 1 | 0.02678 | 27.75 | **0.0012** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Residual | 0.00676 | 7 | 0.00097 | | | |
| Lack of Fit | 0.00668 | 3 | 0.00223 | 111.25 | 0.0003 | significant |
| Pure Error | 0.00008 | 4 | 0.00002 | | | |
| Cor Total | 0.06238 | 16 | | | | |

The Model F-value of 6.40implies the model is significant. There is only a 1.15% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob> F" less than 0.0500 indicate model terms are significant.

**In this case AB, $A^2$, $C^2$ are significant model terms**

**4. Fit Statistics for Asymmetry for DOE**

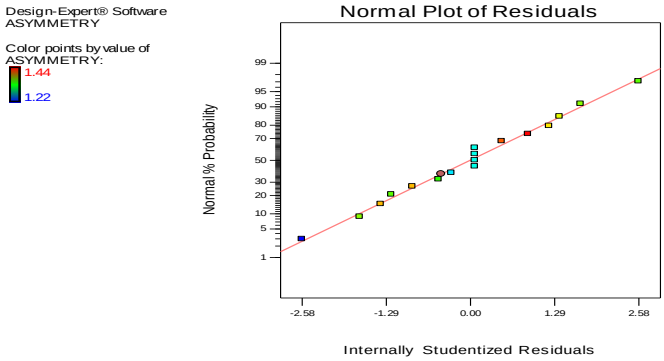
| | | | |
|---|---|---|---|
| Std. Dev. | 0.0311 | R-Squared | 0.8917 |
| Mean | 1.3412 | Adj R-Squared | 0.7525 |
| C.V. % | 2.3162 | Pred R-Squared | -0.7142 |
| PRESS | 0.1069 | Adeq Precision | 6.9779 |

A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model.

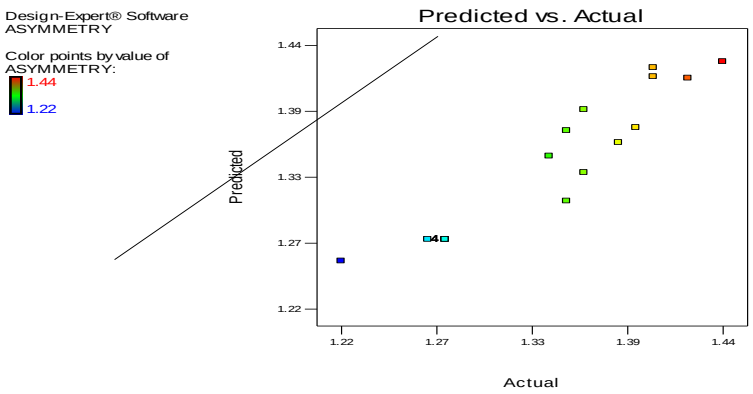
Final Equation in Terms of Coded Factors of Asymmetry for DOE:

| ASYMMETRY | = |
|---|---|
| 1.26 | |
| -0.03075 | * A |
| 0.04175 | * B |
| -0.0125 | * C |
| -0.0475 | * A * B |
| 0.025 | * A * C |
| -0.015 | * B * C |
| 0.03725 | * A^2 |
| 0.01725 | * B^2 |
| 0.07975 | * C^2 |

**5. Graphical Presentation: Diagnostics of Asymmetry for DOE**




**Normal % Probability for DOE of Asymmetry.**




**Predicted Vs Actual for DOE of Asymmetry.**

### 6.6. Results for Theoretical plates DOE

1. **Fit Summary**: After entering the data in Design-Expert software, fit summary applied to the data after which the "Linear Vs Mean and quadratic vs 2FI" was suggested by the software.

**Fit Summary for theoretical plates of DOE**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob> F | |
|---|---|---|---|---|---|---|
| Mean vs Total | 1345431041 | 1 | 1345431041 | | | |
| Linear vs Mean | 34397609.25 | 3 | 11465869.75 | 16.234 | 0.0001 | Suggested |
| 2FI vs Linear | 559281.5 | 3 | 186427.1667 | 0.216 | 0.8829 | |
| Quadratic vs 2FI | 7140868.359 | 3 | 2380289.453 | 11.246 | 0.0046 | Suggested |
| Cubic vs Quadratic | 1480476.75 | 3 | 493492.25 | 1874.258 | < 0.0001 | Aliased |
| Residual | 1053.2 | 4 | 263.3 | | | |
| Total | 1389010330 | 17 | 81706490 | | | |

**2. ANOVA for Theoretical platesof DOE**

Linear Model selected for analysis. The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the **theoretical plates**of DOE are as follows

**ANOVA table for Theoretical plates of DOE**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob> F | |
|---|---|---|---|---|---|---|
| Model | 34013483.13 | 2 | 17006741.56 | 24.89015 | < 0.0001 | significant |
| A-METHANOL | 11558432 | 1 | 11558432 | 16.9163 | 0.0011 | |
| B-FR | 22455051.13 | 1 | 22455051.13 | 32.86401 | < 0.0001 | |
| Residual | 9565805.934 | 14 | 683271.8524 | | | |
| Lack of Fit | 9564752.734 | 10 | 956475.2734 | 3632.644 | < 0.0001 | significant |
| Pure Error | 1053.2 | 4 | 263.3 | | | |
| Cor Total | 43579289.06 | 16 | | | | |

The Model F-value of 24.89015implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.

In this case A, B is significant model terms.

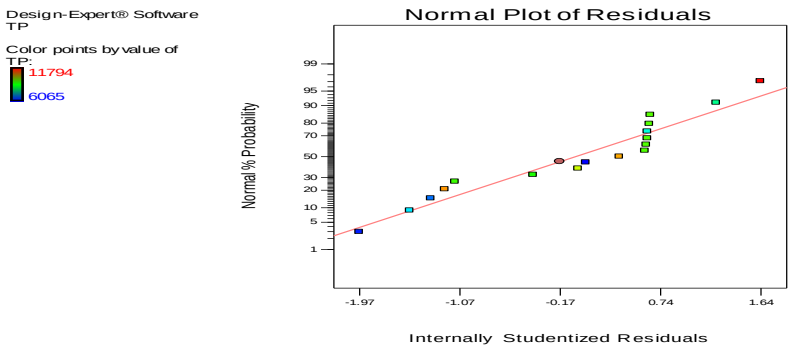
**3. Fit Statistics for** theoretical plates **of DOE**

| | | | |
|---|---|---|---|
| Std. Dev. | 826.603 | R-Squared | 0.780 |
| Mean | 8896.235 | Adj R-Squared | 0.749 |
| C.V. % | 9.292 | Pred R-Squared | 0.656 |
| PRESS | 14985203 | Adeq Precision | 16.573 |

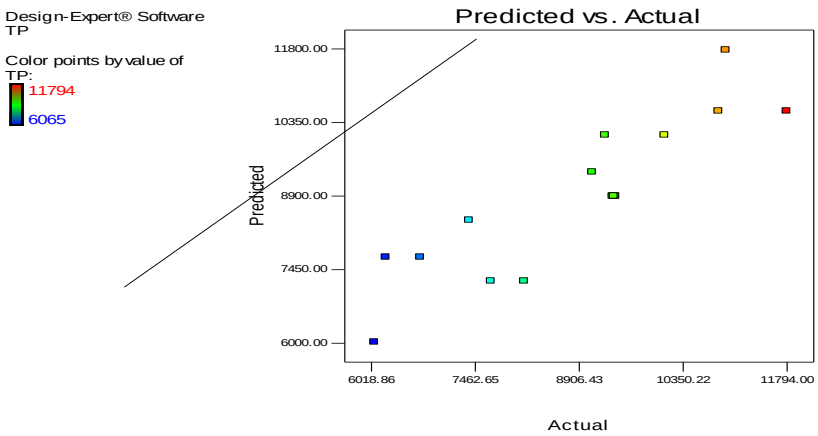
**4. Final Equation in Terms of coded Factors for** theoretical plates **of DOE**

| TP | = |
|---|---|
| 11773.61029 | |
| -1202 | * A |
| -1675.375 | * B |

**5. Graphical Presentation: Diagnostics of theoretical platesfor DOE**




**Normal % Probability for DOE of Theoretical plates.**




**Predicted Vs Actual for DOE of Theoretical plates.**

**Summary of effect of independent variable on dependent variables**

| Sr. No. | Independent variables | Retention time | Asymmetry | Theoretical plates |
|---|---|---|---|---|
| 1 | % Methanol ratio in mobile phase | Inversely proportional (As Methanol increases, R.T. decreases) | Curvature effect (Factor involved in interaction. Individually Methanol don't have effect, acts in interaction) | Inversely proportional (As Methanol increases, Theoretical plates decreases) |
| 2 | Flow rate | Inversely proportional (As Flow rate increases, R.T. decreases) | Curvature effect (Factor involved in interaction. Individually Flow rate don't have effect, acts in interaction) | Inversely proportional (As Flow rate increases, Theoretical plates decreases) |
| 3 | Column oven temperature | Inversely proportional (As COT increases, R.T. decreases) | Curvature effect (Factor involved in interaction. Individually Flow rate don't have effect, acts in interaction) | COT found as Insignificant factor on Theoretical plates in ANOVA |

### 6.7. DOE optimization result

We have selected DOE trial no.02 for validation, which has following parameters.

| Runs | Factor1 | Factor 2 | Factor3 | Response 1 | Response 2 | Response 3 |
|---|---|---|---|---|---|---|
| | A: % Methanol | B:Flow rate | C: COT (°C) | Retention time (RT) | Asymmetry | TP |
| 11 | 70 | 1.0 | 40 | 4.21 | 1.28 | 9394 |

**By entering trial no. 02 results in optimization and checked for solutions as follows**

| Name | Goal | Target value |
|---|---|---|
| A: Methanol | Target-> | 70 |
| B: FR | Target-> | 1 |
| C: C.O.T. | Target-> | 40 |
| R.T. | Target-> | 4.21 |
| Asymmetry | Target-> | 1.28 |
| Theoretical plates | Target-> | 9394 |

**Optimization solutions: Result of optimization for DOE**

| Number | METHANOL | FR | COT | RT | ASYMMETRY | TP | Desirability | |
|---|---|---|---|---|---|---|---|---|
| 1 | 70 | 1 | 40 | 4.22 | 1.28 | 8896 | 0.968 | Selected |
| 2 | 69.91 | 1 | 40 | 4.22 | 1.28 | 8908 | 0.967 | |
| 3 | 68.84 | 1 | 40 | 4.34 | 1.28 | 9036 | 0.962 | |
| 4 | 68.67 | 1 | 40 | 4.35 | 1.28 | 9056 | 0.961 | |
| 5 | 67.76 | 1 | 40 | 4.47 | 1.28 | 9186 | 0.955 | |
| 6 | 61.81 | 1 | 42.5 | 5.22 | 1.34 | 9880 | 0.564 | |

## CONCLUSION

We got the almost same chromatography parameters with its results as that of trial no 2 with the desirability 0.968. Solution no.1 shows almost same parameters with results. (±10%). Hence proposed Box behnken surface methodology model found fit for developed chromatographic method and it can be used to predict dependent variable within a design space.

### 6.5.5. Design space

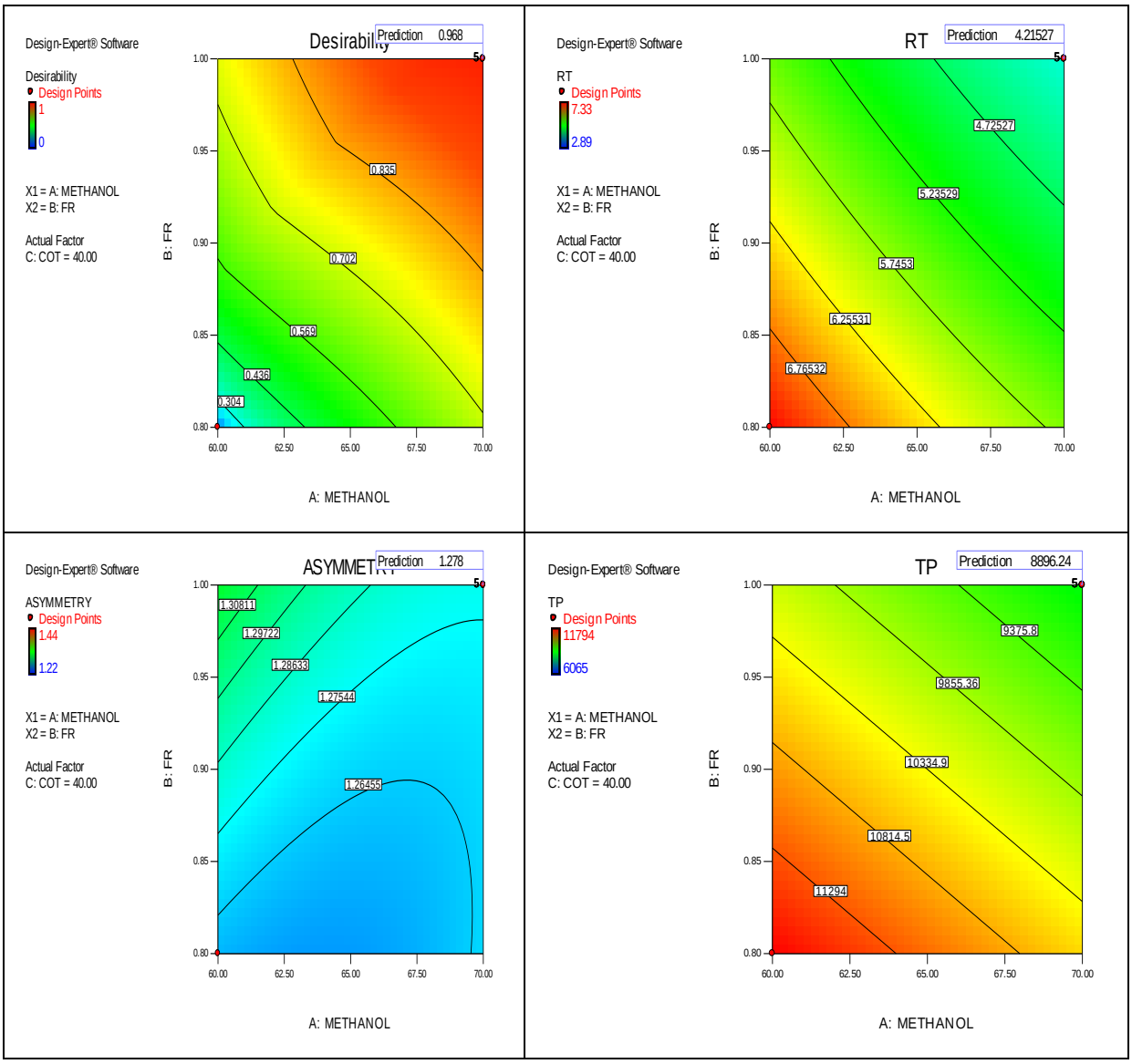



**Fig. No. 42: Design space for Desirability, R.T, Asymmetry and Theoretical plates.**

### 6.5.6. Optimized chromatography method is as follows: subjected for validation.

| Parameter | Description |
|---|---|
| Mode | Isocratic |
| Column Name | BDS Hypersil C8, 250 mm X 4.6mm ID, 5 µm |

| | |
|---|---|
| Detector | UV Detector |
| Injection Volume | 20 µl |
| Wavelength | 271 nm |
| Column Oven temp | 40ºC |
| Mobile Phase | Methanol: Water (70:30 %V/V) |
| Flow Rate | 1 ml/min |
| Run time | 07 Minutes |

**6.7. System suitability test**

**Results for System Suitability Test of Citicoline**

| Sr No. | Standard solution | Area | Asymmetry | Theoretical plates |
|---|---|---|---|---|
| 1 | Standard_1 | 7380314 | 1.27 | 9446 |
| 2 | Standard_2 | 7360348 | 1.27 | 9431 |
| 3 | Standard_3 | 7378410 | 1.27 | 9467 |
| 4 | Standard_4 | 7340064 | 1.28 | 9454 |
| 5 | Standard_5 | 7320146 | 1.27 | 9433 |
| **Mean** | | **7355856** | **1.27** | **9446** |
| **STD Dev** | | **25747.06831** | | |
| **% RSD** | | **0.35** | | |

**System Suitability Acceptance Criteria**

1. Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.

2. Theoretical plates of analyte peak in standard chromatograms should not be less than 2000.

3. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0.

**Data interpretation:** It was observed from the data tabulated above; the method complies with system suitability parameters. Hence, it can be concluded that the chromatographic methodis adequate for intended analysis.

**SUMMARY**

The work describes the Reverse phase high performance liquid chromatographic method for the estimation of citicoline in bulk as well as in tablet dosage form. The estimation was carried out on C18 using the mobile mixture of water and methanol (70:30) as a mobile phase.

All analyte were detected by measuring absorbance at 271 nm with flow rate of 1.0 ml/min. the total run time of the study was 4 min. The method was validated for accuracy, precision,

linearity, specificity, as per ICH guidelines. From the validation Study it was found that the method is specific, rapid, accurate & precise.

Development and validation of RP-HPLC method was found to be linear, accurate, precise, specific and robust according to acceptance criteria and with high level of LOD and LOQ. The results show that the HPLC method presented here can be considered suitable for the analytical determination of Citicoline in bulk and tablet dosage form. The developed method was validated. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The % RSD was also less than 2% showing high degree of precision of the proposed method. The method was successfully applied to the available marketed formulation without any interference due to the excipients and can have an application in the industry.

## CONCLUSION

A simple, sensitive and economical RP-HPLC method has been successfully developed employing the systematic QbD-based approach for quantification of Citicoline in bulk as well as in tablet formulations. The screening and optimization studies employing experimental designs finally embarked upon the selection of optimized condition for optimization and validation. The validation study corroborated excellent linearity, accuracy, precision, specificity and robustness. Further, the experimentally observed value of LOD and LOQ of drug was found to be quite lower. The method demonstrated high degree of practical utility for estimation of Citicoline in pharmaceutical dosage forms.

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