# Application of Design of Experiments for Formulation Development and Mechanistic Evaluation of Iontophoretic Tacrine Hydrochloride Delivery

The objective of this research paper is to develop a mathematical equation to understand the impact of variables and establish statistical control over transdermal iontophoretic delivery of tacrine hydrochloride. Design of experiments (DoE) methodology is implemented to study the effect of independent variables on the dependent variable.

The factors (independent variables) examined are: $X_1$ = current strength (mA), $X_2$ = buffer molarity (mM) and $X_3$ = drug concentration (mg/ml). All the factors are continuous. The response (dependent variable) examined is: Y = tacrine permeation flux (μg/cm$^2$/h). The applied DoE method is Central Composite design.

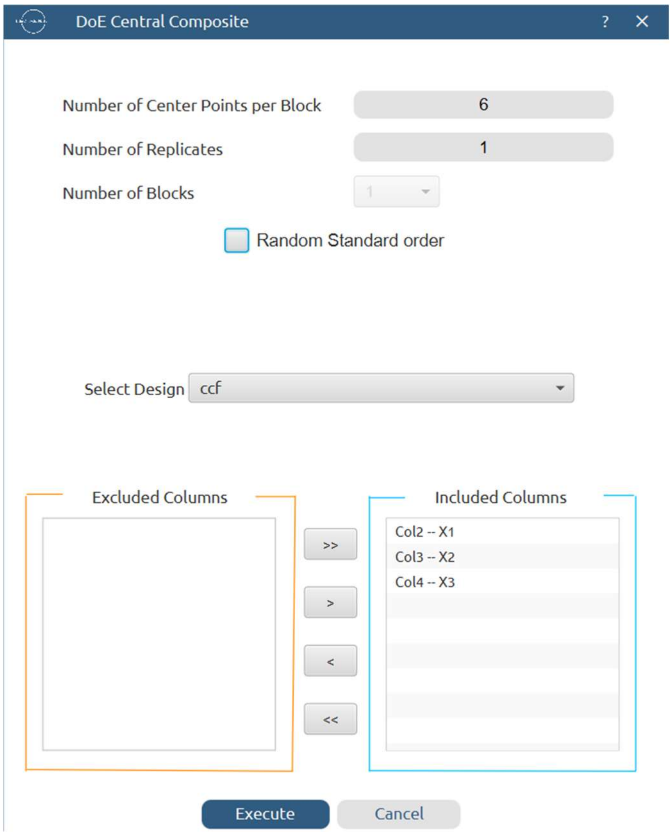
*Isalos version used: 2.0.6*

Scientific article: https://www.tandfonline.com/doi/abs/10.1080/03639045.2016.1181646

## Step 1: Central Composite Design

In the first tab named "Action" define the factors in the column headers and fill each column with the low and high levels of the corresponding factors. This tab can be renamed "Central Composite". Afterwards, apply the Central Composite method: *DOE → Response Surface → Central Composite*

| | Col1 | Col2 (D) | Col3 (I) | Col4 (I) |
|---|---|---|---|---|
| User Header | User Row ID | X1 | X2 | X3 |
| 1 | | 0.1 | 25 | 1 |
| 2 | | 0.3 | 100 | 20 |



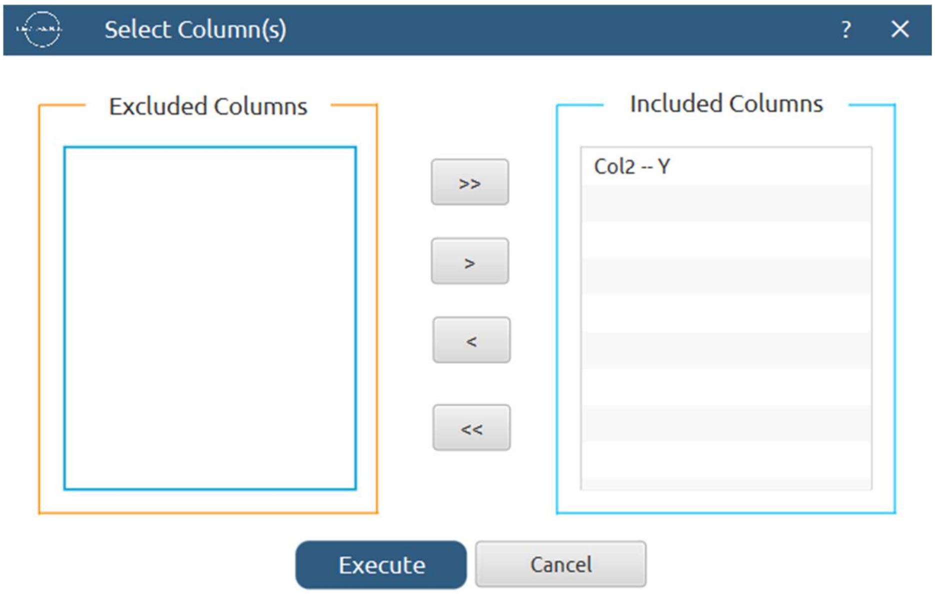

Results (right spreadsheet):

| Standard Order | Block Number | Replicate Number | Point Type | X1 | X2 | X3 |
|---|---|---|---|---|---|---|
| 1 | Block: 1 | Replicate: 1 | Design Point | 0.1 | 25.0 | 1.0 |
| 2 | Block: 1 | Replicate: 1 | Design Point | 0.3 | 25.0 | 1.0 |
| 3 | Block: 1 | Replicate: 1 | Design Point | 0.1 | 100.0 | 1.0 |
| 4 | Block: 1 | Replicate: 1 | Design Point | 0.3 | 100.0 | 1.0 |
| 5 | Block: 1 | Replicate: 1 | Design Point | 0.1 | 25.0 | 20.0 |
| 6 | Block: 1 | Replicate: 1 | Design Point | 0.3 | 25.0 | 20.0 |
| 7 | Block: 1 | Replicate: 1 | Design Point | 0.1 | 100.0 | 20.0 |
| 8 | Block: 1 | Replicate: 1 | Design Point | 0.3 | 100.0 | 20.0 |
| 9 | Block: 1 | Replicate: 1 | Design Point | 0.1 | 62.5 | 10.5 |
| 10 | Block: 1 | Replicate: 1 | Design Point | 0.3 | 62.5 | 10.5 |
| 11 | Block: 1 | Replicate: 1 | Design Point | 0.2 | 25.0 | 10.5 |
| 12 | Block: 1 | Replicate: 1 | Design Point | 0.2 | 100.0 | 10.5 |
| 13 | Block: 1 | Replicate: 1 | Design Point | 0.2 | 62.5 | 1.0 |
| 14 | Block: 1 | Replicate: 1 | Design Point | 0.2 | 62.5 | 20.0 |
| 15 | Block: 1 | ---- | Center Point | 0.2 | 62.5 | 10.5 |
| 16 | Block: 1 | ---- | Center Point | 0.2 | 62.5 | 10.5 |
| 17 | Block: 1 | ---- | Center Point | 0.2 | 62.5 | 10.5 |
| 18 | Block: 1 | ---- | Center Point | 0.2 | 62.5 | 10.5 |
| 19 | Block: 1 | ---- | Center Point | 0.2 | 62.5 | 10.5 |
| 20 | Block: 1 | ---- | Center Point | 0.2 | 62.5 | 10.5 |

# Step 2: Definition of response variables

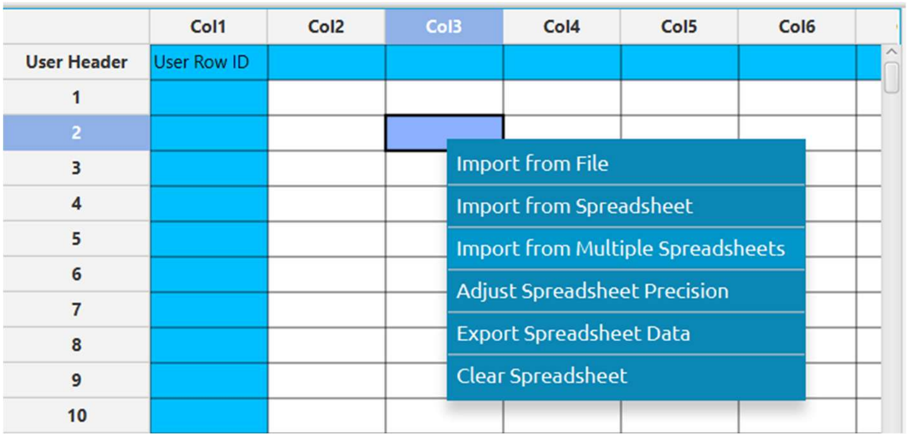
Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the Central Composite method. Then, select all columns to be transferred to the right spreadsheet: *Data Transformation → Data Manipulation → Select Column(s)*

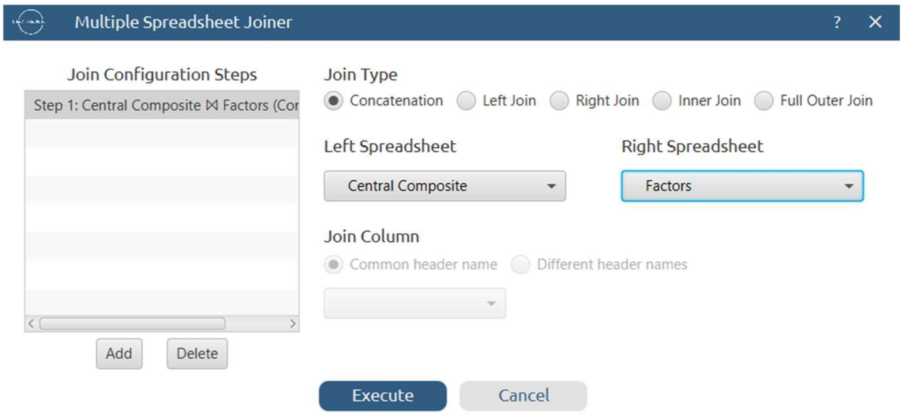
| | Col1 | Col2 (D) |
|---|---|---|
| User Header | User Row ID | Y |
| 1 | | 92.22 |
| 2 | | 219.57 |
| 3 | | 47.84 |
| 4 | | 129.77 |
| 5 | | 229.89 |
| 6 | | 477.09 |
| 7 | | 180.45 |
| 8 | | 405.2 |
| 9 | | 156.68 |
| 10 | | 364.67 |
| 11 | | 312.96 |
| 12 | | 190.79 |
| 13 | | 75.77 |
| 14 | | 315.14 |
| 15 | | 267.38 |
| 16 | | 267.38 |
| 17 | | 278.66 |
| 18 | | 270.89 |
| 19 | | 270.89 |
| 20 | | 278.66 |

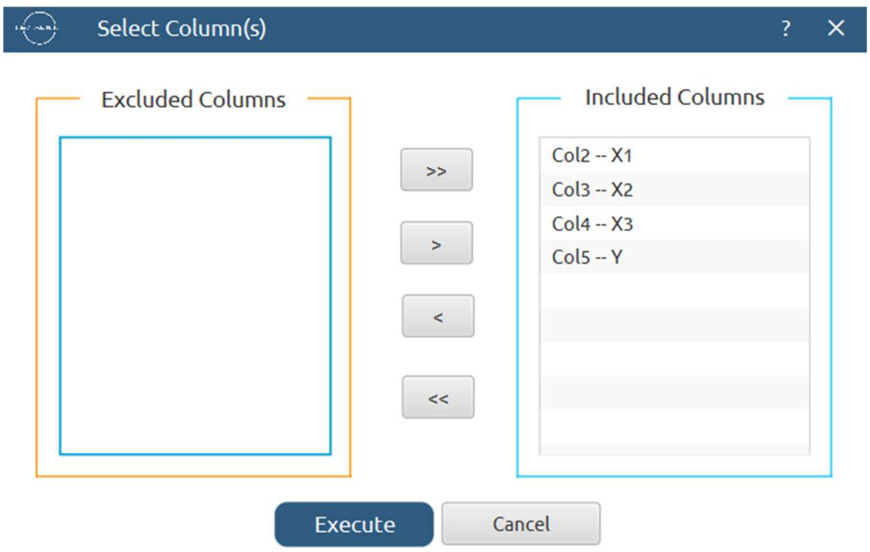


# Step 3: Data isolation

Create a new tab named "Data" and import the results from the "Central Composite" and "Responses" spreadsheets by right clicking on the left spreadsheet. Then, select only the factors and responses columns to be transferred to the right spreadsheet: <u>*Data Transformation → Data Manipulation → Select Column(s)*</u>



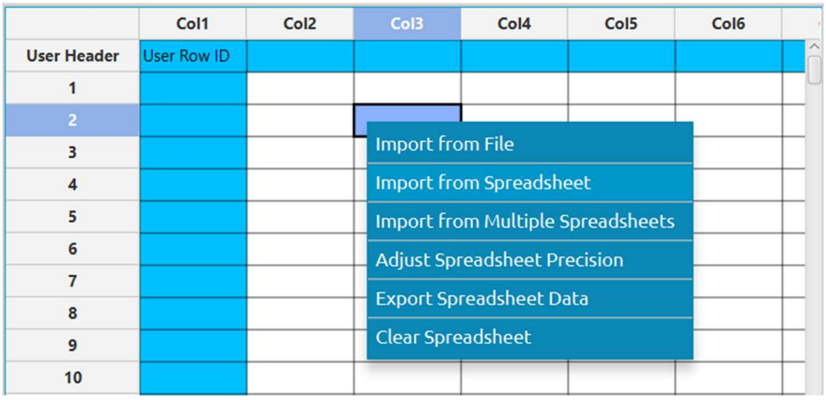



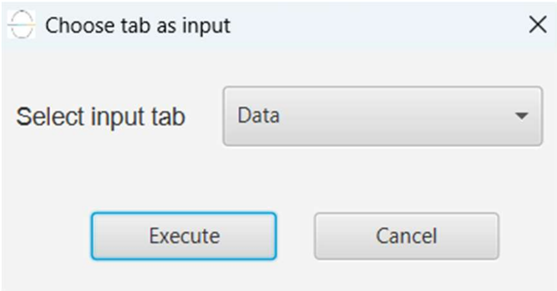
Results:

| | Col1 | Col2 (D) | Col3 (D) | Col4 (D) | Col5 (D) |
|---|---|---|---|---|---|
| User Header | User Row ID | X1 | X2 | X3 | Y |
| 1 | | 0.1 | 25.0 | 1.0 | 92.22 |
| 2 | | 0.3 | 25.0 | 1.0 | 219.57 |
| 3 | | 0.1 | 100.0 | 1.0 | 47.84 |
| 4 | | 0.3 | 100.0 | 1.0 | 129.77 |
| 5 | | 0.1 | 25.0 | 20.0 | 229.89 |
| 6 | | 0.3 | 25.0 | 20.0 | 477.09 |
| 7 | | 0.1 | 100.0 | 20.0 | 180.45 |
| 8 | | 0.3 | 100.0 | 20.0 | 405.2 |
| 9 | | 0.1 | 62.5 | 10.5 | 156.68 |
| 10 | | 0.3 | 62.5 | 10.5 | 364.67 |
| 11 | | 0.2 | 25.0 | 10.5 | 312.96 |
| 12 | | 0.2 | 100.0 | 10.5 | 190.79 |
| 13 | | 0.2 | 62.5 | 1.0 | 75.77 |
| 14 | | 0.2 | 62.5 | 20.0 | 315.14 |
| 15 | | 0.2 | 62.5 | 10.5 | 267.38 |
| 16 | | 0.2 | 62.5 | 10.5 | 267.38 |
| 17 | | 0.2 | 62.5 | 10.5 | 278.66 |
| 18 | | 0.2 | 62.5 | 10.5 | 270.89 |
| 19 | | 0.2 | 62.5 | 10.5 | 270.89 |
| 20 | | 0.2 | 62.5 | 10.5 | 278.66 |

# Step 4: Normalization

Create a new tab named “Normalized data” and import the results from the “Data” spreadsheet. Afterwards, normalize the factor columns to take values in the range [-1, 1]: *Data Transformation → Normalizers → Min-Max*




Results:

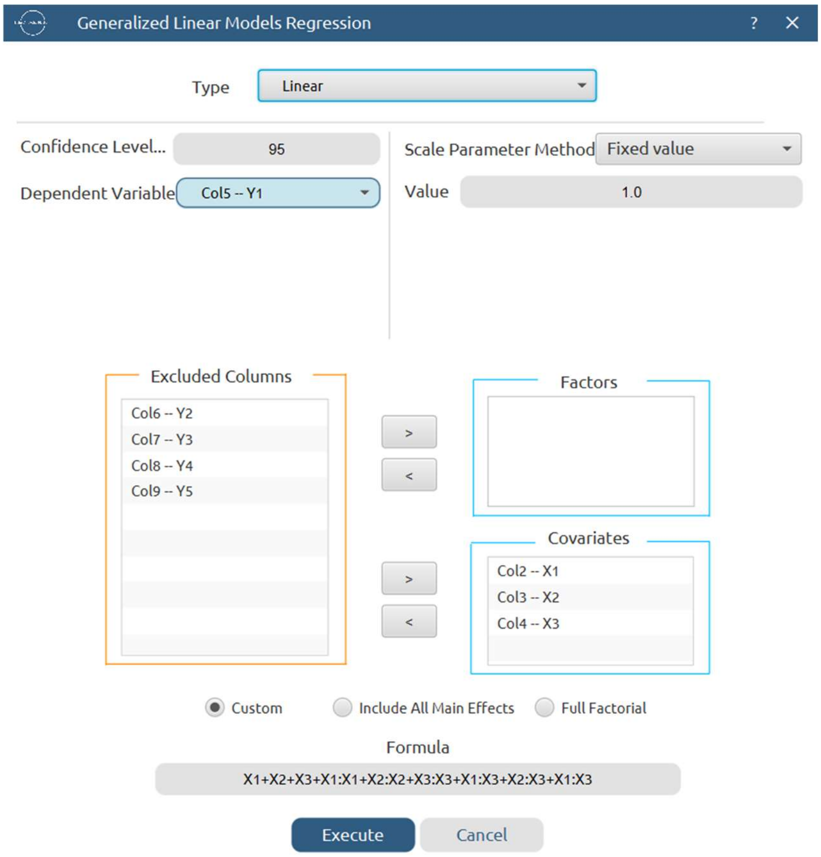
| | Col1 | Col2 (D) | Col3 (D) | Col4 (D) | Col5 (D) |
|---|---|---|---|---|---|
| User Header | User Row ID | X1 | X2 | X3 | Y |
| 1 | | -1.0 | -1.0 | -1.0 | 92.22 |
| 2 | | 1.0 | -1.0 | -1.0 | 219.57 |
| 3 | | -1.0 | 1.0 | -1.0 | 47.84 |
| 4 | | 1.0 | 1.0 | -1.0 | 129.77 |
| 5 | | -1.0 | -1.0 | 1.0 | 229.89 |
| 6 | | 1.0 | -1.0 | 1.0 | 477.09 |
| 7 | | -1.0 | 1.0 | 1.0 | 180.45 |
| 8 | | 1.0 | 1.0 | 1.0 | 405.2 |
| 9 | | -1.0 | 0.0 | 0.0 | 156.68 |
| 10 | | 1.0 | 0.0 | 0.0 | 364.67 |
| 11 | | 0E-7 | -1.0 | 0.0 | 312.96 |
| 12 | | 0E-7 | 1.0 | 0.0 | 190.79 |
| 13 | | 0E-7 | 0.0 | -1.0 | 75.77 |
| 14 | | 0E-7 | 0.0 | 1.0 | 315.14 |
| 15 | | 0E-7 | 0.0 | 0.0 | 267.38 |
| 16 | | 0E-7 | 0.0 | 0.0 | 267.38 |
| 17 | | 0E-7 | 0.0 | 0.0 | 278.66 |
| 18 | | 0E-7 | 0.0 | 0.0 | 270.89 |
| 19 | | 0E-7 | 0.0 | 0.0 | 270.89 |
| 20 | | 0E-7 | 0.0 | 0.0 | 278.66 |

# Step 5: Regression

The goal here is to produce a regression equation that includes main effects, two-factor interactions and quadratic effects for $Y_1$:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2$$

Create a new tab named "Regression – Coded" and import the results from the spreadsheet "Normalized data". Afterwards, fit a generalized linear model to the data: *Analytics → Regression → Statistical fitting → Generalized Linear Models*



Results:

| Y | Prediction |
|---|---|
| 92.22 | 90.0577045 |
| 219.57 | 219.2017045 |
| 47.84 | 28.2767045 |
| 129.77 | 123.4857045 |
| 229.89 | 229.6977045 |
| 477.09 | 490.1767045 |
| 180.45 | 174.3417045 |
| 405.2 | 400.8857045 |
| 156.68 | 184.7061818 |
| 364.67 | 362.5501818 |
| 312.96 | 302.5961818 |
| 190.79 | 227.0601818 |
| 75.77 | 104.1481818 |
| 315.14 | 312.6681818 |
| 267.38 | 263.6745455 |
| 267.38 | 263.6745455 |
| 278.66 | 263.6745455 |
| 270.89 | 263.6745455 |
| 270.89 | 263.6745455 |
| 278.66 | 263.6745455 |

| | |
|---|---|
| Goodness of Fit | |
| | Value |
| Deviance | 4259.2897461 |
| Scaled Deviance | 4259.2897461 |
| Pearson Chi-Square | 4259.2897461 |
| Scaled Pearson Chi-Square | 4259.2897461 |
| Log Likelihood | -2148.0236437 |
| Akaike's Information Criterion (AIC) | 4316.0472875 |
| Finite Sample Corrected AIC (AICC) | 4340.4917319 |
| Bayesian Information Criterion (BIC) | 4326.0046102 |
| Consistent AIC (CAIC) | 4336.0046102 |

| Parameter Estimates | | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Coefficient | Std. Error | Lower CI | Upper CI | Test Statistic | df | p-value |
| intercept | 263.6745455 | 0.3437758 | 263.0007572 | 264.3483337 | 588282.2500979 | 1 | 0.0 |
| X1 | 88.9220000 | 0.3162278 | 88.3022050 | 89.5417950 | 79071.2208400 | 1 | 0.0 |
| X2 | -37.7680000 | 0.3162278 | -38.3877950 | -37.1482050 | 14264.2182400 | 1 | 0.0 |
| X3 | 104.2600000 | 0.3162278 | 103.6402050 | 104.8797950 | 108701.4760000 | 1 | 0.0 |
| X1*X3 | 32.8337500 | 0.3535534 | 32.1407981 | 33.5267019 | 8624.4411125 | 1 | 0.0 |
| X1*X2 | -8.48375 | 0.3535534 | -9.1767019 | -7.7907981 | 575.7921125 | 1 | 0.0 |
| X2*X3 | 1.6062500 | 0.3535534 | 0.9132981 | 2.2992019 | 20.6403125 | 1 | 0.0000055 |
| X1*X1 | 9.9536364 | 0.6030227 | 8.7717336 | 11.1355391 | 272.4559114 | 1 | 0.0 |
| X2*X2 | 1.1536364 | 0.6030227 | -0.0282664 | 2.3355391 | 3.6599114 | 1 | 0.0557366 |
| X3*X3 | -55.2663636 | 0.6030227 | -56.4482664 | -54.0844609 | 8399.5201114 | 1 | 0.0 |

Repeat this step for the uncoded data. Results:

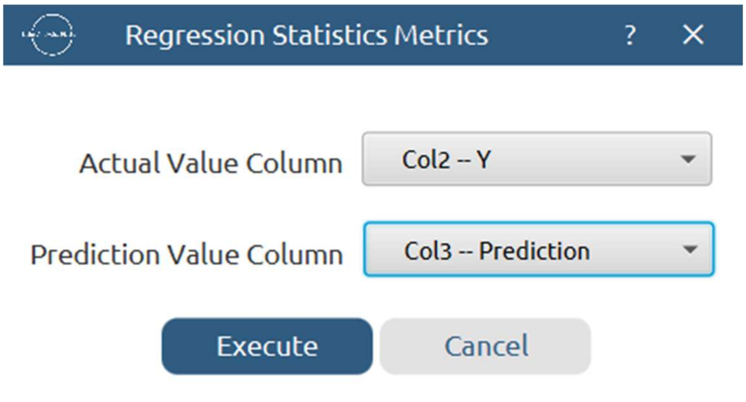
| Y | Prediction |
|---|---|
| 190.79 | 227.0601818 |
| 75.77 | 104.1481818 |
| 267.38 | 263.6745455 |
| 156.68 | 184.7061818 |
| 267.38 | 263.6745455 |
| 364.67 | 362.5501818 |
| 219.57 | 219.2017045 |
| 278.66 | 263.6745455 |
| 229.89 | 229.6977045 |
| 92.22 | 90.0577045 |
| 270.89 | 263.6745455 |
| 477.09 | 490.1767045 |
| 315.14 | 312.6681818 |
| 405.2 | 400.8857045 |
| 270.89 | 263.6745455 |
| 312.96 | 302.5961818 |
| 47.84 | 28.2767045 |
| 129.77 | 123.4857045 |
| 278.66 | 263.6745455 |
| 180.45 | 174.3417045 |

| | |
|---|---|
| Goodness of Fit | |
| | Value |
| Deviance | 4259.2897461 |
| Scaled Deviance | 4259.2897461 |
| Pearson Chi-Square | 4259.2897461 |
| Scaled Pearson Chi-Square | 4259.2897461 |
| Log Likelihood | -2148.023643 7 |
| Akaike's Information Criterion (AIC) | 4316.0472875 |
| Finite Sample Corrected AIC (AICC) | 4340.4917319 |
| Bayesian Information Criterion (BIC) | 4326.0046102 |
| Consistent AIC (CAIC) | 4336.0046102 |

| Parameter Estimates | | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Coefficient | Std. Error | Lower CI | Upper CI | Test Statistic | df | p-value |
| intercept | 56.3073923 | 2.5481407 | 51.3131283 | 61.3016563 | 488.2969565 | 1 | 0.0 |
| X1 | 269.5710367 | 25.3339789 | 219.9173504 | 319.2247229 | 113.2243011 | 1 | 0.0 |
| X2 | -0.7045676 | 0.0583818 | -0.8189937 | -0.5901414 | 145.6434274 | 1 | 0.0 |
| X3 | 16.6403335 | 0.1737354 | 16.2998184 | 16.9808486 | 9173.7621902 | 1 | 0.0 |
| X1*X3 | 34.5618421 | 0.3721615 | 33.8324190 | 35.2912652 | 8624.4411125 | 1 | 0.0 |
| X1*X2 | -2.2623333 | 0.0942809 | -2.4471205 | -2.0775462 | 575.7921125 | 1 | 0.0 |
| X2*X3 | 0.0045088 | 0.0009924 | 0.0025636 | 0.0064539 | 20.6403125 | 1 | 0.0000055 |
| X1*X1 | 995.3636364 | 60.3022689 | 877.1733611 | 1113.5539116 | 272.4559114 | 1 | 0.0 |
| X2*X2 | 0.0008204 | 0.0004288 | -0.0000201 | 0.0016608 | 3.6599114 | 1 | 0.0557366 |
| X3*X3 | -0.6123697 | 0.0066817 | -0.6254656 | -0.5992738 | 8399.5201114 | 1 | 0.0 |

# Step 6: Regression Metrics

Create a tab named "Metrics – Coded" and import the results from the spreadsheet "Regression – Coded". Then, produce the regression metrics for the regression equation of the coded data: *Statistics → Model Metrics → Regression Metrics*




Results:

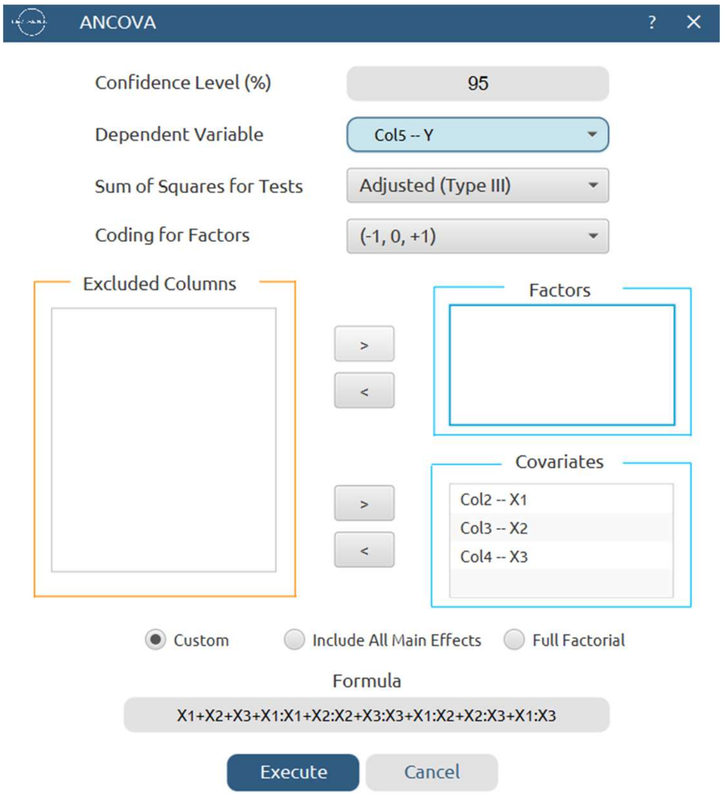
| | Col1 | Col2 (D) | Col3 (D) | Col4 (D) | Col5 (D) |
|---|---|---|---|---|---|
| User Header | User Row ID | Mean Squared Error | Root Mean Squared Error | Mean Absolute Error | R Squared |
| 1 | | 212.9644873 | 14.5933028 | 10.5761250 | 0.9812924 |

Repeat this step for the uncoded data. Results:

| | Col1 | Col2 (D) | Col3 (D) | Col4 (D) | Col5 (D) |
|---|---|---|---|---|---|
| User Header | User Row ID | Mean Squared Error | Root Mean Squared Error | Mean Absolute Error | R Squared |
| 1 | | 212.9644873 | 14.5933028 | 10.5761250 | 0.9812924 |

# Step 7: Analysis of Covariance

Create a new tab named "ANCOVA – Coded" and import the results from the spreadsheet "Normalized data". Afterwards perform analysis of covariance for Y: *Statistics → Analysis of (Co)Variance → ANCOVA*




Results:

| | Col1 | Col2 (S) | Col3 (I) | Col4 (D) | Col5 (D) | Col6 (D) | Col7 (D) |
|---|---|---|---|---|---|---|---|
| User Header | User Row ID | Source | DF | Adj SS | Adj MS | F-Value | P-Value |
| 1 | | X1 | 1 | 2964.5000000 | 2964.5000000 | 7.4798150 | 0.0410390 |
| 2 | | X2 | 1 | 33024.5000000 | 33024.5000000 | 83.3250631 | 0.0002643 |
| 3 | | X3 | 1 | 178802.0 | 178802.0 | 451.1404542 | 0.0000043 |
| 4 | | X1*X1 | 1 | 5712.4102564 | 5712.4102564 | 14.4131461 | 0.0126742 |
| 5 | | X2*X2 | 1 | 9729.6410256 | 9729.6410256 | 24.5491363 | 0.0042676 |
| 6 | | X3*X3 | 1 | 8448.1025641 | 8448.1025641 | 21.3156499 | 0.0057516 |
| 7 | | X1*X2 | 1 | 36.0000000 | 36.0000000 | 0.0908326 | 0.7752529 |
| 8 | | X2*X3 | 1 | 10201.0000000 | 10201.0000000 | 25.7384357 | 0.0038562 |
| 9 | | X1*X3 | 1 | 1089.0000000 | 1089.0000000 | 2.7476871 | 0.1582943 |
| 10 | | Error | 5 | 1981.6666667 | 396.3333333 | | |
| 11 | | Total | 14 | 248864.4000000 | | | |

# References

(1) Patel, N.; Jain, S.; Madan, P.; Lin, S. Application of Design of Experiments for Formulation Development and Mechanistic Evaluation of Iontophoretic Tacrine Hydrochloride Delivery. *Drug Development and Industrial Pharmacy* **2016**, *42* (11), 1894–1902. https://doi.org/10.1080/03639045.2016.1181646.