Development and characterization of miltefosine-loaded polymeric micelles for cancer treatment

This research paper aims to develop a formulation of miltefosine-loaded polymeric micelles of the copolymer Pluronic-F127 to decrease the hemolytic potential of miltefosine. Design of experiments (DoE) methodology is implemented in order to evaluate the effect of the independent variables on the responses.

The factors (independent variables) examined are: X_1 = hydration temperature (°C), X_2 = stirring speed (rpm) and X_3 = stirring time (min). All the factors are continuous. The response (dependent variable) examined is: Y_1 = polydispersity index. The applied DoE method is 2^3 full factorial design.

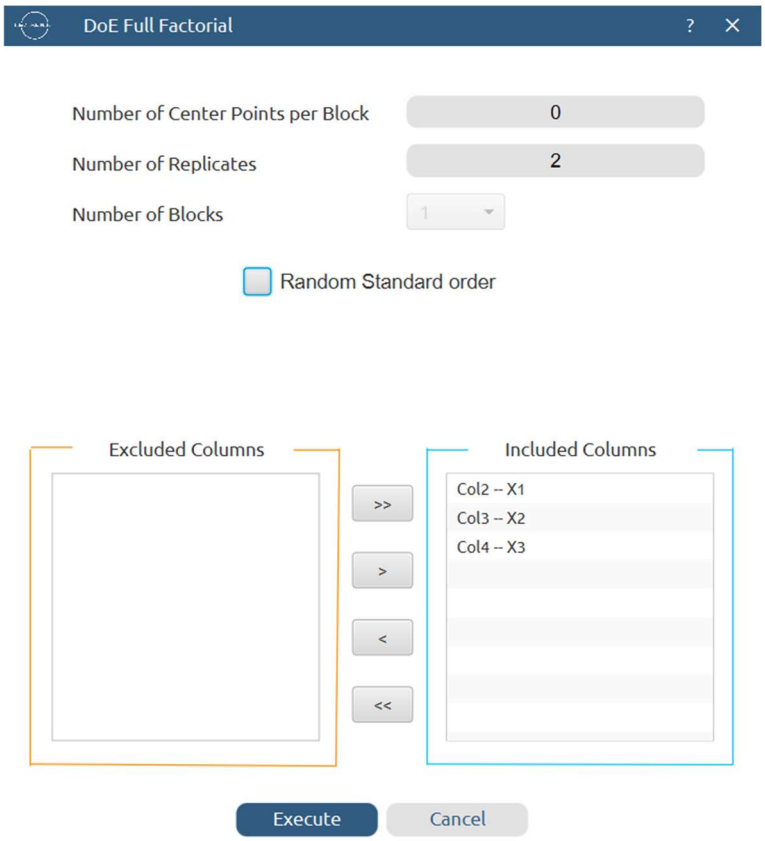
Isalos version used: 2.1.2

Scientific article: https://www.sciencedirect.com/science/article/pii/S0928493117320921

Step 1: Full Factorial 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 “Full Factorial”. Afterwards, apply the full factorial method: *DOE* → *Factorial* → *Full Factorial*

	Col1	Col2 (I)	Col3 (I)	Col4 (I)
User Header	User Row ID	X1	X2	X3
1		30	550	30
2		50	750	60



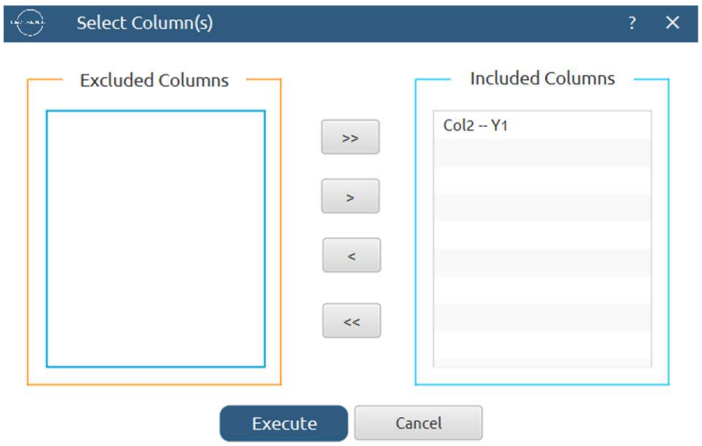
Results (right spreadsheet):

	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	Design Point	30.0	550.0	30.0
2		2	Block: 1	Replicate: 1	Design Point	50.0	550.0	30.0
3		3	Block: 1	Replicate: 1	Design Point	30.0	750.0	30.0
4		4	Block: 1	Replicate: 1	Design Point	50.0	750.0	30.0
5		5	Block: 1	Replicate: 1	Design Point	30.0	550.0	60.0
6		6	Block: 1	Replicate: 1	Design Point	50.0	550.0	60.0
7		7	Block: 1	Replicate: 1	Design Point	30.0	750.0	60.0
8		8	Block: 1	Replicate: 1	Design Point	50.0	750.0	60.0
9		9	Block: 1	Replicate: 2	Design Point	30.0	550.0	30.0
10		10	Block: 1	Replicate: 2	Design Point	50.0	550.0	30.0
11		11	Block: 1	Replicate: 2	Design Point	30.0	750.0	30.0
12		12	Block: 1	Replicate: 2	Design Point	50.0	750.0	30.0
13		13	Block: 1	Replicate: 2	Design Point	30.0	550.0	60.0
14		14	Block: 1	Replicate: 2	Design Point	50.0	550.0	60.0
15		15	Block: 1	Replicate: 2	Design Point	30.0	750.0	60.0
16		16	Block: 1	Replicate: 2	Design Point	50.0	750.0	60.0

Step 2: Definition of response variables

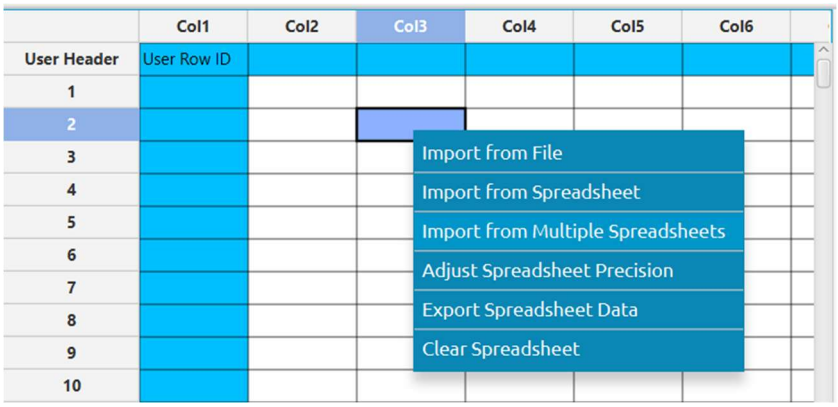
Create a new tab named “Responses” and define the response in the column headers. Fill a column with the values of the response variable that were observed and make sure the values follow the order of the experiments as given by the full factorial method. Then, select the column to be transferred to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

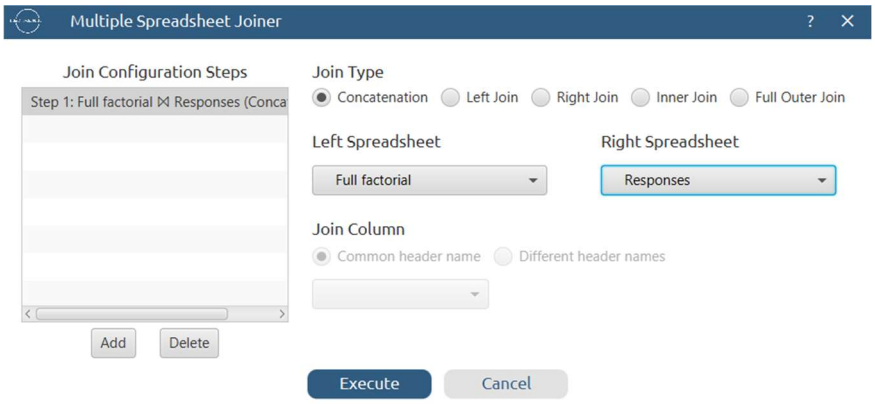
	Col1	Col2 (D)
User Header	User Row ID	Y1
1		8.8
2		4.4
3		5.2
4		5
5		4.5
6		4
7		7.2
8		6.6
9		9
10		4.5
11		4.9
12		5.2
13		4.2
14		4.1
15		6.4
16		6.3

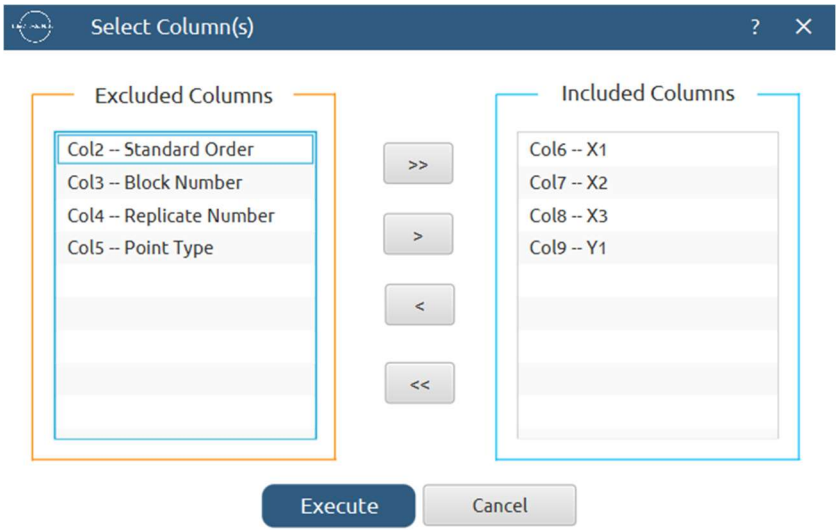


Step 3: Data selection

Create a new tab named “Data” and import the results from the “Full Factorial” and “Responses” spreadsheets by right clicking on the left spreadsheet. Then, select only the factors and response columns to be transferred to the right spreadsheet: *Data Transformation* → *Data Manipulation* → *Select Column(s)*





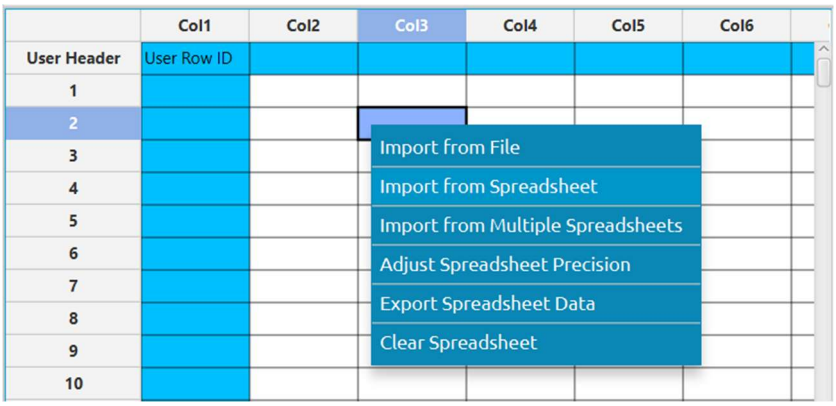


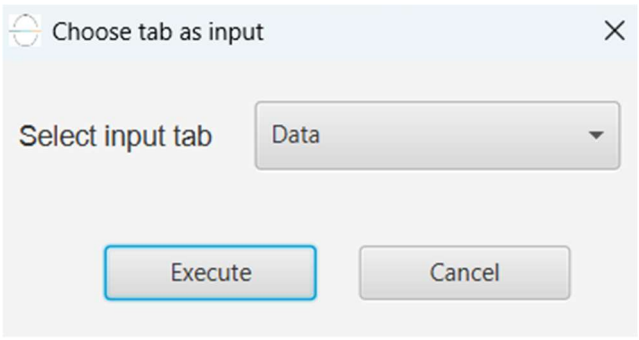
Results:

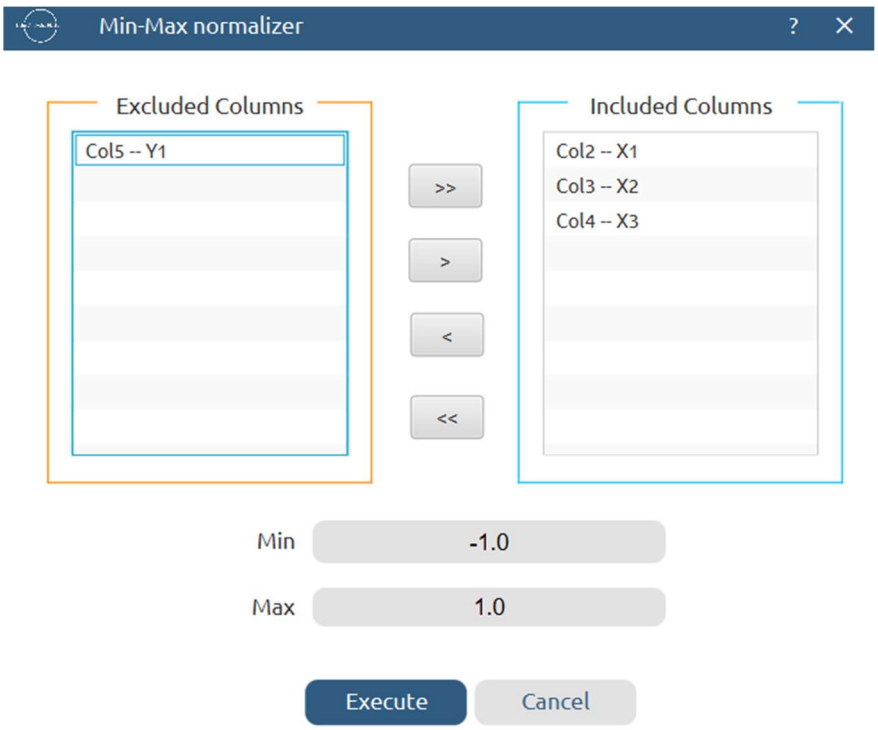
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	X1	X2	X3	Y1
1		30.0	550.0	30.0	8.8
2		50.0	550.0	30.0	4.4
3		30.0	750.0	30.0	5.2
4		50.0	750.0	30.0	5
5		30.0	550.0	60.0	4.5
6		50.0	550.0	60.0	4
7		30.0	750.0	60.0	7.2
8		50.0	750.0	60.0	6.6
9		30.0	550.0	30.0	9
10		50.0	550.0	30.0	4.5
11		30.0	750.0	30.0	4.9
12		50.0	750.0	30.0	5.2
13		30.0	550.0	60.0	4.2
14		50.0	550.0	60.0	4.1
15		30.0	750.0	60.0	6.4
16		50.0	750.0	60.0	6.3

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:

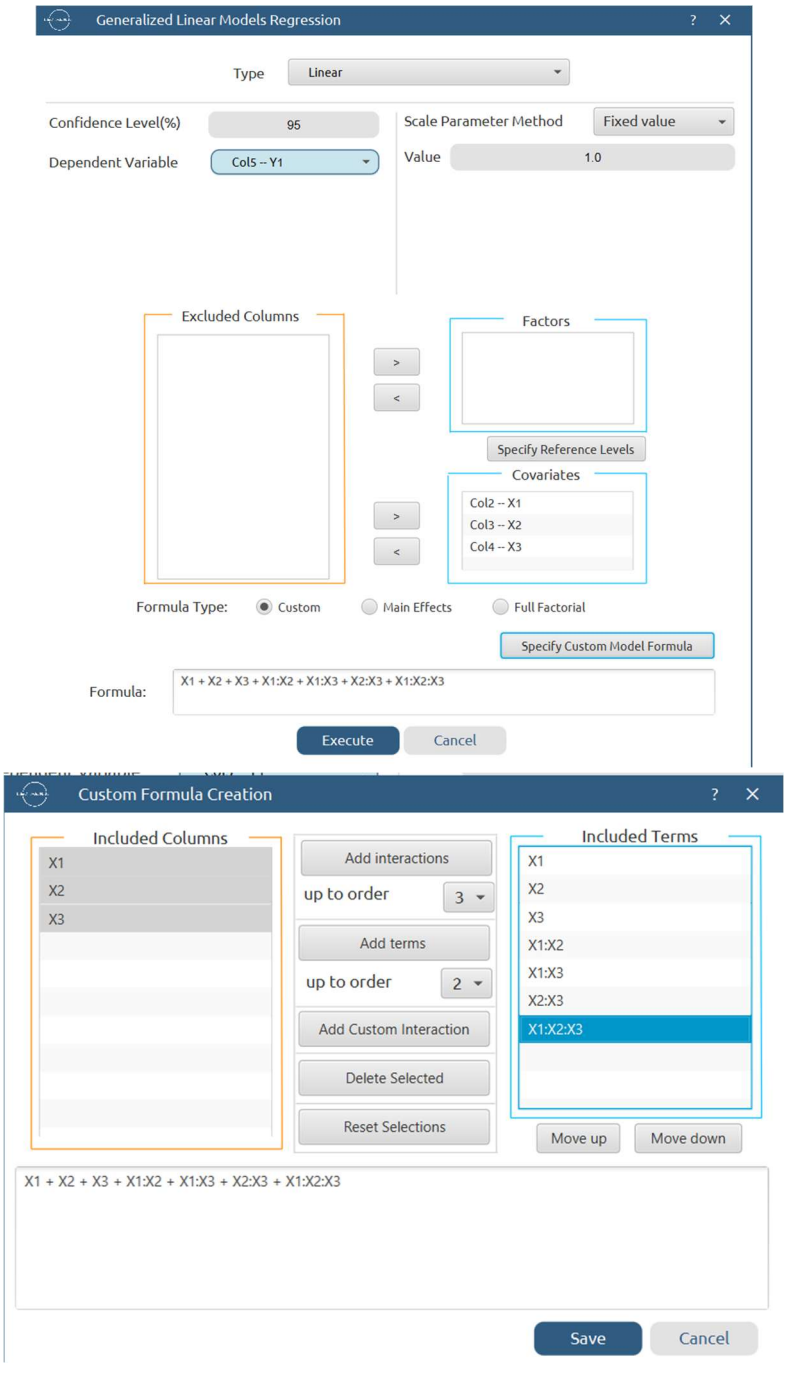
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	X1	X2	X3	Y1
1		-1.0	-1.0	-1.0	8.8
2		1.0	-1.0	-1.0	4.4
3		-1.0	1.0	-1.0	5.2
4		1.0	1.0	-1.0	5.0
5		-1.0	-1.0	1.0	4.5
6		1.0	-1.0	1.0	4.0
7		-1.0	1.0	1.0	7.2
8		1.0	1.0	1.0	6.6
9		-1.0	-1.0	-1.0	9.0
10		1.0	-1.0	-1.0	4.5
11		-1.0	1.0	-1.0	4.9
12		1.0	1.0	-1.0	5.2
13		-1.0	-1.0	1.0	4.2
14		1.0	-1.0	1.0	4.1
15		-1.0	1.0	1.0	6.4
16		1.0	1.0	1.0	6.3

Step 5: Regression

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

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

Create a new tab named “Regression – Y1” 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:

Y1	Prediction
8.8	8.9000000
4.4	4.4500000
5.2	5.0500000
5	5.1000000
4.5	4.3500000
4	4.0500000
7.2	6.8000000
6.6	6.4500000
9	8.9000000
4.5	4.4500000
4.9	5.0500000
5.2	5.1000000
4.2	4.3500000
4.1	4.0500000
6.4	6.8000000
6.3	6.4500000

Goodness of Fit	
	Value
Deviance	0.5050000
Scaled Deviance	0.5050000
Pearson Chi-Square	0.5050000
Scaled Pearson Chi-Square	0.5050000
Log Likelihood	-14.9555165
Akaike's Information Criterion (AIC)	45.9110331
Finite Sample Corrected AIC (AICC)	66.4824616
Bayesian Information Criterion (BIC)	52.0917428
Consistent AIC (CAIC)	60.0917428

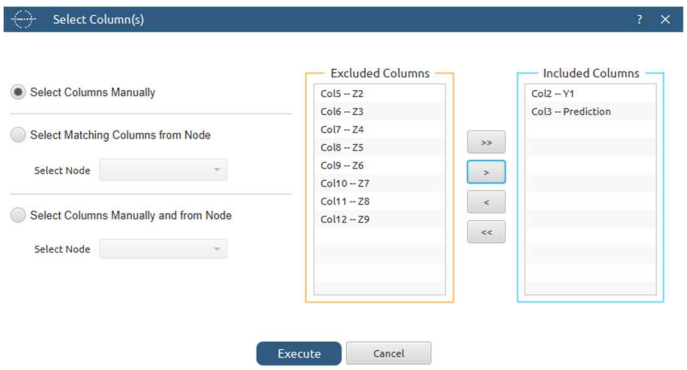
Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	5.64375	0.25	5.1537590	6.1337410	509.6306250	1	0.0
X1	-0.6312500	0.25	-1.1212410	-0.1412590	6.3756250	1	0.0115698
X2	0.2062500	0.25	-0.2837410	0.6962410	0.6806250	1	0.4093716
X3	-0.2312500	0.25	-0.7212410	0.2587410	0.8556250	1	0.3549659
X1*X2	0.55625	0.25	0.0662590	1.0462410	4.9506250	1	0.0260812
X1*X3	0.46875	0.25	-0.0212410	0.9587410	3.515625	1	0.0607927
X2*X3	1.0062500	0.25	0.5162590	1.4962410	16.2006250	1	0.0000570
X1*X2*X3	-0.5687500	0.25	-1.0587410	-0.0787590	5.1756250	1	0.0229059

Step 6: Data selection for Regression Metrics

Create a tab named “Metrics Data – Y1” and import the results from the spreadsheet “Regression – Y1”. Next, assign names to all unnamed columns containing data, using names such as Z1–Z9. Then, select only the response column (Y1) 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	Y1	Prediction	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9
1		8.8	8.9		Goodness of Fit							
2		4.4	4.4500000			Value						
3		5.2	5.05		Deviance	0.5050000						
4		5	5.1000000		Scaled Deviance	0.5050000						
5		4.5	4.3500000		Pearson Chi-Square	0.5050000						
6		4	4.05		Scaled Pearson Chi-Square	0.5050000						
7		7.2	6.8		Log Likelihood	-14.9555165						
8		6.6	6.4500000		Akaike's Information Criterion (AIC)	45.9110331						
9		9	8.9		Finite Sample Corrected AIC (AICC)	66.4824616						
10		4.5	4.4500000		Bayesian Information Criterion (BIC)	52.0917428						
11		4.9	5.05		Consistent AIC (CAIC)	60.0917428						
12		5.2	5.1000000									
13		4.2	4.3500000									
14		4.1	4.05		Parameter Estimates							
15		6.4	6.8		Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
16		6.3	6.4500000		Intercept	5.64375	0.25	5.1537590	6.1337410	509.6306250	1	0.0

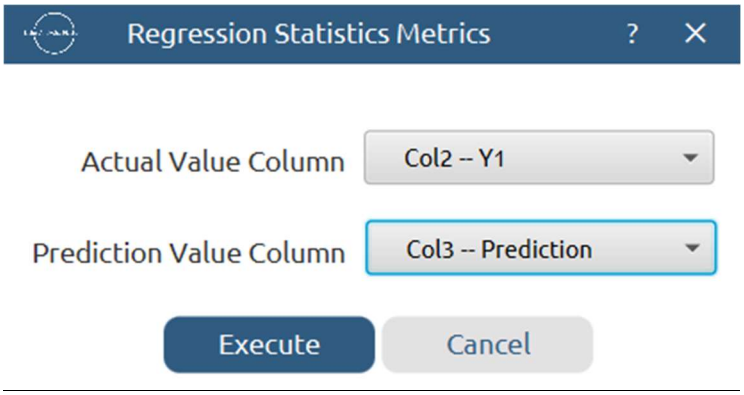
Results:



	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y1	Prediction
1		8.8	8.9
2		4.4	4.4500000
3		5.2	5.05
4		5	5.1000000
5		4.5	4.3500000
6		4	4.05
7		7.2	6.8
8		6.6	6.4500000
9		9	8.9
10		4.5	4.4500000
11		4.9	5.05
12		5.2	5.1000000
13		4.2	4.3500000
14		4.1	4.05
15		6.4	6.8
16		6.3	6.4500000

Step 7: Regression Metrics

Create a tab named “Metrics – Y1” and import the results from the spreadsheet “Metrics Data – Y1”. Then, produce the regression metrics for the Y_1 regression equation: *Statistics* → *Model Metrics* → *Regression Metrics*

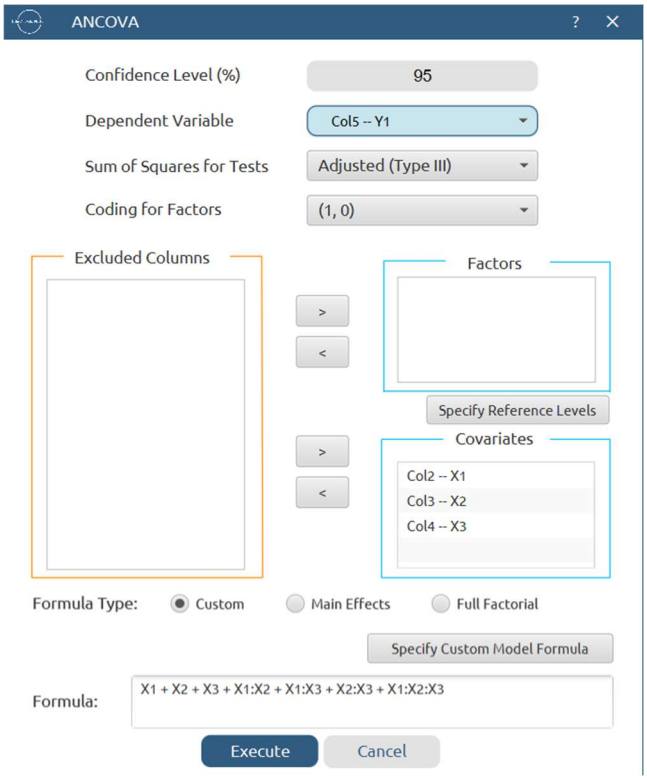


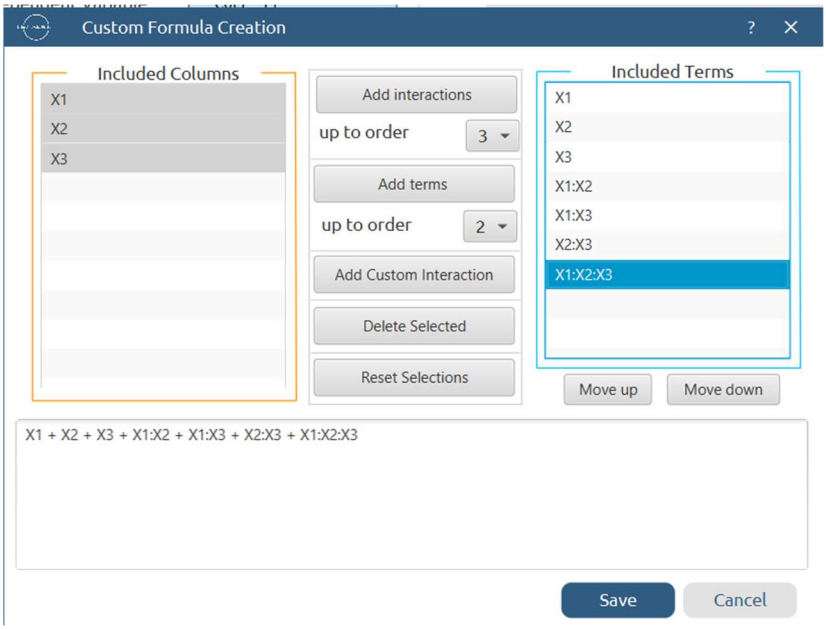
Results:

	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		0.0315625	0.1776584	0.1437500	0.9868006

Step 8: Analysis of Covariance

Create a new tab named “ANCOVA – Y1” and import the results from the spreadsheet “Normalized data”. Afterwards perform analysis of covariance for Y_1: *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	6.3756250	6.3756250	101.0000000	0.0000082
2		X2	1	0.6806250	0.6806250	10.7821782	0.0111275
3		X3	1	0.8556250	0.8556250	13.5544554	0.0062045
4		X1*X2	1	4.9506250	4.9506250	78.4257426	0.0000209
5		X2*X3	1	16.200625	16.200625	256.6435644	2E-7
6		X1*X3	1	3.515625	3.515625	55.6930693	0.0000718
7		X1*X2*X3	1	5.1756250	5.1756250	81.9900990	0.0000177
8		Error	8	0.5050000	0.0631250		
9		Total	15	38.2593750			

Final Isalos Workflow

References

(1) Valenzuela-Oses, J. K.; García, M. C.; Feitosa, V. A.; Pachioni-Vasconcelos, J. A.; Gomes-Filho, S. M.; Lourenço, F. R.; Cerize, N. N. P.; Bassères, D. S.; Rangel-Yagui, C. O. Development and Characterization of Miltefosine-Loaded Polymeric Micelles for Cancer Treatment. *Materials Science and Engineering: C* **2017**, *81*, 327–333. https://doi.org/10.1016/j.msec.2017.07.040.