



Quality by design based silymarin nanoemulsion for enhancement of oral bioavailability

The purpose of this research paper is to develop silymarin loaded nanoemulsions with good oral bioavailability. Design of experiments (DoE) methodology is implemented for the optimization of the silymarin loaded nanoemulsions.

The factors (independent variables) examined are: A = surfactant/cosurfactant mixture concentration (%), B = homogenization pressure (bar) and C = number of homogenization cycles. All the factors are continuous. The responses (dependent variables) examined are: R_1 = globule size (nm), R_2 = polydispersity index, R_3 = transmittance percentage (%) and R_4 = drug release (%). The applied DoE method is Box Behnken design.

Isalos version used: 2.1.2

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

Step 1: Box Behnken 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 “Box Behnken”. Afterwards, apply the Box Behnken method: *DOE* → *Response Surface* → *Box Behnken*

	Col1	Col2 (I)	Col3 (I)	Col4 (I)
User Header	User Row ID	A	B	C
1		30	1000	5
2		40	2500	15

DoE Box Behnken

Number of Center Points per Block
5

Number of Replicates
1

Number of Blocks
1

☐ Random Standard order

Excluded Columns

Included Columns

Col2 -- A
Col3 -- B
Col4 -- C

Execute
Cancel

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	A	B	C
1		1	Block: 1	Replicate: 1	Design Point	30.0	1000.0	10.0
2		2	Block: 1	Replicate: 1	Design Point	40.0	1000.0	10.0
3		3	Block: 1	Replicate: 1	Design Point	30.0	2500.0	10.0
4		4	Block: 1	Replicate: 1	Design Point	40.0	2500.0	10.0
5		5	Block: 1	Replicate: 1	Design Point	30.0	1750.0	5.0
6		6	Block: 1	Replicate: 1	Design Point	40.0	1750.0	5.0
7		7	Block: 1	Replicate: 1	Design Point	30.0	1750.0	15.0
8		8	Block: 1	Replicate: 1	Design Point	40.0	1750.0	15.0
9		9	Block: 1	Replicate: 1	Design Point	35.0	1000.0	5.0
10		10	Block: 1	Replicate: 1	Design Point	35.0	2500.0	5.0
11		11	Block: 1	Replicate: 1	Design Point	35.0	1000.0	15.0
12		12	Block: 1	Replicate: 1	Design Point	35.0	2500.0	15.0
13		13	Block: 1	----	Center Point	35.0	1750.0	10.0
14		14	Block: 1	----	Center Point	35.0	1750.0	10.0
15		15	Block: 1	----	Center Point	35.0	1750.0	10.0
16		16	Block: 1	----	Center Point	35.0	1750.0	10.0
17		17	Block: 1	----	Center Point	35.0	1750.0	10.0

Step 2: Factor selection

Create a new tab named “Factors” and import the results from the “Box Behnken” 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)*

	Col1	Col2	Col3	Col4	Col5	Col6
User Header	User Row ID					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Choose tab as input

Select input tab

Box Behnken

Execute

Cancel

Select Column(s)

Excluded Columns

Col2 -- Standard Order

Col3 -- Block Number

Col4 -- Replicate Number

Col5 -- Point Type

Included Columns

Col6 -- A

Col7 -- B

Col8 -- C

Execute

Cancel

Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	A	B	C
1		30.0	1000.0	10.0
2		40.0	1000.0	10.0
3		30.0	2500.0	10.0
4		40.0	2500.0	10.0
5		30.0	1750.0	5.0
6		40.0	1750.0	5.0
7		30.0	1750.0	15.0
8		40.0	1750.0	15.0
9		35.0	1000.0	5.0
10		35.0	2500.0	5.0
11		35.0	1000.0	15.0
12		35.0	2500.0	15.0
13		35.0	1750.0	10.0
14		35.0	1750.0	10.0
15		35.0	1750.0	10.0
16		35.0	1750.0	10.0
17		35.0	1750.0	10.0

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 Box Behnken design. Then, select all columns to be transferred to the right spreadsheet: *Data Transformation* → *Data Manipulation* → *Select Column(s)*

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	R1	R2	R3	R4
1		317.3	0.87	81.5	65.2
2		125	0.46	97.2	84
3		256.7	0.64	88.5	76.3
4		50.02	0.45	100	90
5		286.2	0.75	85	66.7
6		92	0.46	98	85.3
7		273	0.65	87	68.1
8		70.82	0.5	99.5	88.6
9		230	0.45	90	70
10		145	0.46	97	82.6
11		208.4	0.45	92.5	79.8
12		112	0.46	97.8	85.4
13		180	0.49	99.4	78.5
14		188	0.43	95	78
15		171	0.43	96.2	80.4
16		168	0.43	96.5	77.1
17		175	0.43	96	76.8

Select Column(s)
?
X

Excluded Columns

>>

>

<

<<

Included Columns

Col2 -- R1
Col3 -- R2
Col4 -- R3
Col5 -- R4

Execute

Cancel

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

	Col1	Col2	Col3	Col4	Col5	Col6
User Header	User Row ID					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Multiple Spreadsheet Joiner

Join Configuration Steps

Step 1: Factors & Responses (Concatenate)

Join Type

☒ Concatenation ☐ Left Join ☐ Right Join ☐ Inner Join ☐ Full Outer Join

Left Spreadsheet

Factors

Right Spreadsheet

Responses

Join Column

☒ Common header name ☐ Different header names

Execute Cancel

Min-Max normalizer

Excluded Columns

- Col5 -- R1
- Col6 -- R2
- Col7 -- R3
- Col8 -- R4

Included Columns

- Col2 -- A
- Col3 -- B
- Col4 -- C

>>

>

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<<

Min -1.0

Max 1.0

Execute Cancel

Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	A	B	C	R1	R2	R3	R4
1		-1.0	-1.0	0.0	317.3	0.87	81.5	65.2
2		1.0	-1.0	0.0	125.0	0.46	97.2	84.0
3		-1.0	1.0	0.0	256.7	0.64	88.5	76.3
4		1.0	1.0	0.0	50.02	0.45	100.0	90.0
5		-1.0	0.0	-1.0	286.2	0.75	85.0	66.7
6		1.0	0.0	-1.0	92.0	0.46	98.0	85.3
7		-1.0	0.0	1.0	273.0	0.65	87.0	68.1
8		1.0	0.0	1.0	70.82	0.5	99.5	88.6
9		0.0	-1.0	-1.0	230.0	0.45	90.0	70.0
10		0.0	1.0	-1.0	145.0	0.46	97.0	82.6
11		0.0	-1.0	1.0	208.4	0.45	92.5	79.8
12		0.0	1.0	1.0	112.0	0.46	97.8	85.4
13		0.0	0.0	0.0	180.0	0.49	99.4	78.5
14		0.0	0.0	0.0	188.0	0.43	95.0	78.0
15		0.0	0.0	0.0	171.0	0.43	96.2	80.4
16		0.0	0.0	0.0	168.0	0.43	96.5	77.1
17		0.0	0.0	0.0	175.0	0.43	96.0	76.8

Step 5: Regression

The goal here is to produce a regression equation that includes main effects, two-factor interactions and quadratic effects for R₁:

$$R = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$$

Create a new tab named “Regression – R1” 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

Scale Parameter Method: Fixed value

Value: 1.0

Dependent Variable: Col5 - R1

Excluded Columns: Col6 - R2, Col7 - R3, Col8 - R4

Factors: (empty)

Specify Reference Levels

Covariates: Col2 - A, Col3 - B, Col4 - C

Formula Type: ☒ Custom ☐ Main Effects ☐ Full Factorial

Specify Custom Model Formula

Formula: A + B + C + A:B + A:C + B:C + A:A + B:B + C:C

Execute Cancel

Custom Formula Creation

Included Columns

A

B

C

Add interactions

up to order2

Add terms

up to order2

Add Custom Interaction

Delete Selected

Reset Selections

Included Terms

A

B

C

A:B

A:C

B:C

A:A

B:B

C:C

Move up

Move down

A + B + C + A:B + A:C + B:C + A:A + B:B + C:C

Save

Cancel

Results:

R1	Prediction
317.3	322.7025000
125.0	131.0525000
256.7	250.6475000
50.02	44.6175000
286.2	289.0525000
92.0	94.2025000
273.0	270.7975000
70.82	67.9675
230.0	221.7450000
145.0	148.2000000
208.4	205.2000000
112.0	120.2550000
180.0	176.4000000
188.0	176.4000000
171.0	176.4000000
168.0	176.4000000
175.0	176.4000000

Goodness of Fit	
	Value
Deviance	563.5851000
Scaled Deviance	563.5851000
Pearson Chi-Square	563.5851000
Scaled Pearson Chi-Square	563.5851000
Log Likelihood	-297.4145051
Akaike's Information Criterion (AIC)	614.8290101
Finite Sample Corrected AIC (AICC)	651.4956768
Bayesian Information Criterion (BIC)	623.1611436
Consistent AIC (CAIC)	633.1611436

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Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	176.4000000	0.4472136	175.5234775	177.2765225	155584.8000000	1	0.0
A	-99.4200000	0.3535534	-100.1129519	-98.7270481	79074.6912000	1	0.0
B	-39.6225000	0.3535534	-40.3154519	-38.9295481	12559.5400500	1	0.0
C	-11.1225000	0.3535534	-11.8154519	-10.4295481	989.6800500	1	0.0
A*A	8.7550000	0.4873397	7.7998317	9.7101683	322.7369474	1	0.0
A*B	-3.5950000	0.5	-4.5749820	-2.6150180	51.6961000	1	0E-7
B*B	2.1000000	0.4873397	1.1448317	3.0551683	18.5684211	1	0.0000164
A*C	-1.9950000	0.5	-2.9749820	-1.0150180	15.9201000	1	0.0000661
B*C	-2.8500000	0.5	-3.8299820	-1.8700180	32.4900000	1	0E-7
C*C	-4.6500000	0.4873397	-5.6051683	-3.6948317	91.0421053	1	0.0

Repeat this step for the rest of the response variables. Results, R₂:

R2	Prediction
0.87	0.8187500
0.46	0.4487500
0.64	0.6512500
0.45	0.5012500
0.75	0.7612500
0.46	0.4312500
0.65	0.6787500
0.5	0.4887500
0.45	0.4900000
0.46	0.4375000
0.46	0.4825000
0.46	0.42
0.49	0.4420000
0.43	0.4420000
0.43	0.4420000
0.43	0.4420000
0.43	0.4420000

Goodness of Fit	Value
Deviance	0.0145050
Scaled Deviance	0.0145050
Pearson Chi-Square	0.0145050
Scaled Pearson Chi-Square	0.0145050
Log Likelihood	-15.6292076
Akaike's Information Criterion (AIC)	51.2584151
Finite Sample Corrected AIC (AICC)	87.9250818
Bayesian Information Criterion (BIC)	59.5905486
Consistent AIC (CAIC)	69.5905486

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	0.4420000	0.4472136	-0.4345225	1.3185225	0.9768200	1	0.3229852
A	-0.1300000	0.3535534	-0.8229519	0.5629519	0.1352000	1	0.7131003
B	-0.0287500	0.3535534	-0.7217019	0.6642019	0.0066125	1	0.9351896
C	-0.0062500	0.3535534	-0.6992019	0.6867019	0.0003125	1	0.9858960
A*B	0.0550000	0.5	-0.9249820	1.0349820	0.0121000	1	0.9124094
A*C	0.0350000	0.5	-0.9449820	1.0149820	0.0049000	1	0.9441937
B*C	-0.0025000	0.5	-0.9824820	0.9774820	0.0000250	1	0.9960106
A*A	0.1477500	0.4873397	-0.8074183	1.1029183	0.0919161	1	0.7617553
B*B	0.0152500	0.4873397	-0.9399183	0.9704183	0.0009792	1	0.9750364
C*C	0.0002500	0.4873397	-0.9549183	0.9554183	3E-7	1	0.9995907

Results, R_3 :

R3	Prediction
81.5	81.4000000
97.2	96.675
88.5	89.0250000
100.0	100.1000000
85.0	84.8125000
98.0	98.2375000
87.0	86.7625000
99.5	99.6875000
90.0	90.2875
97.0	96.6625000
92.5	92.8375000
97.8	97.5125000
99.4	96.62
95.0	96.62
96.2	96.62
96.5	96.62
96.0	96.62

Goodness of Fit	
	Value
Deviance	12.0755000
Scaled Deviance	12.0755000
Pearson Chi-Square	12.0755000
Scaled Pearson Chi-Square	12.0755000
Log Likelihood	-21.6597051
Akaike's Information Criterion (AIC)	63.3194101
Finite Sample Corrected AIC (AICC)	99.9860768
Bayesian Information Criterion (BIC)	71.6515436
Consistent AIC (CAIC)	81.6515436

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	96.62	0.4472136	95.7434775	97.4965225	46677.1220000	1	0.0
A	6.5875000	0.3535534	5.8945481	7.2804519	347.1612500	1	0.0
B	2.7625000	0.3535534	2.0695481	3.4554519	61.0512500	1	0E-7
C	0.8500000	0.3535534	0.1570481	1.5429519	5.7800000	1	0.0162095
A*A	-3.3850000	0.4873397	-4.3401683	-2.4298317	48.2451579	1	0E-7
A*B	-1.0500000	0.5	-2.0299820	-0.0700180	4.4100000	1	0.0357288
B*B	-1.4350000	0.4873397	-2.3901683	-0.4798317	8.6704211	1	0.0032342
A*C	-0.1250000	0.5	-1.1049820	0.8549820	0.0625000	1	0.8025873
B*C	-0.4250000	0.5	-1.4049820	0.5549820	0.7225000	1	0.3953251
C*C	-0.8600000	0.4873397	-1.8151683	0.0951683	3.1141053	1	0.0776171

Results, R₄:

R4	Prediction
65.2	64.2375000
84.0	84.6875
76.3	75.6125000
90.0	90.9625
66.7	66.5375000
85.3	83.4875000
68.1	69.9125
88.6	88.7625000
70.0	71.125
82.6	83.4500000
79.8	78.9500000
85.4	84.275
78.5	78.1600000
78.0	78.1600000
80.4	78.1600000
77.1	78.1600000
76.8	78.1600000

Goodness of Fit	
	Value
Deviance	21.5295000
Scaled Deviance	21.5295000
Pearson Chi-Square	21.5295000
Scaled Pearson Chi-Square	21.5295000
Log Likelihood	-26.3867051
Akaike's Information Criterion (AIC)	72.7734101
Finite Sample Corrected AIC (AICC)	109.4400768
Bayesian Information Criterion (BIC)	81.1055436
Consistent AIC (CAIC)	91.1055436

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	78.1600000	0.4472136	77.2834775	79.0365225	30544.9280000	1	0.0
A	8.9500000	0.3535534	8.2570481	9.6429519	640.8200000	1	0.0
B	4.4125000	0.3535534	3.7195481	5.1054519	155.7612500	1	0.0
C	2.1625000	0.3535534	1.4695481	2.8554519	37.4112500	1	0E-7
A*A	-0.7800000	0.4873397	-1.7351683	0.1751683	2.5616842	1	0.1094819
A*B	-1.2750000	0.5	-2.2549820	-0.2950180	6.5025000	1	0.0107723
B*B	1.4950000	0.4873397	0.5398317	2.4501683	9.4106316	1	0.0021573
A*C	0.4750000	0.5	-0.5049820	1.4549820	0.9025000	1	0.3421123
B*C	-1.7500000	0.5	-2.7299820	-0.7700180	12.2500000	1	0.0004653
C*C	-0.2050000	0.4873397	-1.1601683	0.7501683	0.1769474	1	0.6740099

Step 6: Data selection for Regression Metrics

Create a tab named “Metrics Data – R1” and import the results from the spreadsheet “Regression – R1”. Next, assign names to all unnamed columns containing data, using names such as Z1–Z9. Then, select only the response column (R1) 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	R1	Prediction	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9
1		317.3	322.7025000		Goodness of Fit							
2		125	131.0525000			Value						
3		256.7	250.6475000		Deviance	563.5851000						
4		50.02	44.6175000		Scaled Deviance	563.5851000						
5		286.2	289.0525000		Pearson Chi-Square	563.5851000						
6		92	94.2025		Scaled Pearson Chi-Square	563.5851000						
7		273	270.7975000		Log Likelihood	-297.4145051						
8		70.82	67.9675000		Akaike's Information Criterion (AIC)	614.8290101						
9		230	221.7450000		Finite Sample Corrected AIC (AICC)	651.4956768						
10		145	148.2000000		Bayesian Information Criterion (BIC)	623.1611436						
11		208.4	205.2000000		Consistent AIC (CAIC)	633.1611436						
12		112	120.2550000									
13		180	176.4000000									
14		188	176.4000000		Parameter Estimates							
15		171	176.4000000		Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
16		168	176.4000000		intercept	176.4000000	0.4472136	175.5234775	177.2765225	155584.8000000	1	0.0

Results:

Select Column(s)

Select Columns Manually

Select Matching Columns from Node

Select Columns Manually and from Node

Select Node

Select Node

Excluded Columns

Col5 -- Z2

Col6 -- Z3

Col7 -- Z4

Col8 -- Z5

Col9 -- Z6

Col10 -- Z7

Col11 -- Z8

Col12 -- Z9

>>

>

<

<<

Included Columns

Col2 -- R1

Col3 -- Prediction

Execute

Cancel

	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	R1	Prediction
1		317.3	322.7025000
2		125	131.0525000
3		256.7	250.6475000
4		50.02	44.6175000
5		286.2	289.0525000
6		92	94.2025
7		273	270.7975000
8		70.82	67.9675000
9		230	221.7450000
10		145	148.2000000
11		208.4	205.2000000
12		112	120.2550000
13		180	176.4000000
14		188	176.4000000
15		171	176.4000000
16		168	176.4000000
17		175	176.4000000

Repeat this step for the rest of the response variables.

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Step 7: Regression Metrics

Create a tab named “Metrics – R1” and import the results from the spreadsheet “Metrics Data – R1”. Then, produce the regression metrics for the R_1 regression equation: *Statistics* → *Model Metrics* → *Regression Metrics*

Regression Statistics Metrics
?
✕

Actual Value Column Col2 -- R1

Prediction Value Column Col3 -- Prediction

Execute
Cancel

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		33.1520647	5.7577830	5.0782353	0.9939857

Repeat this step for the rest of the response variables. Results, R_2 :

	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		0.0008532	0.0292102	0.0250588	0.9459125

Results, R_3 :

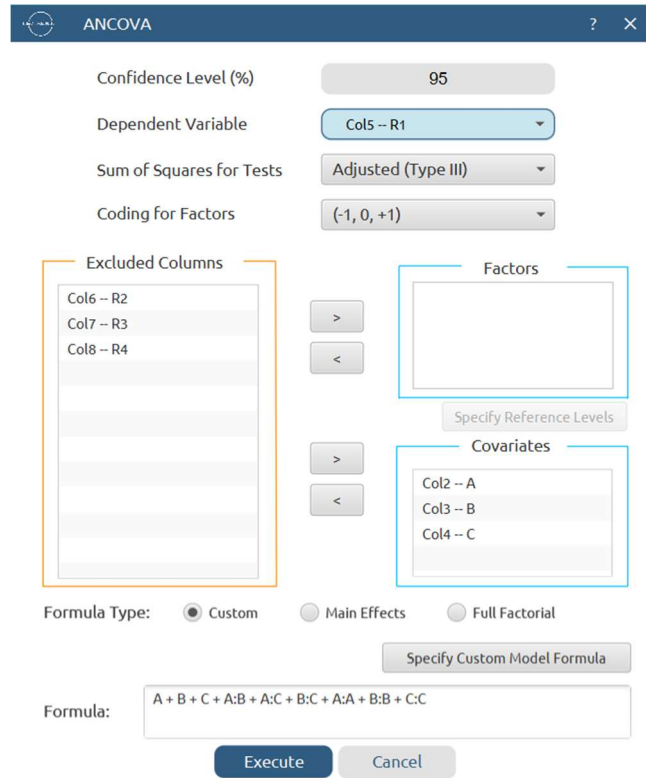
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.7103235	0.8428069	0.5241176	0.9756485

Results, R_4 :

	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		1.2664412	1.1253627	0.9623529	0.9757217

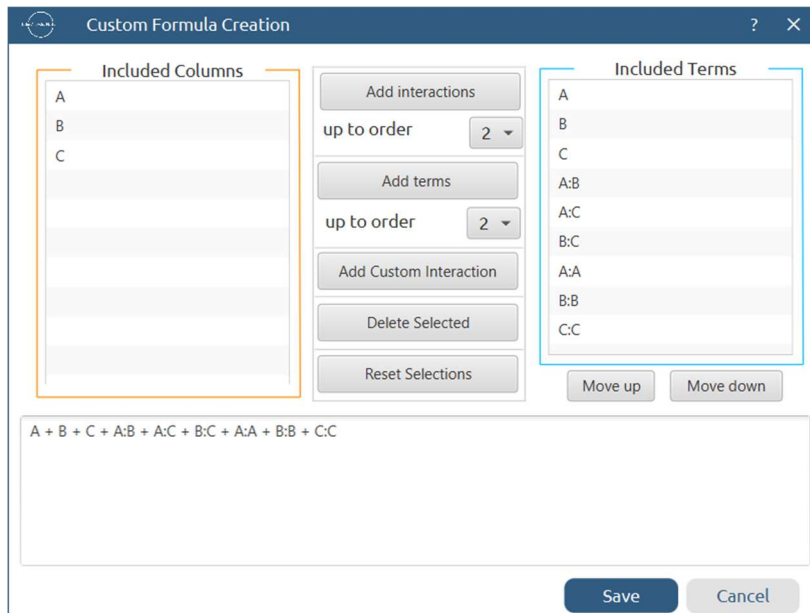
Step 8: Analysis of Covariance

Create a new tab named “ANCOVA – R1” and import the results from the spreadsheet “Normalized data”. Afterwards perform analysis of covariance for R₁: Statistics → Analysis of (Co)Variance → ANCOVA



The ANCOVA dialog box is shown with the following settings:

- Confidence Level (%): 95
- Dependent Variable: Col5 -- R1
- Sum of Squares for Tests: Adjusted (Type III)
- Coding for Factors: (-1, 0, +1)
- Excluded Columns: Col6 -- R2, Col7 -- R3, Col8 -- R4
- Factors: (Empty)
- Covariates: Col2 -- A, Col3 -- B, Col4 -- C
- Formula Type: ☒ Custom, ☐ Main Effects, ☐ Full Factorial
- Specify Custom Model Formula: A + B + C + A:B + A:C + B:C + A:A + B:B + C:C
- Buttons: Execute, Cancel



The Custom Formula Creation dialog box is shown with the following settings:

- Included Columns: A, B, C
- Add interactions: up to order 2
- Add terms: up to order 2
- Add Custom Interaction: (Empty)
- Delete Selected: (Empty)
- Reset Selections: (Empty)
- Included Terms: A, B, C, A:B, A:C, B:C, A:A, B:B, C:C
- Buttons: Move up, Move down
- Formula: A + B + C + A:B + A:C + B:C + A:A + B:B + C:C
- Buttons: Save, Cancel

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		A	1	79074.6912000	79074.6912000	982.1459765	0E-7
2		B	1	12559.5400500	12559.5400500	155.9955725	0.0000049
3		C	1	989.6800500	989.6800500	12.2923057	0.0099121
4		A*A	1	322.7369474	322.7369474	4.0085493	0.0853500
5		B*B	1	18.5684211	18.5684211	0.2306288	0.6457021
6		C*C	1	91.0421053	91.0421053	1.1307871	0.3229093
7		A*B	1	51.6961000	51.6961000	0.6420906	0.4492947
8		A*C	1	15.9201000	15.9201000	0.1977354	0.6699789
9		B*C	1	32.4900000	32.4900000	0.4035415	0.5454631
10		Error	7	563.5851000	80.5121571		
11		Total	16	93707.392			

Repeat this step for the rest of the response variables. Results, R₂:

	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		A	1	0.1352000	0.1352000	65.2464667	0.0000857	Yes
2		B	1	0.0066125	0.0066125	3.1911410	0.1171972	No
3		C	1	0.0003125	0.0003125	0.1508101	0.7093030	No
4		A*B	1	0.0121000	0.0121000	5.8393657	0.0463311	Yes
5		A*C	1	0.0049000	0.0049000	2.3647018	0.1679942	No
6		B*C	1	0.0000250	0.0000250	0.0120648	0.9156190	No
7		A*A	1	0.0919161	0.0919161	44.3579709	0.0002878	Yes
8		B*B	1	0.0009792	0.0009792	0.4725594	0.5139363	No
9		C*C	1	3E-7	3E-7	0.0001270	0.9913230	No
10		Error	7	0.0145050	0.0020721			
11		Total	16	0.2681765				

Results, R₃:

	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		A	1	347.1612500	347.1612500	201.2445654	0.0000021
2		B	1	61.0512500	61.0512500	35.3905635	0.0005706
3		C	1	5.7800000	5.7800000	3.3505859	0.1098711
4		A*A	1	48.2451579	48.2451579	27.9670494	0.0011376
5		B*B	1	8.6704211	8.6704211	5.0261229	0.0599096
6		C*C	1	3.1141053	3.1141053	1.8052037	0.2210047
7		A*B	1	4.4100000	4.4100000	2.5564159	0.1538799
8		A*C	1	0.0625000	0.0625000	0.0362304	0.8544429
9		B*C	1	0.7225000	0.7225000	0.4188232	0.5381606
10		Error	7	12.0755000	1.7250714		
11		Total	16	495.8823529			

Results, R₄:

	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		A	1	640.8200000	640.8200000	208.3531898	0.0000018
2		B	1	155.7612500	155.7612500	50.6434776	0.0001909
3		C	1	37.4112500	37.4112500	12.1637172	0.0101608
4		A*A	1	2.5616842	2.5616842	0.8328939	0.3917841
5		B*B	1	9.4106316	9.4106316	3.0597283	0.1237359
6		C*C	1	0.1769474	0.1769474	0.0575318	0.8173127
7		A*B	1	6.5025000	6.5025000	2.1141922	0.1892593
8		A*C	1	0.9025000	0.9025000	0.2934346	0.6048330
9		B*C	1	12.2500000	12.2500000	3.9829072	0.0861612
10		Error	7	21.5295000	3.0756429		
11		Total	16	886.7800000			

References

- (1) Nagi, A.; Iqbal, B.; Kumar, S.; Sharma, S.; Ali, J.; Baboota, S. Quality by Design Based Silymarin Nanoemulsion for Enhancement of Oral Bioavailability. *Journal of Drug Delivery Science and Technology* **2017**, *40*, 35–44. <https://doi.org/10.1016/j.jddst.2017.05.019>.