Formulation development and evaluation of a novel bi-dependent clarithromycin gastroretentive drug delivery system using Box-Behnken design

The objective of this research paper is to develop a novel bi-dependent gastroretentive tablet (BDGRT) formulation containing clarithromycin and to evaluate pharmacokinetics in beagle dogs. Design of experiments (DoE) methodology is implemented for the optimization of the BDGRT formulation.

The factors (independent variables) examined are: X_1 = amount of HPMC K4M (mg), X_2 = amount of $NaHCO_3$ (%) and X_3 = amount of camphor (%). All the factors are continuous. The responses (dependent variables) examined are: Y_1 = floating lag time (sec), Y_2 = friability (%), Y_3 = tablet crushing strength (kg/cm^2), Y_4 = cumulative drug release at 5th h (%) and Y_5 = cumulative drug release at 10th h (%). The applied DoE method is Box Behnken design.

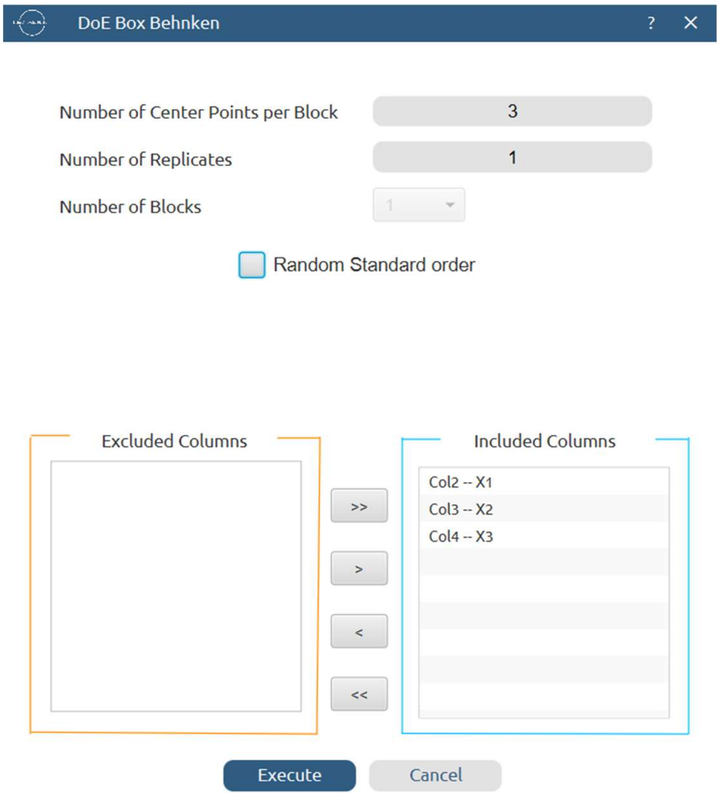
Isalos version used: 2.0.6

Scientific article: https://www.sciencedirect.com/science/article/abs/pii/S1773224716301800

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	X1	X2	X3
1		50	8	8
2		150	12	14



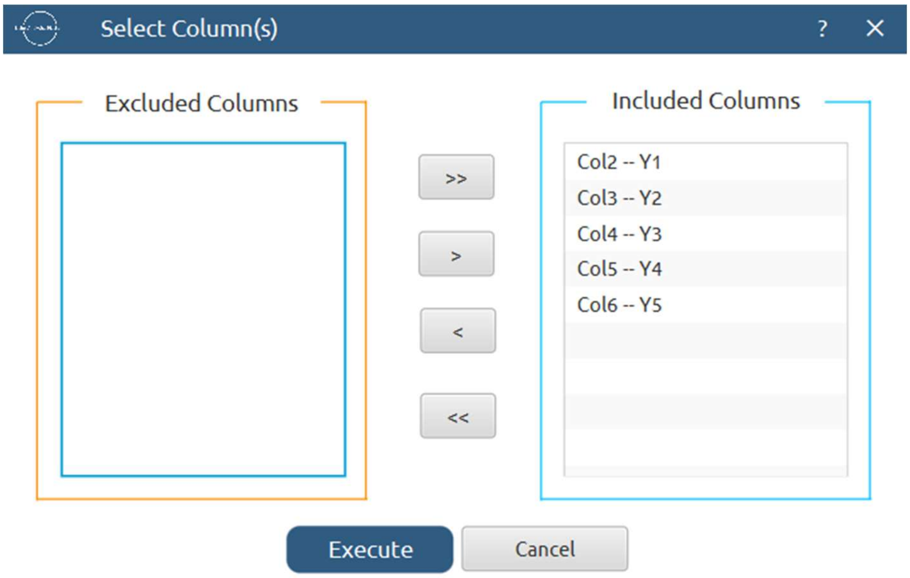
Results (right spreadsheet):

	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	50.0	8.0	11.0
2		2	Block: 1	Replicate: 1	Design Point	150.0	8.0	11.0
3		3	Block: 1	Replicate: 1	Design Point	50.0	12.0	11.0
4		4	Block: 1	Replicate: 1	Design Point	150.0	12.0	11.0
5		5	Block: 1	Replicate: 1	Design Point	50.0	10.0	8.0
6		6	Block: 1	Replicate: 1	Design Point	150.0	10.0	8.0
7		7	Block: 1	Replicate: 1	Design Point	50.0	10.0	14.0
8		8	Block: 1	Replicate: 1	Design Point	150.0	10.0	14.0
9		9	Block: 1	Replicate: 1	Design Point	100.0	8.0	8.0
10		10	Block: 1	Replicate: 1	Design Point	100.0	12.0	8.0
11		11	Block: 1	Replicate: 1	Design Point	100.0	8.0	14.0
12		12	Block: 1	Replicate: 1	Design Point	100.0	12.0	14.0
13		13	Block: 1	----	Center Point	100.0	10.0	11.0
14		14	Block: 1	----	Center Point	100.0	10.0	11.0
15		15	Block: 1	----	Center Point	100.0	10.0	11.0

Step 2: Definition of response variables

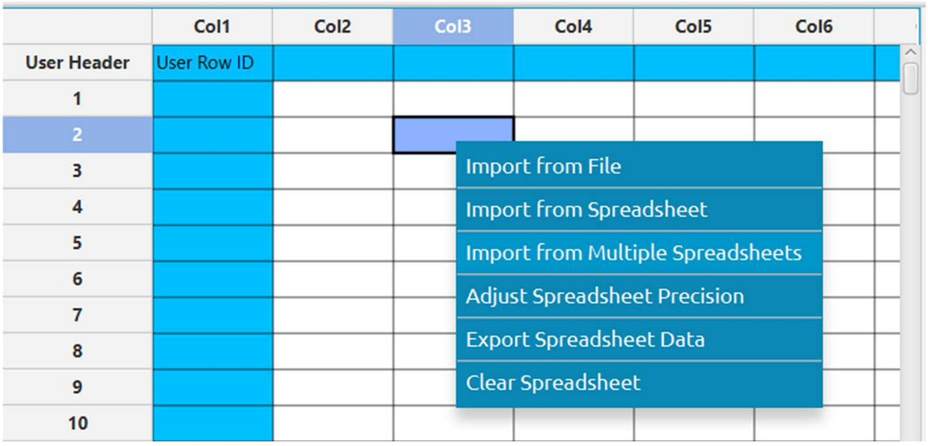
Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the Box Behnken method. Then, select all columns to be transferred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

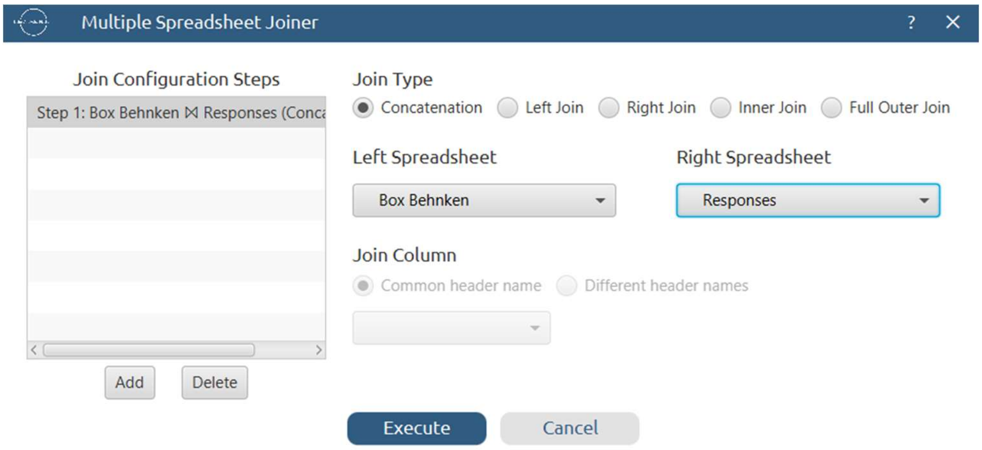
	Col1	Col2 (I)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)
User Header	User Row ID	Y1	Y2	Y3	Y4	Y5
1		250	0.7	3.03	60.5	90.25
2		200	0.6	4.03	44.31	82.1
3		88	0.8	3.5	69.3	98.32
4		50	0.9	3.23	45.05	84.9
5		320	0.5	4.87	59.9	93.85
6		254	0.6	5.03	40.1	82.93
7		0	1.4	2.2	64.36	94.15
8		0	1.6	2.5	43.94	86.78
9		412	0.6	4.7	45.5	72.03
10		210	0.5	5.2	52.1	90.2
11		0	1.8	2.4	44.44	93.19
12		0	1.8	2.2	53.31	84.82
13		65	0.9	3.3	55.01	86.99
14		54	0.8	3.5	56.36	84.29
15		50	0.8	3.57	51.58	87.13

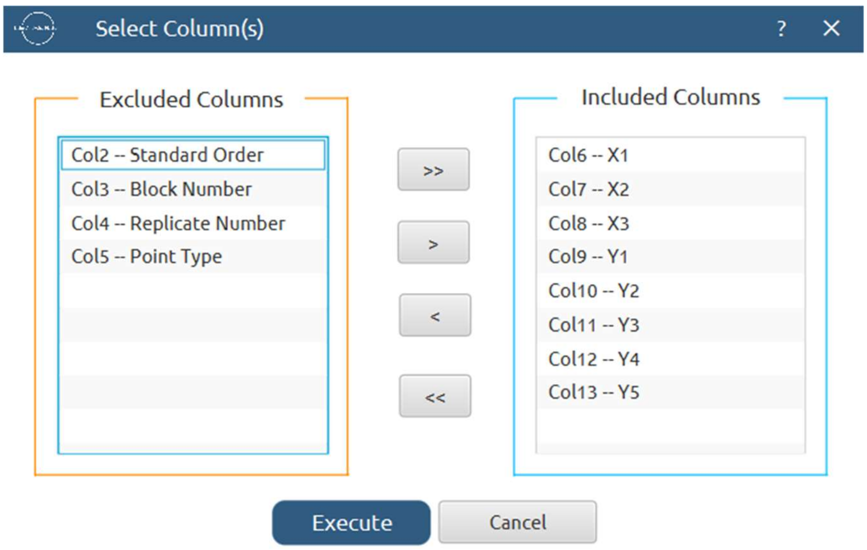


Step 3: Data isolation

Create a new tab named "Data" and import the results from the "Box Behnken" 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)_





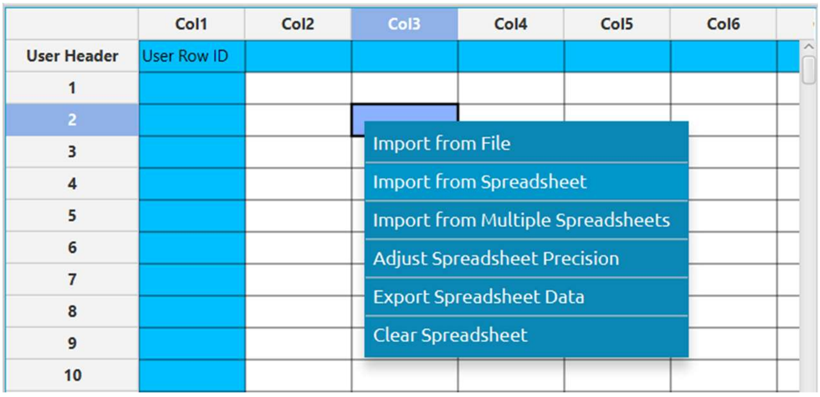


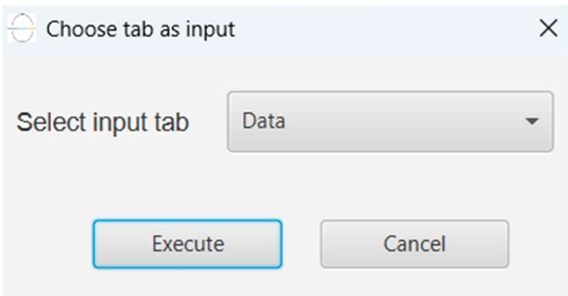
Results:

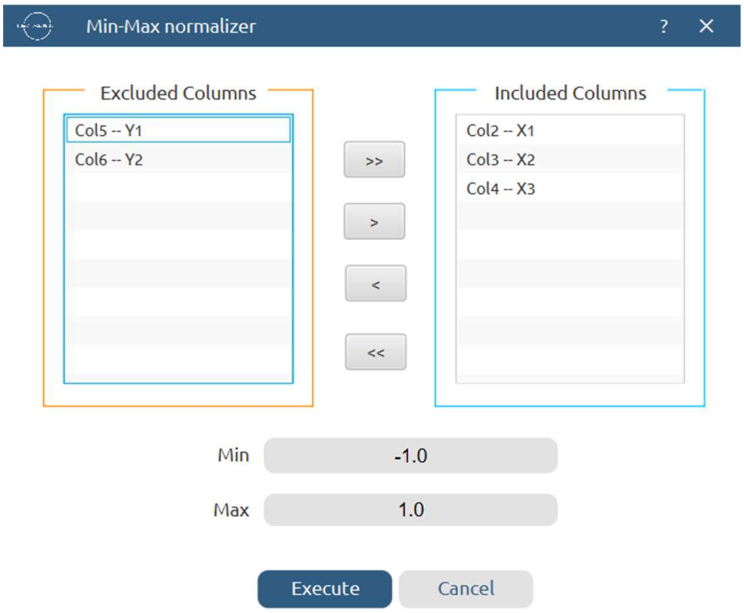
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (I)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	Y1	Y2	Y3	Y4	Y5
1		50.0	8.0	11.0	250	0.7	3.03	60.5	90.25
2		150.0	8.0	11.0	200	0.6	4.03	44.31	82.1
3		50.0	12.0	11.0	88	0.8	3.5	69.3	98.32
4		150.0	12.0	11.0	50	0.9	3.23	45.05	84.9
5		50.0	10.0	8.0	320	0.5	4.87	59.9	93.85
6		150.0	10.0	8.0	254	0.6	5.03	40.1	82.93
7		50.0	10.0	14.0	0	1.4	2.2	64.36	94.15
8		150.0	10.0	14.0	0	1.6	2.5	43.94	86.78
9		100.0	8.0	8.0	412	0.6	4.7	45.5	72.03
10		100.0	12.0	8.0	210	0.5	5.2	52.1	90.2
11		100.0	8.0	14.0	0	1.8	2.4	44.44	93.19
12		100.0	12.0	14.0	0	1.8	2.2	53.31	84.82
13		100.0	10.0	11.0	65	0.9	3.3	55.01	86.99
14		100.0	10.0	11.0	54	0.8	3.5	56.36	84.29
15		100.0	10.0	11.0	50	0.8	3.57	51.58	87.13

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:

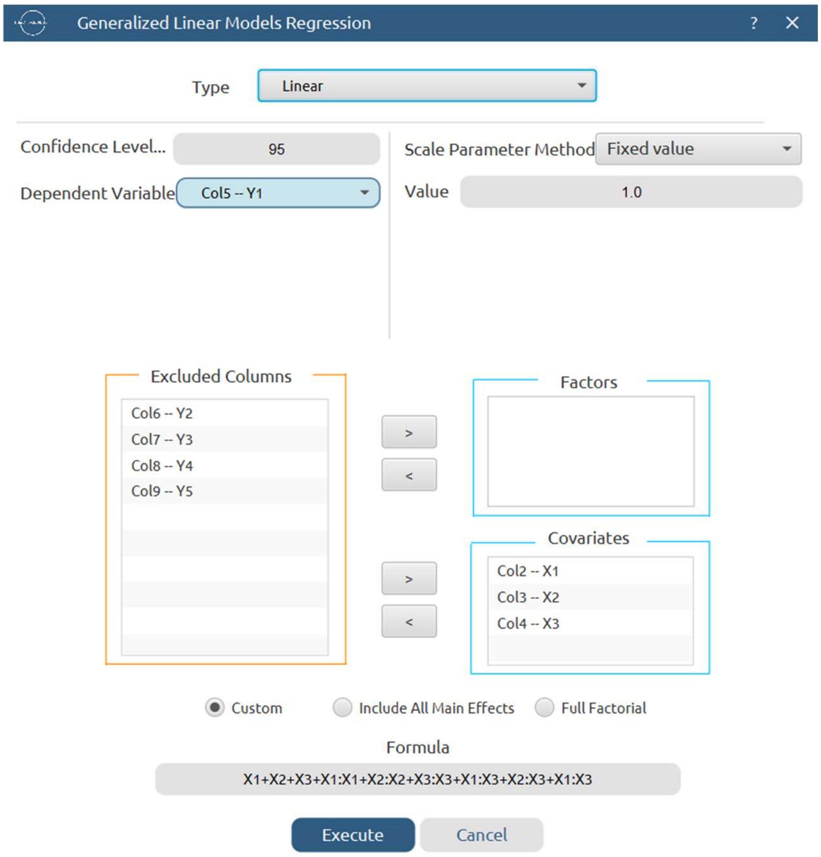
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	Y1	Y2	Y3	Y4	Y5
1		-1.0	-1.0	0.0	250.0	0.7	3.03	60.5	90.25
2		1.0	-1.0	0.0	200.0	0.6	4.03	44.31	82.1
3		-1.0	1.0	0.0	88.0	0.8	3.5	69.3	98.32
4		1.0	1.0	0.0	50.0	0.9	3.23	45.05	84.9
5		-1.0	0.0	-1.0	320.0	0.5	4.87	59.9	93.85
6		1.0	0.0	-1.0	254.0	0.6	5.03	40.1	82.93
7		-1.0	0.0	1.0	0.0	1.4	2.2	64.36	94.15
8		1.0	0.0	1.0	0.0	1.6	2.5	43.94	86.78
9		0.0	-1.0	-1.0	412.0	0.6	4.7	45.5	72.03
10		0.0	1.0	-1.0	210.0	0.5	5.2	52.1	90.2
11		0.0	-1.0	1.0	0.0	1.8	2.4	44.44	93.19
12		0.0	1.0	1.0	0.0	1.8	2.2	53.31	84.82
13		0.0	0.0	0.0	65.0	0.9	3.3	55.01	86.99
14		0.0	0.0	0.0	54.0	0.8	3.5	56.36	84.29
15		0.0	0.0	0.0	50.0	0.8	3.57	51.58	87.13

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 – 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
250.0	230.5000000
200.0	192.0000000
88.0	102.0000000
50.0	63.5000000
320.0	328.75
254.0	257.25
0.0	-3.2500000
0.0	-8.7500000
412.0	419.7500000
210.0	190.2500000
0.0	19.7500000
0.0	-7.7500000
65.0	56.3333333
54.0	56.3333333
50.0	56.3333333

Goodness of Fit	
	Value
Deviance	2017.6666667
Scaled Deviance	2017.6666667
Pearson Chi-Square	2017.6666667
Scaled Pearson Chi-Square	2017.6666667
Log Likelihood	-1022.6174113
Akaike's Information Criterion (AIC)	2063.2348227
Finite Sample Corrected AIC (AICC)	2099.2348227
Bayesian Information Criterion (BIC)	2069.6072745
Consistent AIC (CAIC)	2078.6072745

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	56.3333333	0.5773503	55.2017476	57.4649191	9520.3333333	1	0.0
X1	-19.2500000	0.3535534	-19.9429519	-18.5570481	2964.5000000	1	0.0
X2	-64.2500000	0.3535534	-64.9429519	-63.5570481	33024.5000000	1	0.0
X3	-149.5	0.3535534	-150.1929519	-148.8070481	178802.0000000	1	0.0
X1*X3	16.5000000	0.5	15.5200180	17.4799820	1089.0000000	1	0.0
X2*X3	50.5	0.5	49.5200180	51.4799820	10201.0	1	0.0
X1*X1	39.3333333	0.5204165	38.3133357	40.3533309	5712.4102564	1	0.0
X2*X2	51.3333333	0.5204165	50.3133357	52.3533309	9729.6410256	1	0.0
X3*X3	47.8333333	0.5204165	46.8133357	48.8533309	8448.1025641	1	0.0

Repeat this step for the rest of the response variables. Results, Y_2:

Y2	Prediction
0.7	0.7250000
0.6	0.7
0.8	0.7000000
0.9	0.8750000
0.5	0.4625000
0.6	0.4875
1.4	1.5125000
1.6	1.6375000
0.6	0.6125
0.5	0.6375
1.8	1.6625
1.8	1.7875000
0.9	0.8333333
0.8	0.8333333
0.8	0.8333333

Goodness of Fit	
	Value
Deviance	0.0941667
Scaled Deviance	0.0941667
Pearson Chi-Square	0.0941667
Scaled Pearson Chi-Square	0.0941667
Log Likelihood	-13.8311613
Akaike's Information Criterion (AIC)	47.6623227
Finite Sample Corrected AIC (AICC)	102.6623227
Bayesian Information Criterion (BIC)	54.7428247
Consistent AIC (CAIC)	64.7428247

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	0.8333333	0.5773503	-0.2982524	1.9649191	2.0833333	1	0.1489147
X1	0.0375000	0.3535534	-0.6554519	0.7304519	0.0112500	1	0.9155300
X2	0.0375000	0.3535534	-0.6554519	0.7304519	0.0112500	1	0.9155300
X3	0.5500000	0.3535534	-0.1429519	1.2429519	2.4200000	1	0.1197949
X1*X3	0.0250000	0.5	-0.9549820	1.0049820	0.0025000	1	0.9601224
X1*X2	0.0500000	0.5	-0.9299820	1.0299820	0.0100000	1	0.9203443
X2*X3	0.0250000	0.5	-0.9549820	1.0049820	0.0025000	1	0.9601224
X1*X1	-0.1166667	0.5204165	-1.1366643	0.9033309	0.0502564	1	0.8226177
X2*X2	0.0333333	0.5204165	-0.9866643	1.0533309	0.0041026	1	0.9489294
X3*X3	0.3083333	0.5204165	-0.7116643	1.3283309	0.3510256	1	0.5535331

Results, Y_3:

Y3	Prediction
3.03	2.9850000
4.03	3.9175
3.5	3.6125000
3.23	3.275
4.87	4.8487500
5.03	5.07625
2.2	2.1537500
2.5	2.5212500
4.7	4.7662500
5.2	5.10875
2.4	2.4912500
2.2	2.13375
3.3	3.4566667
3.5	3.4566667
3.57	3.4566667

Goodness of Fit	
	Value
Deviance	0.0992417
Scaled Deviance	0.0992417
Pearson Chi-Square	0.0992417
Scaled Pearson Chi-Square	0.0992417
Log Likelihood	-13.8336988
Akaike's Information Criterion (AIC)	47.6673977
Finite Sample Corrected AIC (AICC)	102.6673977
Bayesian Information Criterion (BIC)	54.7478997
Consistent AIC (CAIC)	64.7478997

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	3.4566667	0.5773503	2.3250809	4.5882524	35.8456333	1	0E-7
X1	0.1487500	0.3535534	-0.5442019	0.8417019	0.1770125	1	0.6739533
X2	-0.0037500	0.3535534	-0.6967019	0.6892019	0.0001125	1	0.9915373
X3	-1.3125000	0.3535534	-2.0054519	-0.6195481	13.7812500	1	0.0002054
X1*X3	0.0350000	0.5	-0.9449820	1.0149820	0.0049000	1	0.9441937
X1*X2	-0.3175000	0.5	-1.2974820	0.6624820	0.4032250	1	0.5254284
X2*X3	-0.1750000	0.5	-1.1549820	0.8049820	0.1225000	1	0.7263387
X1*X1	0.0079167	0.5204165	-1.0120809	1.0279143	0.0002314	1	0.9878629
X2*X2	-0.0170833	0.5204165	-1.0370809	1.0029143	0.0010776	1	0.9738131
X3*X3	0.1854167	0.5204165	-0.8345809	1.2054143	0.1269391	1	0.7216270

Results, Y_4:

Y4	Prediction
60.5	59.7312500
44.31	43.5962500
69.3	70.0137500
45.05	45.8187500
59.9	60.9462500
40.1	41.0912500
64.36	63.3687500
43.94	42.8937500
45.5	45.2225
52.1	50.3400000
44.44	46.2000000
53.31	53.5875000
55.01	54.3166667
56.36	54.3166667
51.58	54.3166667

Goodness of Fit	
	Value
Deviance	24.8497417
Scaled Deviance	24.8497417
Pearson Chi-Square	24.8497417
Scaled Pearson Chi-Square	24.8497417
Log Likelihood	-26.2089488
Akaike's Information Criterion (AIC)	72.4178977
Finite Sample Corrected AIC (AICC)	127.4178977
Bayesian Information Criterion (BIC)	79.4983997
Consistent AIC (CAIC)	89.4983997

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	54.3166667	0.5773503	53.1850809	55.4482524	8850.9008333	1	0.0
X1	-10.0825000	0.3535534	-10.7754519	-9.3895481	813.2544500	1	0.0
X2	3.1262500	0.3535534	2.4332981	3.8192019	78.1875125	1	0.0
X3	1.0562500	0.3535534	0.3632981	1.7492019	8.9253125	1	0.0028125
X1*X3	-0.1550000	0.5	-1.1349820	0.8249820	0.0961000	1	0.7565610
X1*X2	-2.0150000	0.5	-2.9949820	-1.0350180	16.2409000	1	0.0000558
X2*X3	0.5675000	0.5	-0.4124820	1.5474820	1.2882250	1	0.2563753
X1*X1	1.8554167	0.5204165	0.8354191	2.8754143	12.7110314	1	0.0003635
X2*X2	-1.3820833	0.5204165	-2.4020809	-0.3620857	7.0528776	1	0.0079138
X3*X3	-4.0970833	0.5204165	-5.1170809	-3.0770857	61.9794160	1	0E-7

Results, Y_5:

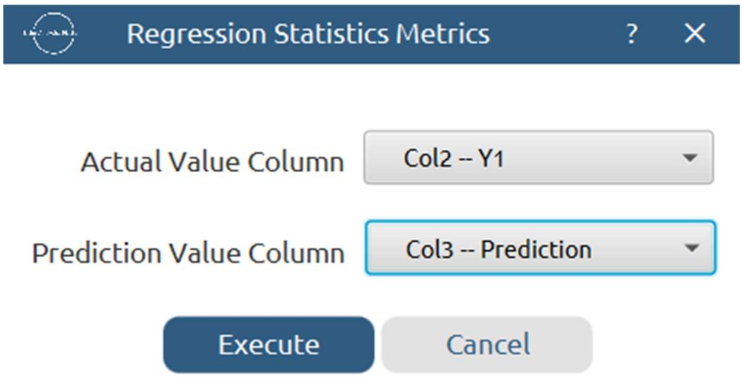
Y5	Prediction
90.25	89.9737500
82.1	82.6437500
98.32	97.7762500
84.9	85.17625
93.85	92.80625
82.93	81.06625
94.15	96.0137500
86.78	87.8237500
72.03	73.3500000
90.2	91.7875000
93.19	91.6025000
84.82	83.5000000
86.99	86.1366667
84.29	86.1366667
87.13	86.1366667

Goodness of Fit	
	Value
Deviance	23.5200917
Scaled Deviance	23.5200917
Pearson Chi-Square	23.5200917
Scaled Pearson Chi-Square	23.5200917
Log Likelihood	-25.5441238
Akaike's Information Criterion (AIC)	71.0882477
Finite Sample Corrected AIC (AICC)	126.0882477
Bayesian Information Criterion (BIC)	78.1687497
Consistent AIC (CAIC)	88.1687497

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	86.1366667	0.5773503	85.0050809	87.2682524	22258.5760333	1	0.0
X1	-4.9825000	0.3535534	-5.6754519	-4.2895481	198.6024500	1	0.0
X2	2.5837500	0.3535534	1.8907981	3.2767019	53.4061125	1	0E-7
X3	2.4912500	0.3535534	1.7982981	3.1842019	49.6506125	1	0E-7
X1*X3	0.8875000	0.5	-0.0924820	1.8674820	3.1506250	1	0.0758979
X1*X2	-1.3175000	0.5	-2.2974820	-0.3375180	6.9432250	1	0.0084137
X2*X3	-6.6350000	0.5	-7.6149820	-5.6550180	176.0929000	1	0.0
X1*X1	3.5616667	0.5204165	2.5416691	4.5816643	46.8386564	1	0E-7
X2*X2	-0.8058333	0.5204165	-1.8258309	0.2141643	2.3976641	1	0.1215166
X3*X3	-0.2708333	0.5204165	-1.2908309	0.7491643	0.2708333	1	0.6027733

Step 6: 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*



	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		134.5111111	11.5978925	10.0888889	0.9918925

Repeat this step for the rest of the response variables. Results, Y_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		0.0062778	0.0792324	0.0655556	0.9683721

Results, Y_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		0.0066161	0.0813395	0.0718889	0.9932575

Results, Y_4:

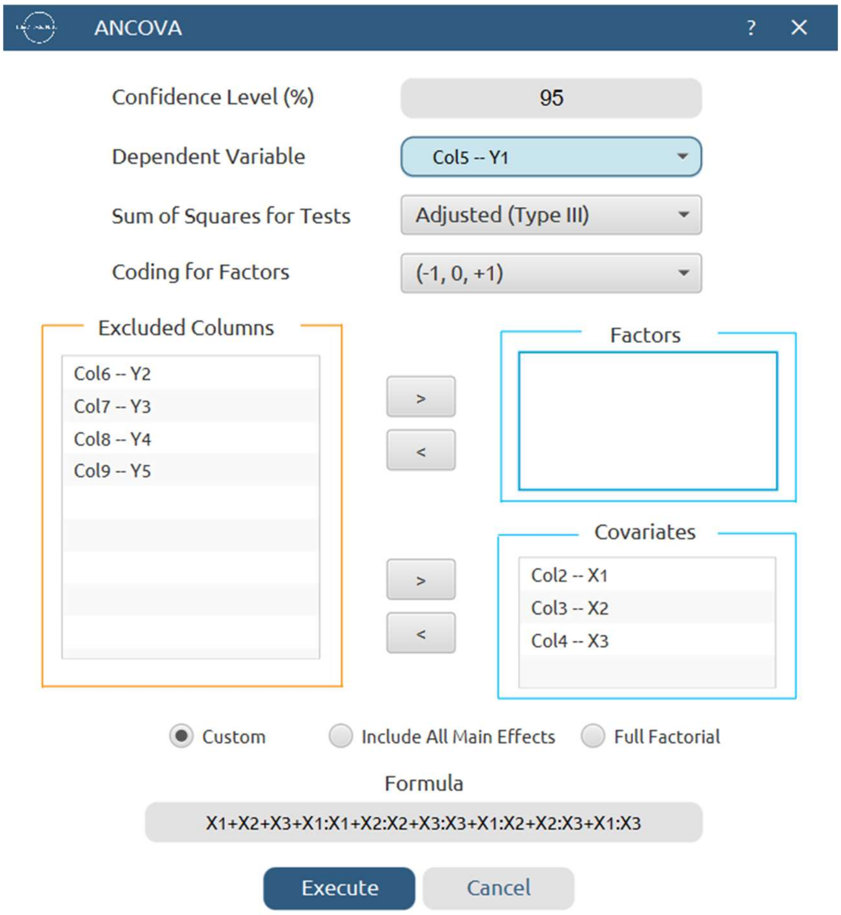
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		1.6566494	1.2871089	1.1058889	0.9758246

Results, Y_5:

	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		1.5680061	1.2522005	1.1308889	0.9582497

Step 7: 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	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			

Repeat this step for the rest of the response variables. Results, Y_2:

	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	0.0112500	0.0112500	0.5973451	0.4745247
2		X2	1	0.0112500	0.0112500	0.5973451	0.4745247
3		X3	1	2.4200000	2.4200000	128.4955752	0.0000934
4		X1*X1	1	0.0502564	0.0502564	2.6684820	0.1632817
5		X2*X2	1	0.0041026	0.0041026	0.2178353	0.6603242
6		X3*X3	1	0.3510256	0.3510256	18.6385296	0.0075904
7		X1*X2	1	0.0100000	0.0100000	0.5309735	0.4988816
8		X2*X3	1	0.0025000	0.0025000	0.1327434	0.7305054
9		X1*X3	1	0.0025000	0.0025000	0.1327434	0.7305054
10		Error	5	0.0941667	0.0188333		
11		Total	14	2.9773333			

Results, Y_3:

	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	0.1770125	0.1770125	8.9182551	0.0305757
2		X2	1	0.0001125	0.0001125	0.0056680	0.9429066
3		X3	1	13.7812500	13.7812500	694.3278193	0.0000015
4		X1*X1	1	0.0002314	0.0002314	0.0116589	0.9182134
5		X2*X2	1	0.0010776	0.0010776	0.0542899	0.8249979
6		X3*X3	1	0.1269391	0.1269391	6.3954540	0.0525990
7		X1*X2	1	0.4032250	0.4032250	20.3153078	0.0063570
8		X2*X3	1	0.1225000	0.1225000	6.1718028	0.0555468
9		X1*X3	1	0.0049000	0.0049000	0.2468721	0.6403586
10		Error	5	0.0992417	0.0198483		
11		Total	14	14.7188933			

Results, Y_4:

	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	813.2544500	813.2544500	163.6343872	0.0000520
2		X2	1	78.1875125	78.1875125	15.7320574	0.0106748
3		X3	1	8.9253125	8.9253125	1.7958562	0.2378861
4		X1*X1	1	12.7110314	12.7110314	2.5575782	0.1706616
5		X2*X2	1	7.0528776	7.0528776	1.4191048	0.2870111
6		X3*X3	1	61.9794160	61.9794160	12.4708371	0.0167147
7		X1*X2	1	16.2409000	16.2409000	3.2678207	0.1304561
8		X2*X3	1	1.2882250	1.2882250	0.2592029	0.6323335
9		X1*X3	1	0.0961000	0.0961000	0.0193362	0.8948342
10		Error	5	24.8497417	4.9699483		
11		Total	14	1027.8957600			

Results, Y_5:

	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	198.6024500	198.6024500	42.2197440	0.0012887
2		X2	1	53.4061125	53.4061125	11.3532960	0.0199013
3		X3	1	49.6506125	49.6506125	10.5549360	0.0227252
4		X1*X1	1	46.8386564	46.8386564	9.9571586	0.0252225
5		X2*X2	1	2.3976641	2.3976641	0.5097055	0.5071985
6		X3*X3	1	0.2708333	0.2708333	0.0575749	0.8198976
7		X1*X2	1	6.9432250	6.9432250	1.4760200	0.2786353
8		X2*X3	1	176.0929000	176.0929000	37.4345692	0.0016913
9		X1*X3	1	3.1506250	3.1506250	0.6697731	0.4503589
10		Error	5	23.5200917	4.7040183		
11		Total	14	563.3520400			

References

(1) Malladi, M.; Jukanti, R. Formulation Development and Evaluation of a Novel Bi-Dependent Clarithromycin Gastroretentive Drug Delivery System Using Box-Behnken Design. *Journal of Drug Delivery Science and Technology* **2016**, *35*, 134–145. https://doi.org/10.1016/j.jddst.2016.06.003.