Development of nanostructured lipid carriers containing salicyclic acid for dermal use based on the Quality by Design method

This research paper aims to evaluate the applicability of the Quality by Design (QbD) methodology in the development and optimalization of nanostructured lipid carriers containing salicyclic acid (NLC SA). Design of experiments (DoE) methodology is implemented in order to evaluate the role of the independent and dependent variables.

The factors (independent variables) examined are: X_1 = surfactant concentration (w/w %), X_2 = solid/liquid lipid ratio and X_3 = ultrasonication time (min). All the factors are continuous. The responses (dependent variables) examined are: Y_1 = particle size (μm) and Y_2 = particle size distribution. The applied DoE method is 2^3 full factorial design.

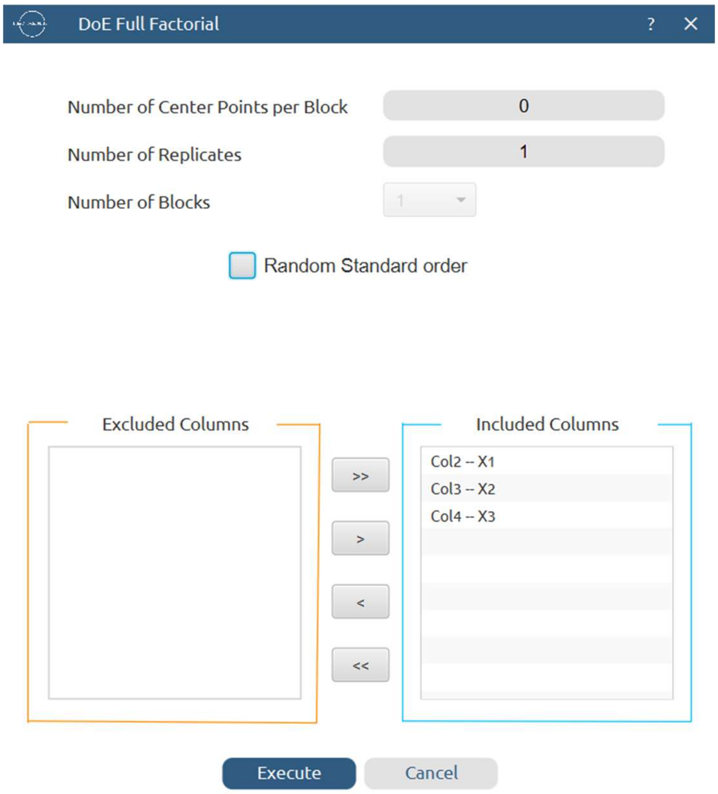
Isalos version used: 2.0.6

Scientific article: https://www.sciencedirect.com/science/article/abs/pii/S0928098716305607

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 (D)	Col4 (I)
User Header	User Row ID	X1	X2	X3
1		1	2.333	10
2		5	9	20

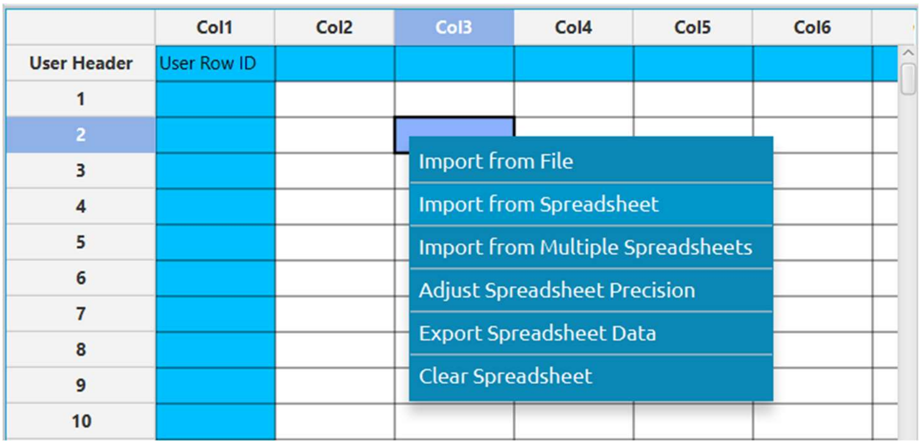


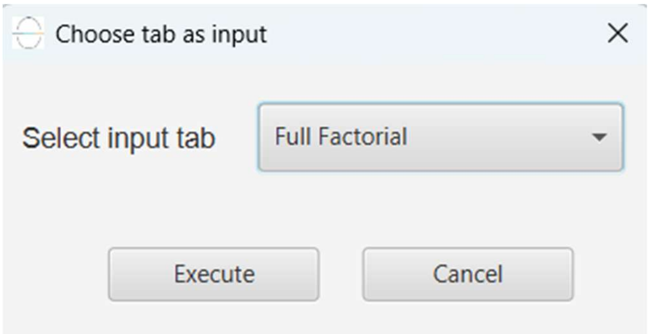
Results (right spreadsheet):

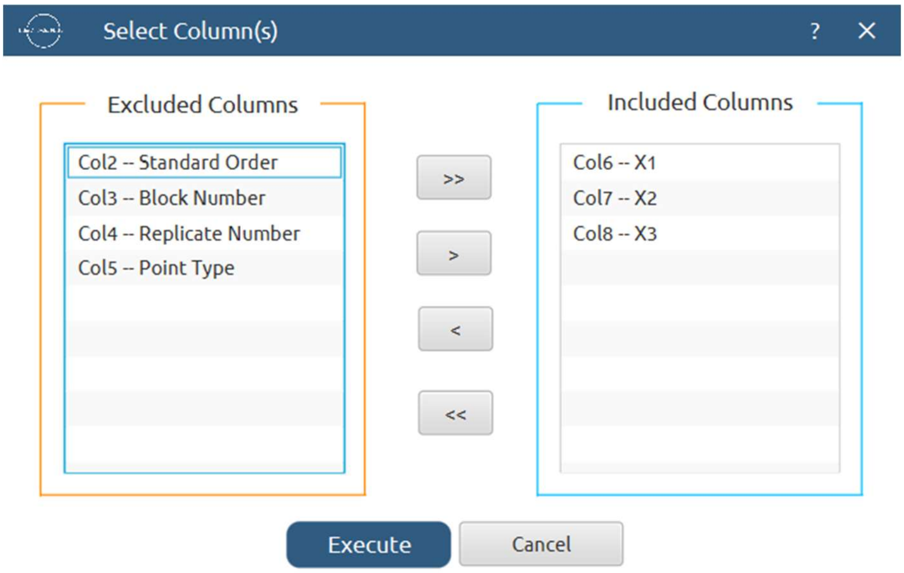
Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3
1	Block: 1	Replicate: 1	Design Point	1.0	2.333	10.0
2	Block: 1	Replicate: 1	Design Point	5.0	2.333	10.0
3	Block: 1	Replicate: 1	Design Point	1.0	9.0	10.0
4	Block: 1	Replicate: 1	Design Point	5.0	9.0	10.0
5	Block: 1	Replicate: 1	Design Point	1.0	2.333	20.0
6	Block: 1	Replicate: 1	Design Point	5.0	2.333	20.0
7	Block: 1	Replicate: 1	Design Point	1.0	9.0	20.0
8	Block: 1	Replicate: 1	Design Point	5.0	9.0	20.0

Step 2: Factor isolation

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







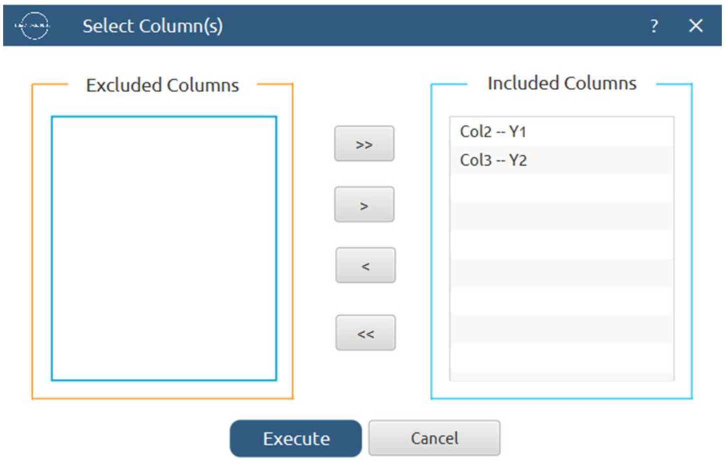
Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	X1	X2	X3
1		1.0	2.333	10.0
2		5.0	2.333	10.0
3		1.0	9.0	10.0
4		5.0	9.0	10.0
5		1.0	2.333	20.0
6		5.0	2.333	20.0
7		1.0	9.0	20.0
8		5.0	9.0	20.0

Step 3: Definition of response variables

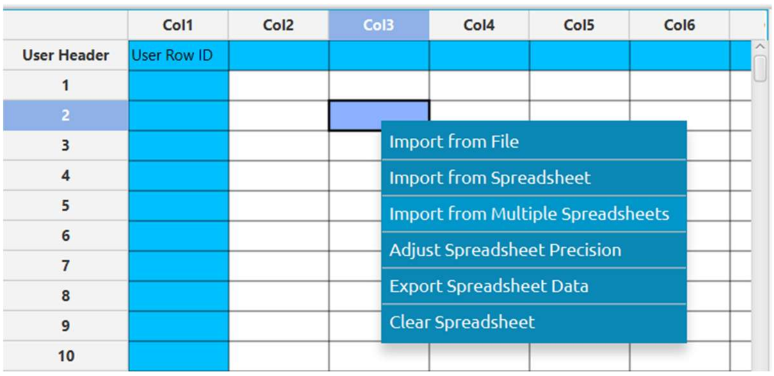
Create a new tab named "Responses" and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the full factorial design. Then, select all columns to be transferred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

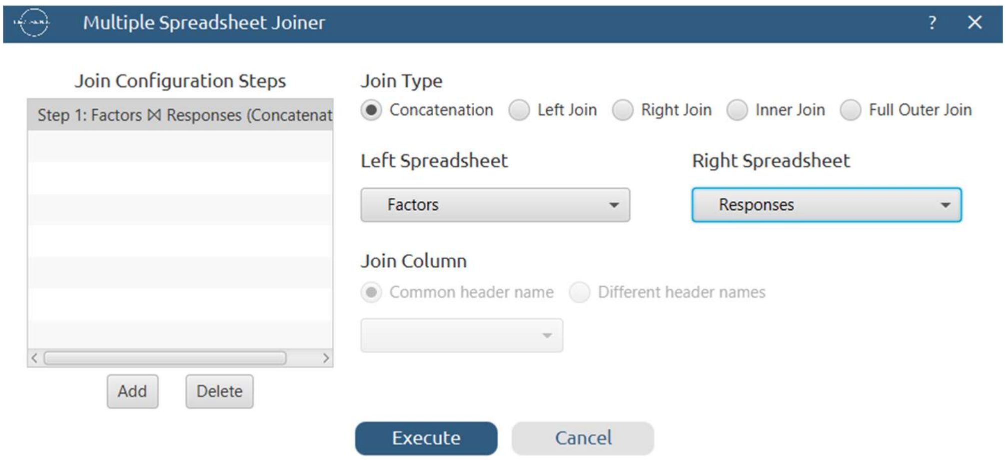
	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y1	Y2
1		7.669	5.279
2		0.118	1.034
3		12.735	3.023
4		0.121	1.074
5		14.954	5.646
6		0.116	0.874
7		21.574	3.032
8		0.121	1.025

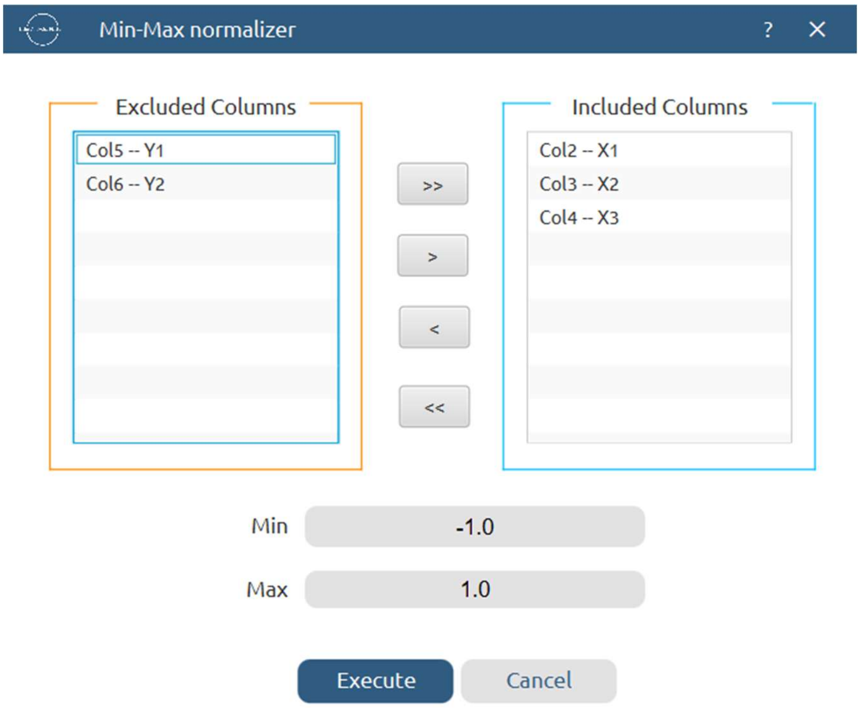


Step 4: Normalization

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





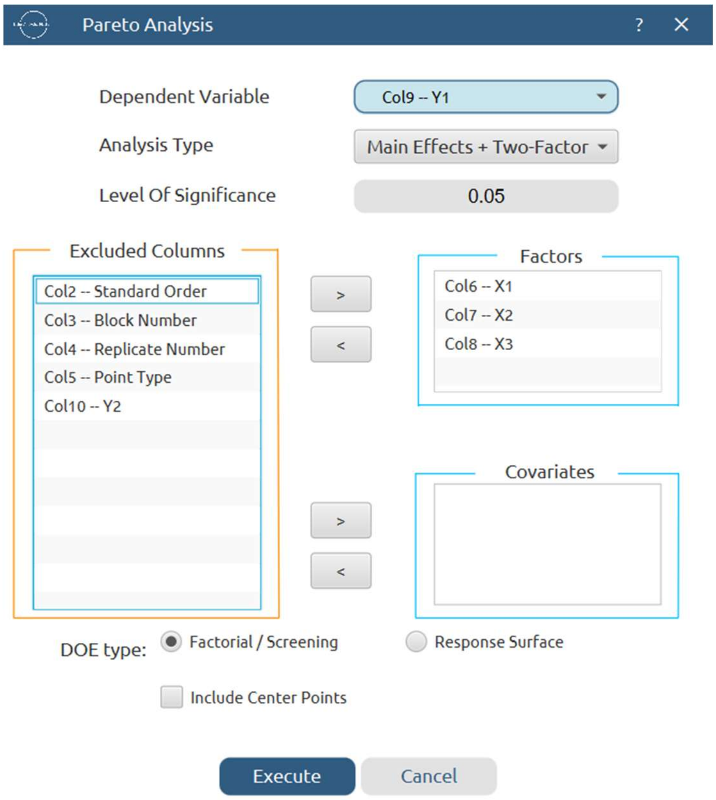


Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)
User Header	User Row ID	X1	X2	X3	Y1	Y2
1		-1.0	-1.0	-1.0	7.669	5.279
2		1.0	-1.0	-1.0	0.118	1.034
3		-1.0	1.0	-1.0	12.735	3.023
4		1.0	1.0	-1.0	0.121	1.074
5		-1.0	-1.0	1.0	14.954	5.646
6		1.0	-1.0	1.0	0.116	0.874
7		-1.0	1.0	1.0	21.574	3.032
8		1.0	1.0	1.0	0.121	1.025

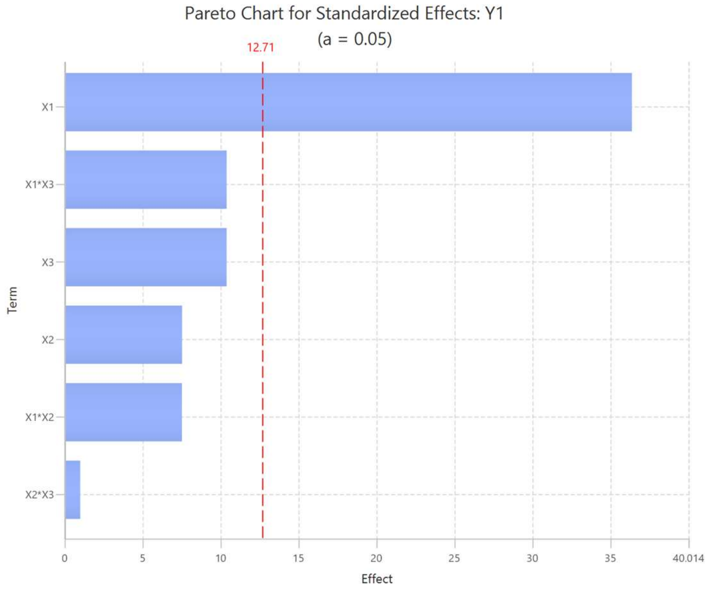
Step 5: Pareto analysis

Create a new tab named “Pareto Analysis – Y1” and import the results from the spreadsheet “Normalized data”. Then, conduct pareto analysis for the first response variable, Y_1: *DOE → Post DoE Analysis → Pareto Analysis*



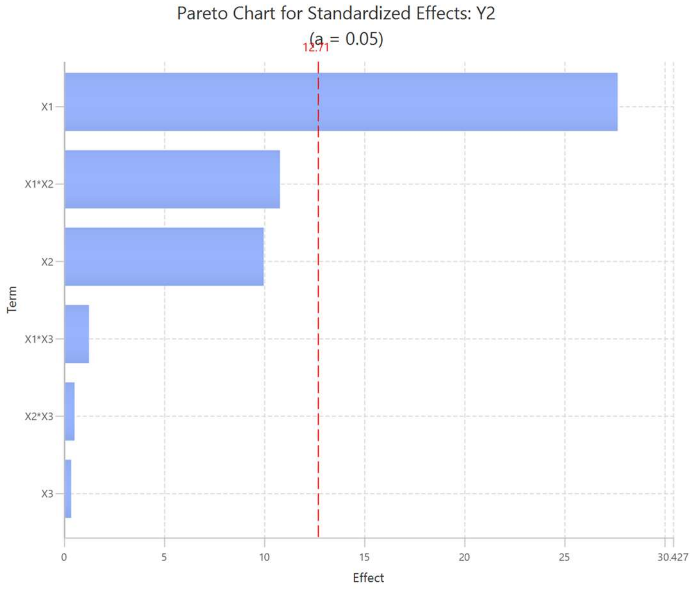
Results:

	Col1	Col2 (S)	Col3 (S)
User Header	User Row ID	Pareto Analysis of :	Standardized Effects
1		Variable	Effect
2		X1	36.3762887
3		X1*X3	10.3904639
4		X3	10.3878866
5		X2	7.5347938
6		X1*X2	7.5244845
7		X2*X3	1.0025773
8		Significance Value	12.7062047



Repeat this step for the second response variable, Y_2. Results:

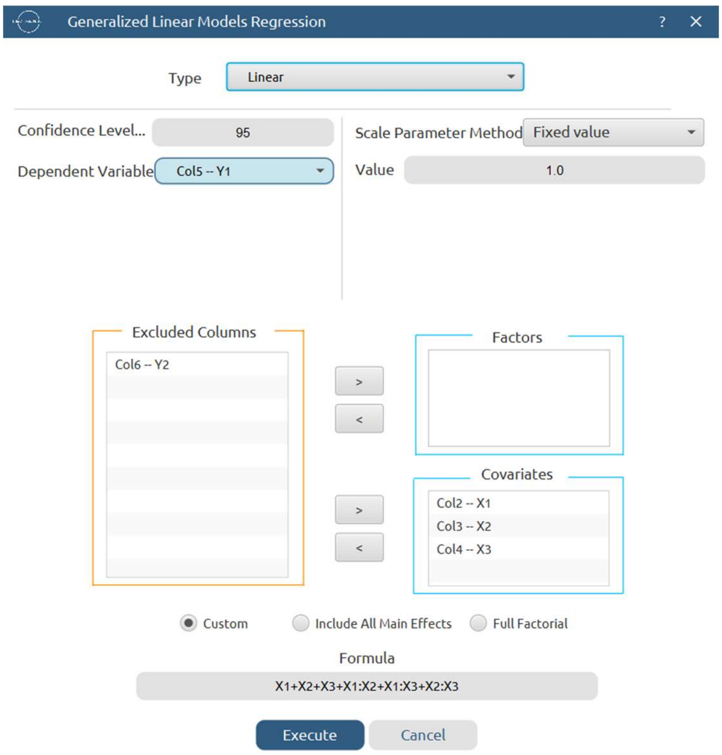
	Col1	Col2 (S)	Col3 (S)
User Header	User Row ID	Pareto Analysis of :	Standardized Effects
1		Variable	Effect
2		X1	27.6609808
3		X1*X2	10.7910448
4		X2	9.9765458
5		X1*X3	1.2473348
6		X2*X3	0.5266525
7		X3	0.3560768
8		Significance Value	12.7062047



Step 6: Regression

The goal here is to produce a regression equation that includes main effects and two-factor interactions for Y_1: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$

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
7.669	7.4750000
0.118	0.3120000
12.735	12.9290000
0.121	-0.0730000
14.954	15.1480000
0.116	-0.0780000
21.574	21.3800000
0.121	0.3150000

Goodness of Fit	
	Value
Deviance	0.3010880
Scaled Deviance	0.3010880
Pearson Chi-Square	0.3010880
Scaled Pearson Chi-Square	0.3010880
Log Likelihood	-7.5020523
Akaike's Information Criterion (AIC)	29.0041045
Finite Sample Corrected AIC (AICC)	Infinity
Bayesian Information Criterion (BIC)	29.5601953
Consistent AIC (CAIC)	36.5601953

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	7.1760000	0.3535534	6.4830481	7.8689519	411.959808	1	0.0
X1	-7.057	0.3535534	-7.7499519	-6.3640481	398.409992	1	0.0
X2	1.4617500	0.3535534	0.7687981	2.1547019	17.0937045	1	0.0000356
X3	2.0152500	0.3535534	1.3222981	2.7082019	32.4898605	1	0E-7
X1*X3	-2.0157500	0.3535534	-2.7087019	-1.3227981	32.5059845	1	0E-7
X1*X2	-1.4597500	0.3535534	-2.1527019	-0.7667981	17.0469605	1	0.0000365
X2*X3	0.1945000	0.3535534	-0.4984519	0.8874519	0.3026420	1	0.5822308

Repeat this step for the second response variable, Y_2. Results:

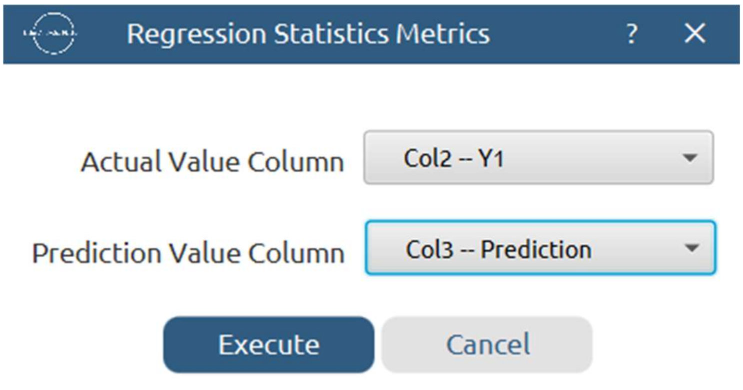
Y2	Prediction
5.279	5.3376250
1.034	0.9753750
3.023	2.964375
1.074	1.1326250
5.646	5.587375
0.874	0.9326250
3.032	3.0906250
1.025	0.9663750

Goodness of Fit	
	Value
Deviance	0.0274951
Scaled Deviance	0.0274951
Pearson Chi-Square	0.0274951
Scaled Pearson Chi-Square	0.0274951
Log Likelihood	-7.3652558
Akaike's Information Criterion (AIC)	28.7305117
Finite Sample Corrected AIC (AICC)	Infinity
Bayesian Information Criterion (BIC)	29.2866024
Consistent AIC (CAIC)	36.2866024

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	2.6233750	0.3535534	1.9304231	3.3163269	55.0567711	1	0E-7
X1	-1.6216250	0.3535534	-2.3145769	-0.9286731	21.0373411	1	0.0000045
X2	-0.5848750	0.3535534	-1.2778269	0.1080769	2.7366301	1	0.0980714
X3	0.0208750	0.3535534	-0.6720769	0.7138269	0.0034861	1	0.9529175
X1*X3	-0.0731250	0.3535534	-0.7660769	0.6198269	0.0427781	1	0.8361436
X1*X2	0.6326250	0.3535534	-0.0603269	1.3255769	3.2017151	1	0.0735611
X2*X3	-0.0308750	0.3535534	-0.7238269	0.6620769	0.0076261	1	0.9304110

Step 7: Regression Metrics

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



Results:

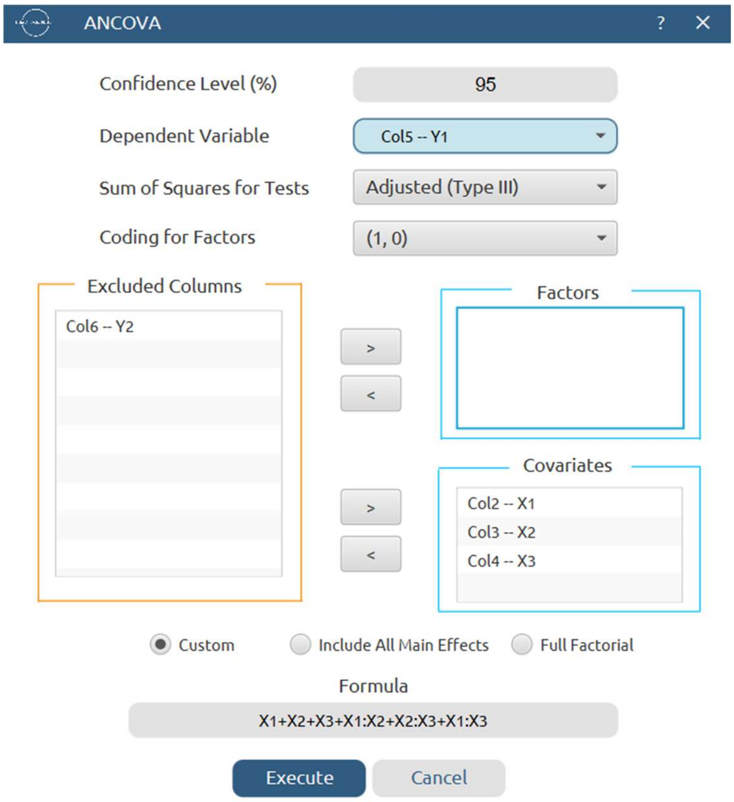
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0376360	0.1940000	0.1940000	0.9993956

Repeat this step for the second response variable, Y_2. 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		0.0034369	0.0586250	0.0586250	0.9989838

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:

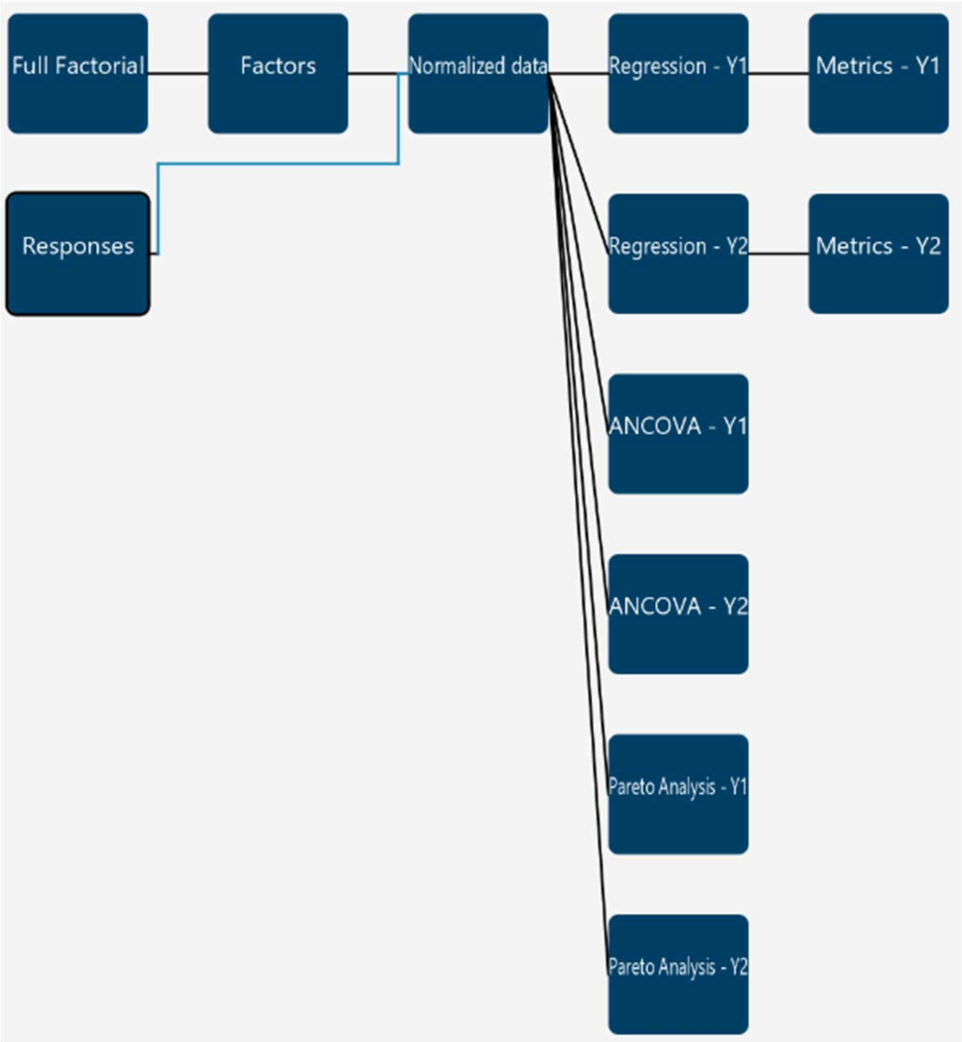
	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	398.4099920	398.4099920	1323.2343767	0.0174965
2		X2	1	17.0937045	17.0937045	56.7731178	0.0839998
3		X3	1	32.4898605	32.4898605	107.9081880	0.0610966
4		X1*X2	1	17.0469605	17.0469605	56.6178675	0.0841135
5		X2*X3	1	0.3026420	0.3026420	1.0051613	0.4991807
6		X1*X3	1	32.5059845	32.5059845	107.9617404	0.0610815
7		Error	1	0.3010880	0.3010880		
8		Total	7	498.1502320			

Repeat this step for the second response variable, Y_2. 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	21.0373411	21.0373411	765.1298594	0.0230051
2		X2	1	2.7366301	2.7366301	99.5314669	0.0635992
3		X3	1	0.0034861	0.0034861	0.1267907	0.7822263
4		X1*X2	1	3.2017151	3.2017151	116.4466474	0.0588272
5		X2*X3	1	0.0076261	0.0076261	0.2773628	0.6914040
6		X1*X3	1	0.0427781	0.0427781	1.5558440	0.4302164
7		Error	1	0.0274951	0.0274951		
8		Total	7	27.0570719			

Final Isalos Workflow

The final workflow is presented below:



References

(1) Kovács, A.; Berkó, Sz.; Csányi, E.; Csóka, I. Development of Nanostructured Lipid Carriers Containing Salicyclic Acid for Dermal Use Based on the Quality by Design Method. *European Journal of Pharmaceutical Sciences* **2017**, *99*, 246–257. https://doi.org/10.1016/j.ejps.2016.12.020.