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.

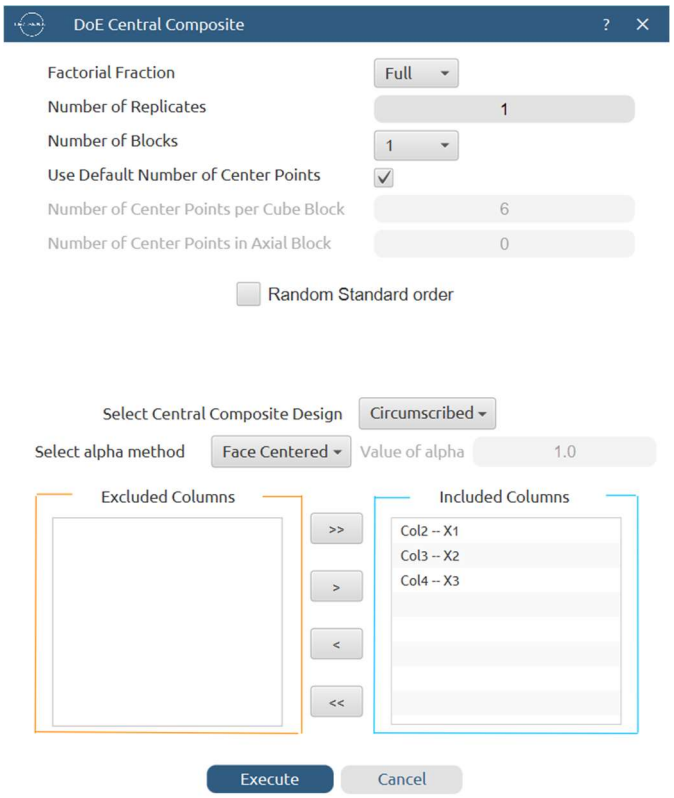
Isalos version used: 2.1.2

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



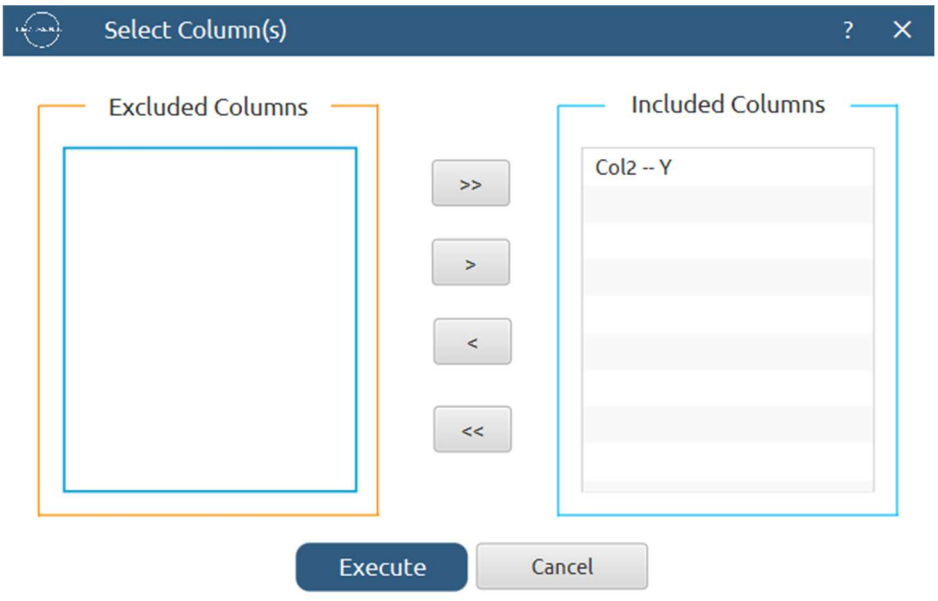
Results (right spreadsheet):

	Col1	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3
1		1	Block: 1	Replicate: 1	Cube Point	0.1	25.0	1.0
2		2	Block: 1	Replicate: 1	Cube Point	0.3	25.0	1.0
3		3	Block: 1	Replicate: 1	Cube Point	0.1	100.0	1.0
4		4	Block: 1	Replicate: 1	Cube Point	0.3	100.0	1.0
5		5	Block: 1	Replicate: 1	Cube Point	0.1	25.0	20.0
6		6	Block: 1	Replicate: 1	Cube Point	0.3	25.0	20.0
7		7	Block: 1	Replicate: 1	Cube Point	0.1	100.0	20.0
8		8	Block: 1	Replicate: 1	Cube Point	0.3	100.0	20.0
9		9	Block: 1	----	Center Point	0.2	62.5	10.5
10		10	Block: 1	----	Center Point	0.2	62.5	10.5
11		11	Block: 1	----	Center Point	0.2	62.5	10.5
12		12	Block: 1	----	Center Point	0.2	62.5	10.5
13		13	Block: 1	----	Center Point	0.2	62.5	10.5
14		14	Block: 1	----	Center Point	0.2	62.5	10.5
15		15	Block: 1	Replicate: 1	Axial Point	0.1	62.5	10.5
16		16	Block: 1	Replicate: 1	Axial Point	0.3	62.5	10.5
17		17	Block: 1	Replicate: 1	Axial Point	0.2	25.0	10.5
18		18	Block: 1	Replicate: 1	Axial Point	0.2	100.0	10.5
19		19	Block: 1	Replicate: 1	Axial Point	0.2	62.5	1.0
20		20	Block: 1	Replicate: 1	Axial Point	0.2	62.5	20.0

Step 2: Definition of response variables

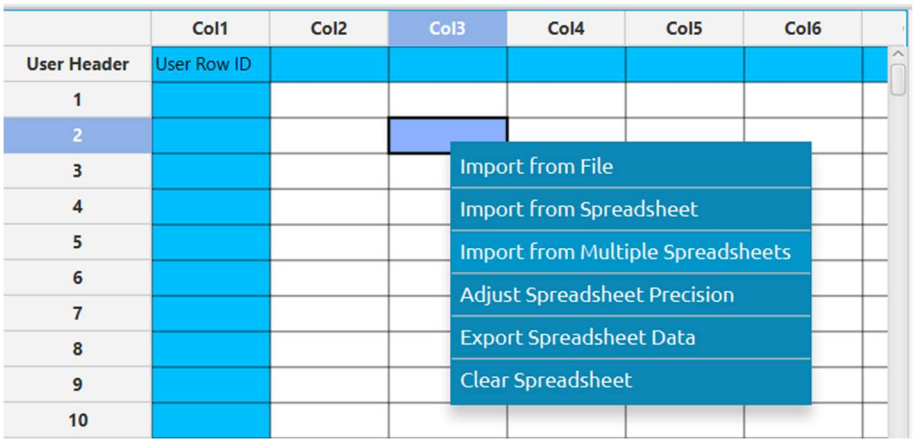
Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the Central Composite method. Then, select all columns to be transferred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

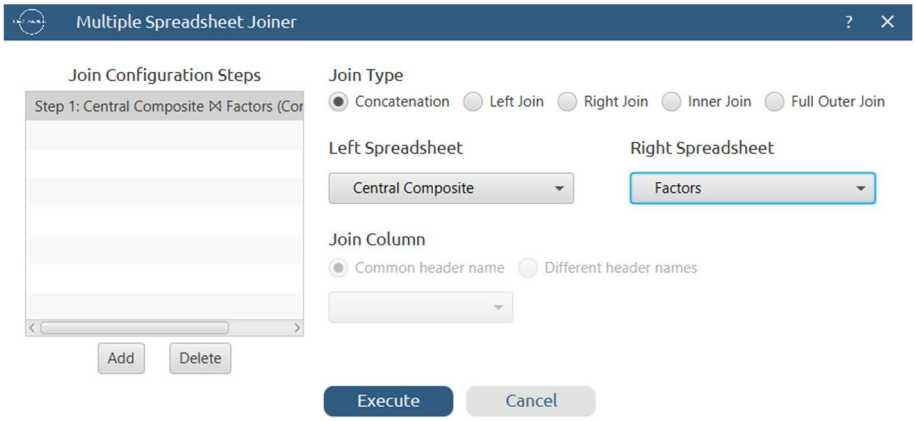
	Col1	Col2 (D)
User Header	User Row ID	Y
1		92.22
2		219.57
3		47.84
4		129.77
5		229.89
6		477.09
7		180.45
8		405.2
9		267.38
10		267.38
11		278.66
12		270.89
13		270.89
14		278.66
15		156.68
16		364.67
17		312.96
18		190.79
19		75.77
20		315.14

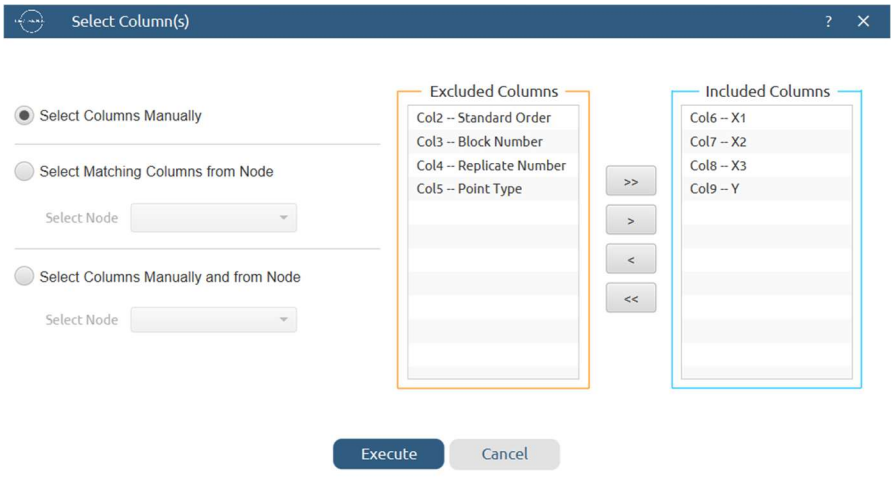


Step 3: Data selection

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: *Data Transformation* → *Data Manipulation* → *Select Column(s)*





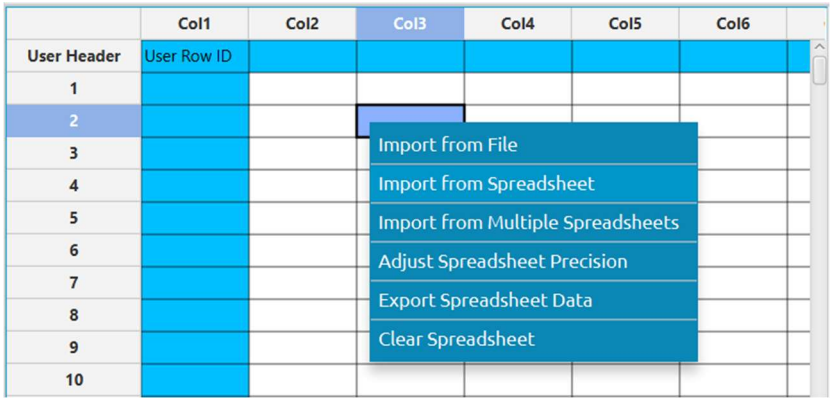


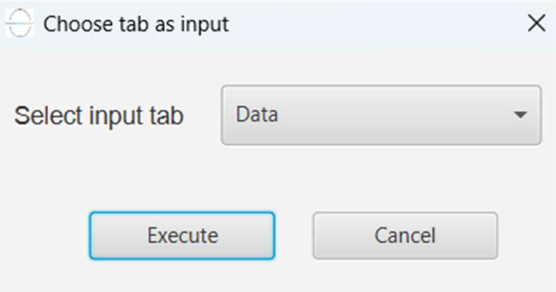
Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
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		0.2	62.5	10.5	267.38
10		0.2	62.5	10.5	267.38
11		0.2	62.5	10.5	278.66
12		0.2	62.5	10.5	270.89
13		0.2	62.5	10.5	270.89
14		0.2	62.5	10.5	278.66
15		0.1	62.5	10.5	156.68
16		0.3	62.5	10.5	364.67
17		0.2	25.0	10.5	312.96
18		0.2	100.0	10.5	190.79
19		0.2	62.5	1.0	75.77
20		0.2	62.5	20.0	315.14

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:

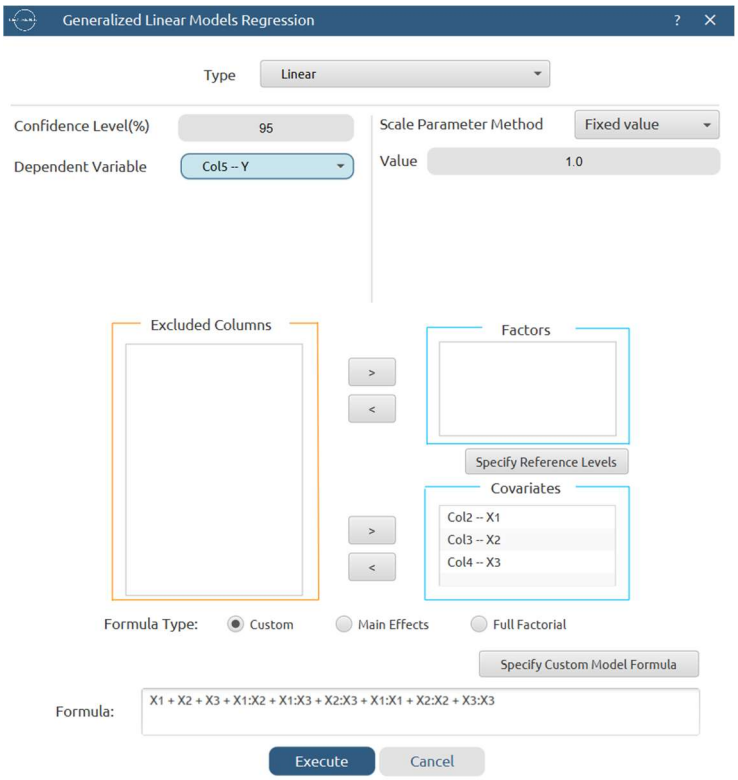
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	X1	X2	X3	Y
1		-1.0	-1.0	-1.0	92.22
2		1.0	-1.0	-1.0	219.57
3		-1.0	1.0	-1.0	47.84
4		1.0	1.0	-1.0	129.77
5		-1.0	-1.0	1.0	229.89
6		1.0	-1.0	1.0	477.09
7		-1.0	1.0	1.0	180.45
8		1.0	1.0	1.0	405.2
9		0E-7	0.0	0.0	267.38
10		0E-7	0.0	0.0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

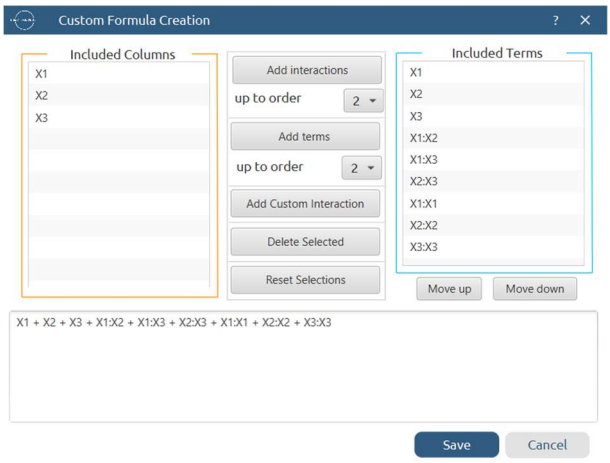
Step 5: Regression

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*


Generalized Linear Models Regression
Type
Linear
Confidence Level(%)
95
Dependent Variable
Col5 -- Y
Scale Parameter Method
Fixed value
Value
1.0
Excluded Columns
Factors
Specify Reference Levels
Covariates
Col2 -- X1
Col3 -- X2
Col4 -- X3
Formula Type:
Custom
Main Effects
Full Factorial
Specify Custom Model Formula
Formula:
X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X1 + X2:X2 + X3:X3
Execute
Cancel


Custom Formula Creation
Included Columns
X1
X2
X3
Add interactions
up to order
2
Add terms
up to order
2
Add Custom Interaction
Delete Selected
Reset Selections
Included Terms
X1
X2
X3
X1:X2
X1:X3
X2:X3
X1:X1
X2:X2
X3:X3
Move up
Move down
X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X1 + X2:X2 + X3:X3
Save
Cancel

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
267.38	263.6745455
267.38	263.6745455
278.66	263.6745455
270.89	263.6745455
270.89	263.6745455
278.66	263.6745455
156.68	184.7061818
364.67	362.5501818
312.96	302.5961818
190.79	227.0601818
75.77	104.1481818
315.14	312.6681818

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	104.26	0.3162278	103.6402050	104.8797950	108701.476000	1	0.0
X1*X2	-8.4837500	0.3535534	-9.1767019	-7.7907981	575.7921125	1	0.0
X1*X3	32.83375	0.3535534	32.1407981	33.5267019	8624.4411125	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
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
267.38	263.6745455
267.38	263.6745455
278.66	263.6745455
270.89	263.6745455
270.89	263.6745455
278.66	263.6745455
156.68	184.7061818
364.67	362.5501818
312.96	302.5961818
190.79	227.0601818
75.77	104.1481818
315.14	312.6681818

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

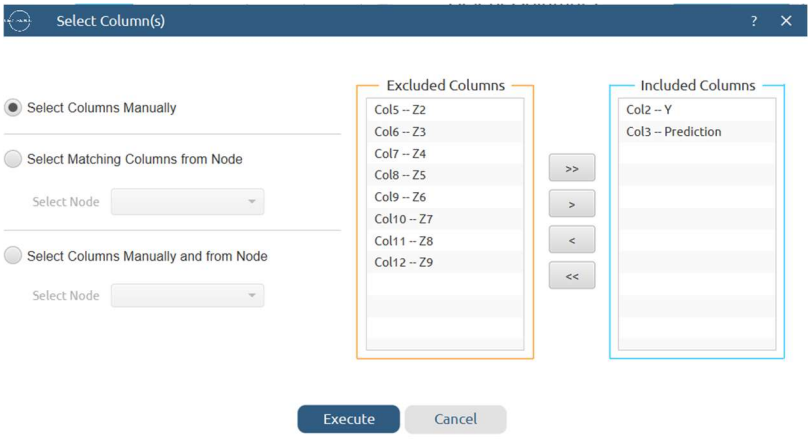
Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	56.3073923	2.5481407	51.3131283	61.3016563	488.2969565	1	0.0
X1	269.5710367	25.3339789	219.9173504	319.2247229	113.2243011	1	0.0
X2	-0.7045676	0.0583818	-0.8189937	-0.5901414	145.6434274	1	0.0
X3	16.6403335	0.1737354	16.2998184	16.9808486	9173.7621902	1	0.0
X1*X2	-2.2623333	0.0942809	-2.4471205	-2.0775462	575.7921125	1	0.0
X1*X3	34.5618421	0.3721615	33.8324190	35.2912652	8624.4411125	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: Data selection for Regression Metrics

Create a tab named “Metrics Data – Coded” and import the results from the spreadsheet “Regression – Coded”. Next, assign names to all unnamed columns containing data, using names such as Z1–Z9. Then, select only the response column (Y) and the “Prediction” column to be transferred to the right spreadsheet: *Data Transformation* → *Data Manipulation* → *Select Column(s)*

	Col1	Col2 (D)	Col3 (D)	Col4	Col5 (S)	Col6 (S)	Col7 (S)	Col8 (S)	Col9 (S)	Col10 (S)	Col11 (S)	Col12 (S)
User Header	User Row ID	Y	Prediction	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9
1		92.22	90.0577045		Goodness of Fit							
2		219.57	219.2017045			Value						
3		47.84	28.2767045		Deviance	4259.2897461						
4		129.77	123.4857045		Scaled Deviance	4259.2897461						
5		229.89	229.6977045		Pearson Chi-Square	4259.2897461						
6		477.09	490.1767045		Scaled Pearson Chi-Square	4259.2897461						
7		180.45	174.3417045		Log Likelihood	-2148.0236437						
8		405.2	400.8857045		Akaike's Information Criterion (AIC)	4316.0472875						
9		267.38	263.6745455		Finite Sample Corrected AIC (AICC)	4340.4917319						
10		267.38	263.6745455		Bayesian Information Criterion (BIC)	4326.0046102						
11		278.66	263.6745455		Consistent AIC (CAIC)	4336.0046102						
12		270.89	263.6745455									
13		270.89	263.6745455									
14		278.66	263.6745455		Parameter Estimates							
15		156.68	184.7061818		Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
16		364.67	362.5501818		intercept	263.6745455	0.3437758	263.0007572	264.3483337	588282.2500979	1	0.0

Results:

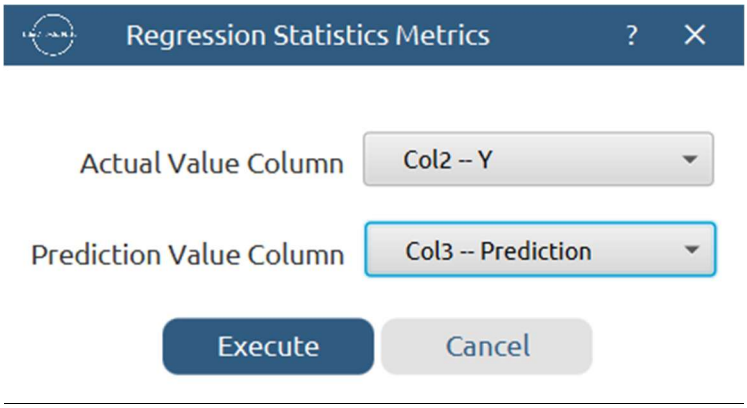


	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y	Prediction
1		92.22	90.0577045
2		219.57	219.2017045
3		47.84	28.2767045
4		129.77	123.4857045
5		229.89	229.6977045
6		477.09	490.1767045
7		180.45	174.3417045
8		405.2	400.8857045
9		267.38	263.6745455
10		267.38	263.6745455
11		278.66	263.6745455
12		270.89	263.6745455
13		270.89	263.6745455
14		278.66	263.6745455
15		156.68	184.7061818
16		364.67	362.5501818
17		312.96	302.5961818
18		190.79	227.0601818
19		75.77	104.1481818
20		315.14	312.6681818

Repeat this process for the uncoded data as well.

Step 7: Regression Metrics

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



Results:

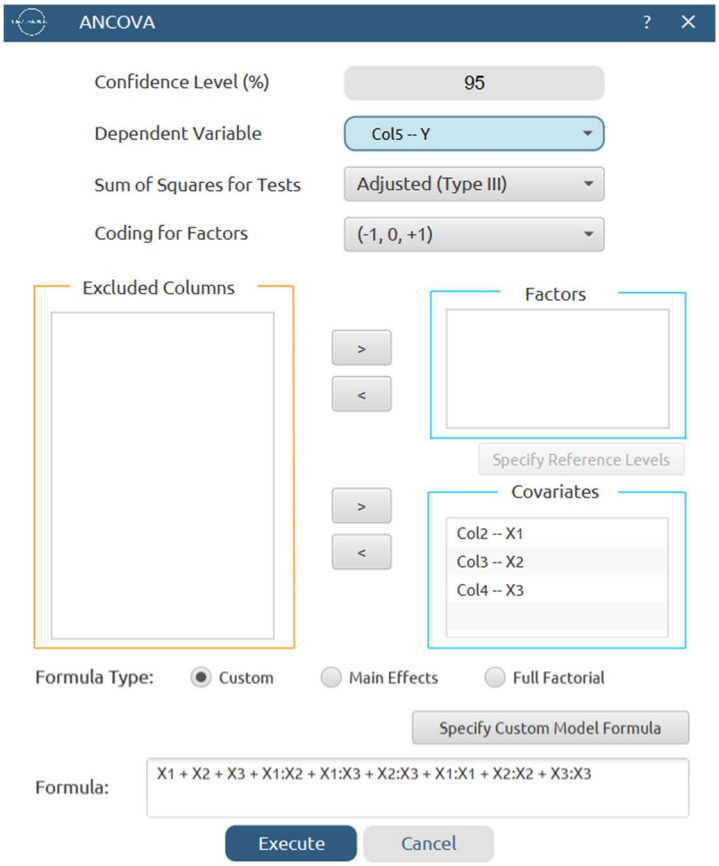
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		212.9644873	14.5933028	10.5761250	0.9812924

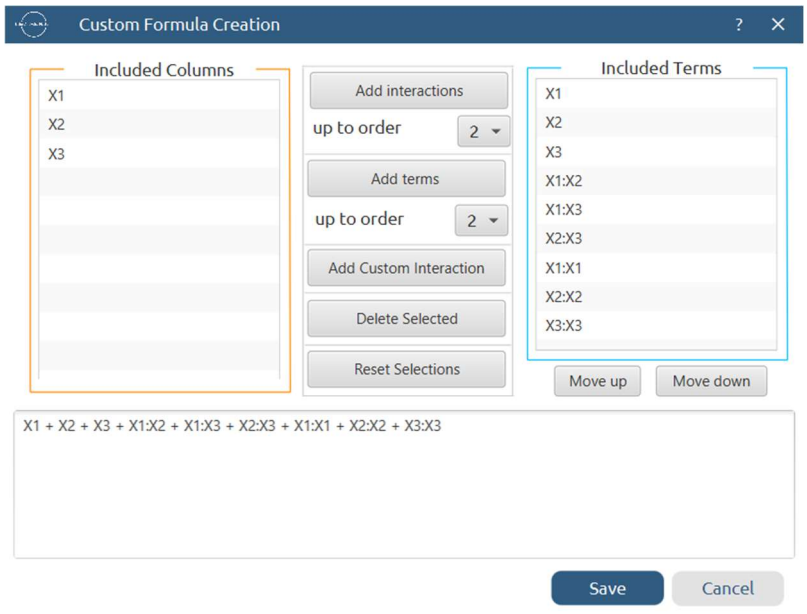
Repeat this step for the uncoded data. Results:

	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 8: 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)	Col8 (S)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value	Significant
1		X1	1	79071.22084	79071.22084	185.6441462	1E-7	Yes
2		X2	1	14264.2182400	14264.2182400	33.4896640	0.0001760	Yes
3		X3	1	108701.476000	108701.476000	255.2103343	0E-7	Yes
4		X1*X2	1	575.7921125	575.7921125	1.3518501	0.2719503	No
5		X1*X3	1	8624.4411125	8624.4411125	20.2485429	0.0011434	Yes
6		X2*X3	1	20.6403125	20.6403125	0.0484595	0.8301948	No
7		X1*X1	1	272.4559114	272.4559114	0.6396745	0.4424130	No
8		X2*X2	1	3.6599114	3.6599114	0.0085928	0.9279749	No
9		X3*X3	1	8399.5201114	8399.5201114	19.7204713	0.0012530	Yes
10		Error	10	4259.2897461	425.9289746			
11		Total	19	227676.707700				

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.