Development of pellets for oral lysozyme delivery by using a quality by design approach

The objectives of this research paper are to identify how the critical process parameters influence the critical quality attributes of a lysozyme-containing multiparticulate dosage form, and to determine the critical points of API activity preservation. Design of experiments (DoE) methodology is implemented in order to determine the effects of the adjustable critical process parameters.

The factors (independent variables) examined are: X_1 = impeller speed (rpm), X_2 = liquid addition (ml/min), X_3 = extrusion speed (rpm), X_4 = spheronizer speed (rpm) and X_5 = spheronization time (min). All the factors are continuous. The responses (dependent variables) examined are: Y_1 = activity (%), Y_2 = hardness (N) and Y_3 = roundness. The applied DoE method is 2^5 full factorial design.

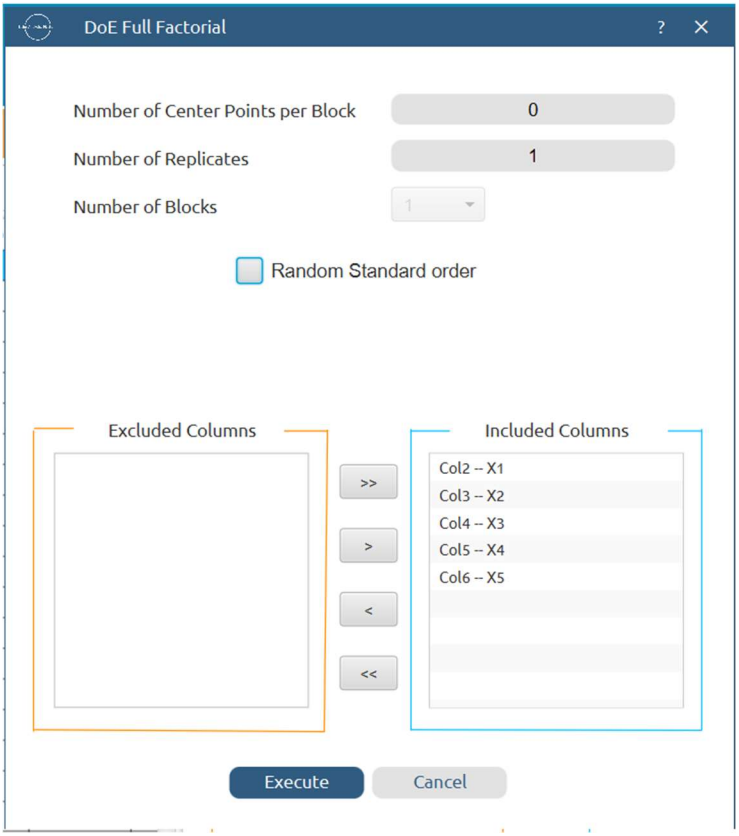
Isalos version used: 2.1.0

Scientific article: https://www.sciencedirect.com/science/article/abs/pii/S0263876215004918

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)	Col5 (I)	Col6 (I)
User Header	User Row ID	X1	X2	X3	X4	X5
1		500	5	70	1000	15
2		1500	10	120	2000	30



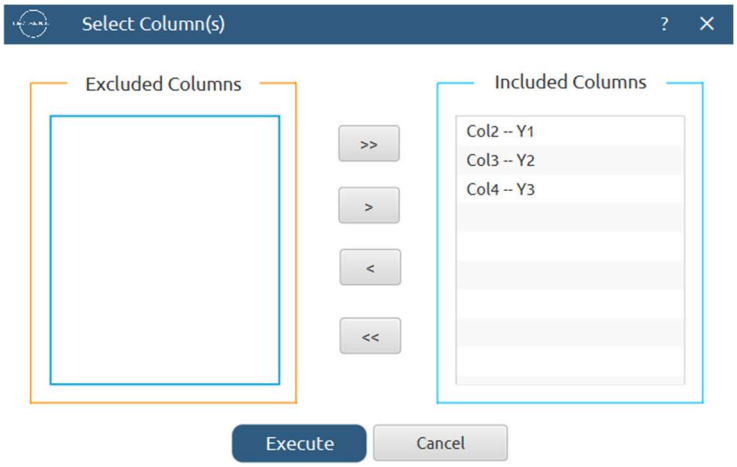
Results (right spreadsheet):

	Col1	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)	Col10 (D)
User Header	User Row ID	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3	X4	X5
1		1	Block: 1	Replicate: 1	Design Point	1500.0	10.0	120.0	1000.0	15.0
2		2	Block: 1	Replicate: 1	Design Point	500.0	10.0	120.0	1000.0	15.0
3		3	Block: 1	Replicate: 1	Design Point	1500.0	5.0	120.0	1000.0	15.0
4		4	Block: 1	Replicate: 1	Design Point	500.0	5.0	120.0	1000.0	15.0
5		5	Block: 1	Replicate: 1	Design Point	1500.0	10.0	70.0	1000.0	15.0
6		6	Block: 1	Replicate: 1	Design Point	500.0	10.0	70.0	1000.0	15.0
7		7	Block: 1	Replicate: 1	Design Point	1500.0	5.0	70.0	1000.0	15.0
8		8	Block: 1	Replicate: 1	Design Point	500.0	5.0	70.0	1000.0	15.0
9		9	Block: 1	Replicate: 1	Design Point	1500.0	10.0	120.0	2000.0	15.0
10		10	Block: 1	Replicate: 1	Design Point	500.0	10.0	120.0	2000.0	15.0
11		11	Block: 1	Replicate: 1	Design Point	1500.0	5.0	120.0	2000.0	15.0
12		12	Block: 1	Replicate: 1	Design Point	500.0	5.0	120.0	2000.0	15.0
13		13	Block: 1	Replicate: 1	Design Point	1500.0	10.0	70.0	2000.0	15.0
14		14	Block: 1	Replicate: 1	Design Point	500.0	10.0	70.0	2000.0	15.0
15		15	Block: 1	Replicate: 1	Design Point	1500.0	5.0	70.0	2000.0	15.0
16		16	Block: 1	Replicate: 1	Design Point	500.0	5.0	70.0	2000.0	15.0
17		17	Block: 1	Replicate: 1	Design Point	1500.0	10.0	120.0	1000.0	30.0
18		18	Block: 1	Replicate: 1	Design Point	500.0	10.0	120.0	1000.0	30.0
19		19	Block: 1	Replicate: 1	Design Point	1500.0	5.0	120.0	1000.0	30.0
20		20	Block: 1	Replicate: 1	Design Point	500.0	5.0	120.0	1000.0	30.0
21		21	Block: 1	Replicate: 1	Design Point	1500.0	10.0	70.0	1000.0	30.0

Step 2: Definition of response variables

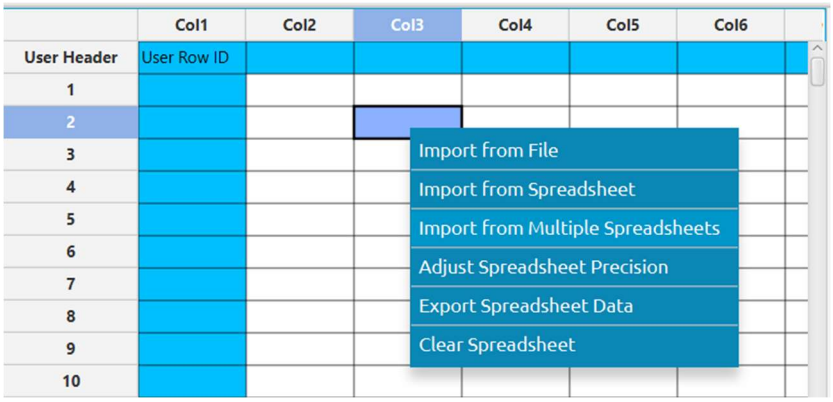
Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the full factorial method. Then, select all columns to be transferred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

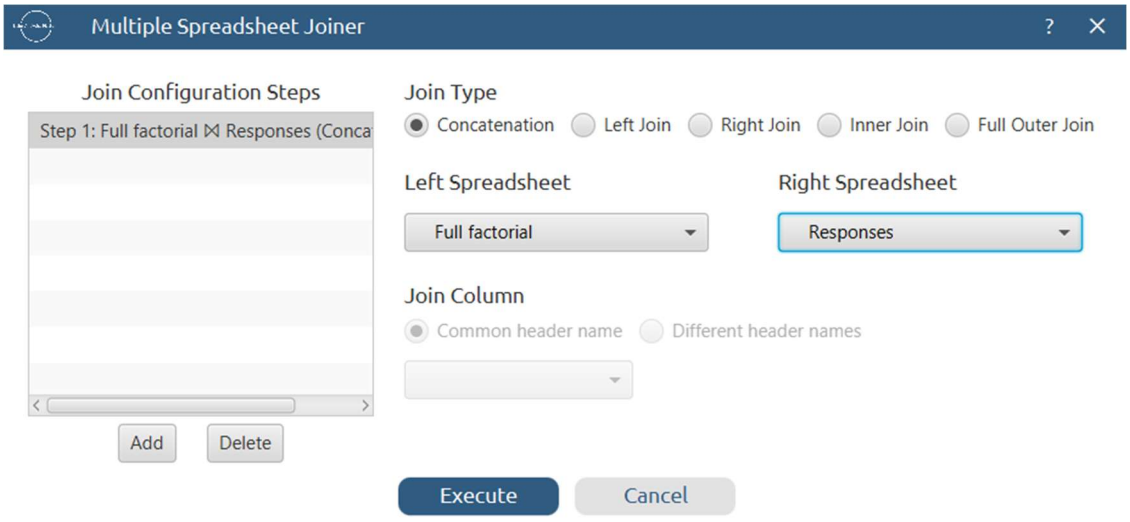
	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	Y1	Y2	Y3
1		67.87565	8.87	1.38
2		84.45596	5.71	1.34
3		79.79275	4.45	1.39
4		71.50259	5.94	1.2
5		70.46632	5.29	1.4
6		96.89119	2.42	1.3
7		69.43005	4.99	1.37
8		68.39378	9.18	1.27
9		47.15026	6.57	1.27
10		65.80311	3.14	1.34
11		81.34715	3.48	1.28
12		77.72021	3.86	1.17
13		83.93782	5.36	1.23
14		71.50259	3.01	1.22
15		75.12953	3.87	1.27
16		70.46632	6.39	1.16
17		74.09326	4.9	1.4
18		78.75648	2.42	1.29
19		72.53886	3.48	1.42
20		67.87565	6.82	1.22
21		56.47668	7.71	1.39



Step 3: Data isolation

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 responses columns to be transferred to the right spreadsheet: *Data Transformation → Data Manipulation → Select Column(s)*



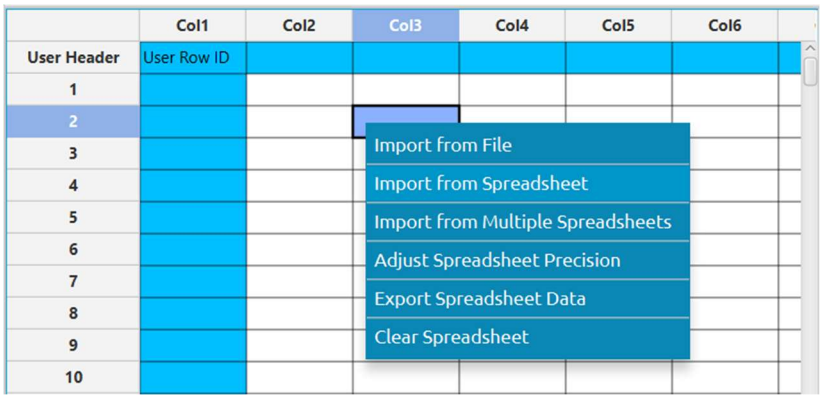


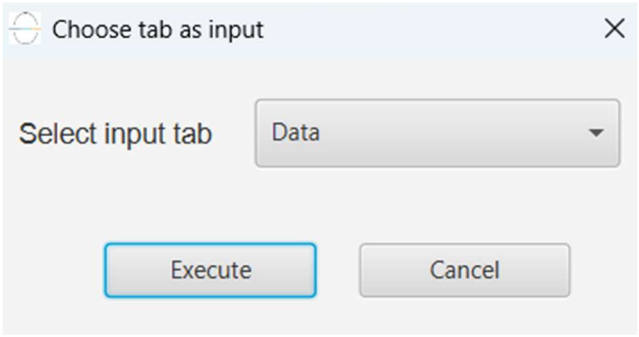
Results:

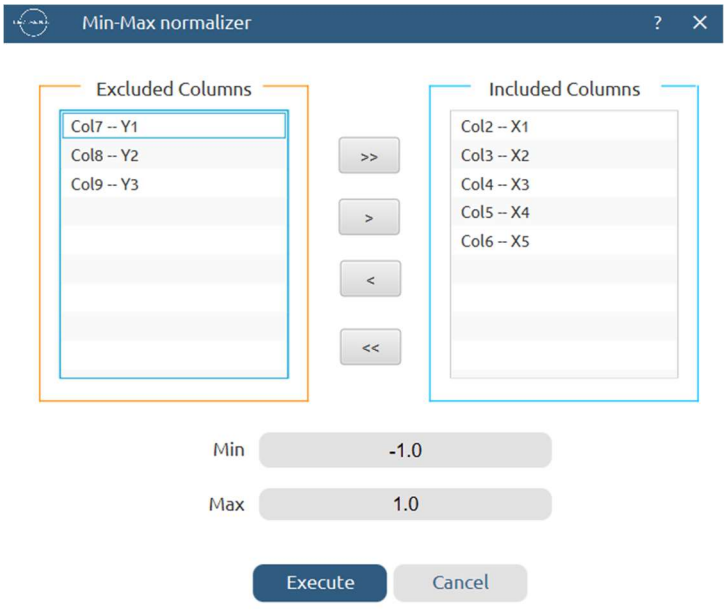
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	X4	X5	Y1	Y2	Y3
1		1500.0	10.0	120.0	1000.0	15.0	67.87565	8.87	1.38
2		500.0	10.0	120.0	1000.0	15.0	84.45596	5.71	1.34
3		1500.0	5.0	120.0	1000.0	15.0	79.79275	4.45	1.39
4		500.0	5.0	120.0	1000.0	15.0	71.50259	5.94	1.2
5		1500.0	10.0	70.0	1000.0	15.0	70.46632	5.29	1.4
6		500.0	10.0	70.0	1000.0	15.0	96.89119	2.42	1.3
7		1500.0	5.0	70.0	1000.0	15.0	69.43005	4.99	1.37
8		500.0	5.0	70.0	1000.0	15.0	68.39378	9.18	1.27
9		1500.0	10.0	120.0	2000.0	15.0	47.15026	6.57	1.27
10		500.0	10.0	120.0	2000.0	15.0	65.80311	3.14	1.34
11		1500.0	5.0	120.0	2000.0	15.0	81.34715	3.48	1.28
12		500.0	5.0	120.0	2000.0	15.0	77.72021	3.86	1.17
13		1500.0	10.0	70.0	2000.0	15.0	83.93782	5.36	1.23
14		500.0	10.0	70.0	2000.0	15.0	71.50259	3.01	1.22
15		1500.0	5.0	70.0	2000.0	15.0	75.12953	3.87	1.27
16		500.0	5.0	70.0	2000.0	15.0	70.46632	6.39	1.16
17		1500.0	10.0	120.0	1000.0	30.0	74.09326	4.9	1.4
18		500.0	10.0	120.0	1000.0	30.0	78.75648	2.42	1.29
19		1500.0	5.0	120.0	1000.0	30.0	72.53886	3.48	1.42
20		500.0	5.0	120.0	1000.0	30.0	67.87565	6.82	1.22
21		1500.0	10.0	70.0	1000.0	30.0	56.47668	7.71	1.39
22		500.0	10.0	70.0	1000.0	30.0	86.5285	2.55	1.2
23		1500.0	5.0	70.0	1000.0	30.0	62.6943	4.79	1.4
24		500.0	5.0	70.0	1000.0	30.0	72.53886	6.39	1.25
25		1500.0	10.0	120.0	2000.0	30.0	66.83938	7.18	1.35
26		500.0	10.0	120.0	2000.0	30.0	62.6943	3.78	1.32
27		1500.0	5.0	120.0	2000.0	30.0	84.97409	5.41	1.39
28		500.0	5.0	120.0	2000.0	30.0	80.82902	4.24	1.31
29		1500.0	10.0	70.0	2000.0	30.0	50.7772	6	1.29
30		500.0	10.0	70.0	2000.0	30.0	94.81865	3.16	1.27
31		1500.0	5.0	70.0	2000.0	30.0	77.20207	3.27	1.29
32		500.0	5.0	70.0	2000.0	30.0	75.64767	6.48	1.14

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:

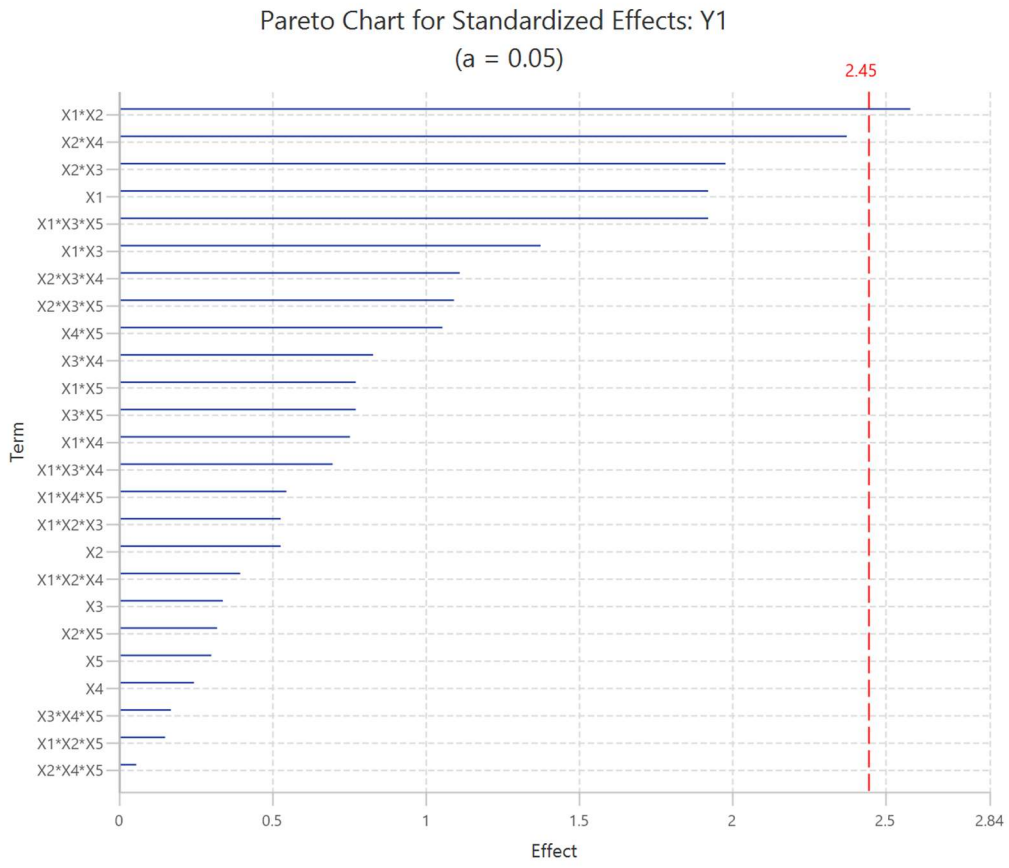
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	X4	X5	Y1	Y2	Y3
1		1.0	1.0	1.0	-1.0	-1.0	67.87565	8.87	1.38
2		-1.0	1.0	1.0	-1.0	-1.0	84.45596	5.71	1.34
3		1.0	-1.0	1.0	-1.0	-1.0	79.79275	4.45	1.39
4		-1.0	-1.0	1.0	-1.0	-1.0	71.50259	5.94	1.2
5		1.0	1.0	-1.0	-1.0	-1.0	70.46632	5.29	1.4
6		-1.0	1.0	-1.0	-1.0	-1.0	96.89119	2.42	1.3
7		1.0	-1.0	-1.0	-1.0	-1.0	69.43005	4.99	1.37
8		-1.0	-1.0	-1.0	-1.0	-1.0	68.39378	9.18	1.27
9		1.0	1.0	1.0	1.0	-1.0	47.15026	6.57	1.27
10		-1.0	1.0	1.0	1.0	-1.0	65.80311	3.14	1.34
11		1.0	-1.0	1.0	1.0	-1.0	81.34715	3.48	1.28
12		-1.0	-1.0	1.0	1.0	-1.0	77.72021	3.86	1.17
13		1.0	1.0	-1.0	1.0	-1.0	83.93782	5.36	1.23
14		-1.0	1.0	-1.0	1.0	-1.0	71.50259	3.01	1.22
15		1.0	-1.0	-1.0	1.0	-1.0	75.12953	3.87	1.27
16		-1.0	-1.0	-1.0	1.0	-1.0	70.46632	6.39	1.16
17		1.0	1.0	1.0	-1.0	1.0	74.09326	4.9	1.4
18		-1.0	1.0	1.0	-1.0	1.0	78.75648	2.42	1.29
19		1.0	-1.0	1.0	-1.0	1.0	72.53886	3.48	1.42
20		-1.0	-1.0	1.0	-1.0	1.0	67.87565	6.82	1.22
21		1.0	1.0	-1.0	-1.0	1.0	56.47668	7.71	1.39
22		-1.0	1.0	-1.0	-1.0	1.0	86.5285	2.55	1.2
23		1.0	-1.0	-1.0	-1.0	1.0	62.6943	4.79	1.4
24		-1.0	-1.0	-1.0	-1.0	1.0	72.53886	6.39	1.25
25		1.0	1.0	1.0	1.0	1.0	66.83938	7.18	1.35
26		-1.0	1.0	1.0	1.0	1.0	62.6943	3.78	1.32
27		1.0	-1.0	1.0	1.0	1.0	84.97409	5.41	1.39
28		-1.0	-1.0	1.0	1.0	1.0	80.82902	4.24	1.31
29		1.0	1.0	-1.0	1.0	1.0	50.7772	6	1.29
30		-1.0	1.0	-1.0	1.0	1.0	94.81865	3.16	1.27
31		1.0	-1.0	-1.0	1.0	1.0	77.20207	3.27	1.29
32		-1.0	-1.0	-1.0	1.0	1.0	75.64767	6.48	1.14

Step 5: Pareto analysis

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

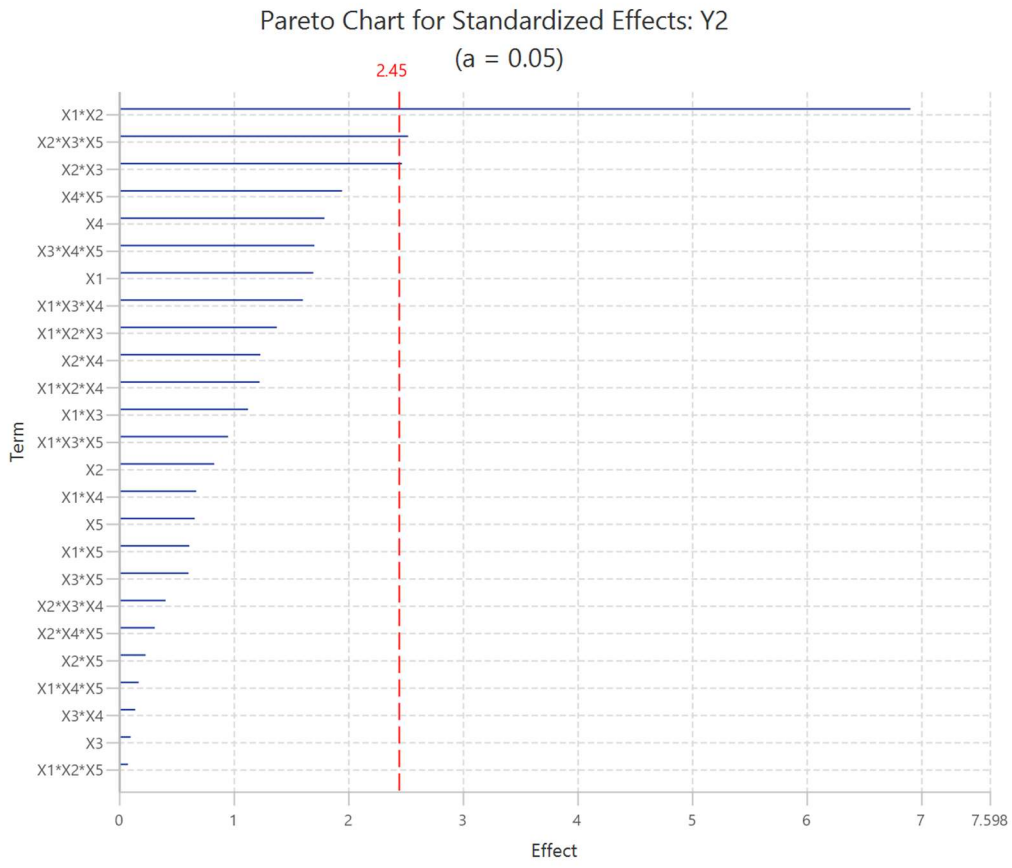
Results:

	Col1	Col2 (S)	Col3 (S)
User Header	User Row ID	Pareto Analysis of :	Standardized Effects
1		Variable	Effect
2		X1*X2	2.5818438
3		X2*X4	2.3745425
4		X2*X3	1.9787854
5		X1	1.9222492
6		X1*X3*X5	1.9222484
7		X1*X3	1.3757270
8		X2*X3*X4	1.1118888
9		X2*X3*X5	1.0930434
10		X4*X5	1.0553522
11		X3*X4	0.8292052
12		X1*X5	0.7726690
13		X3*X5	0.7726683
14		X1*X4	0.7538229
15		X1*X3*X4	0.6972863
16		X1*X4*X5	0.5465213
17		X1*X2*X3	0.5276762
18		X2	0.5276762
19		X1*X2*X4	0.3957577
20		X3	0.3392196
21		X2*X5	0.3203750
22		X5	0.3015292
23		X4	0.2449926
24		X3*X4*X5	0.1696103
25		X1*X2*X5	0.1507645
26		X2*X4*X5	0.0565368
27		Significance Value	2.4469119



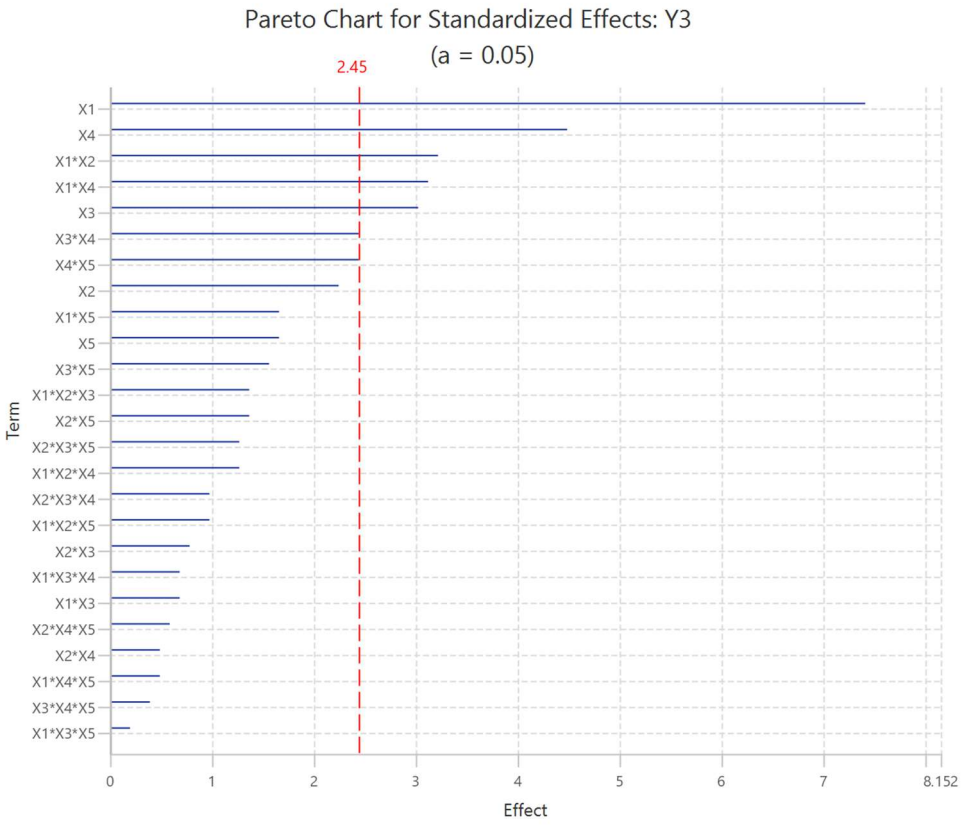
Repeat this step for the rest of the response variables. Results, Y_2:

	Col1	Col2 (S)	Col3 (S)
User Header	User Row ID	Pareto Analysis of :	Standardized Effects
1		Variable	Effect
2		X1*X2	6.9072096
3		X2*X3*X5	2.5234339
4		X2*X3	2.4698507
5		X4*X5	1.9474145
6		X4	1.7933628
7		X3*X4*X5	1.7062901
8		X1	1.6962432
9		X1*X3*X4	1.6058216
10		X1*X2*X3	1.3780930
11		X2*X4	1.2340881
12		X1*X2*X4	1.2273902
13		X1*X3	1.1269217
14		X1*X3*X5	0.9527763
15		X2	0.8322141
16		X1*X4	0.6748134
17		X5	0.6614176
18		X1*X5	0.6145323
19		X3*X5	0.6078344
20		X2*X3*X4	0.4068974
21		X2*X4*X5	0.3131268
22		X2*X5	0.2327520
23		X1*X4*X5	0.1724709
24		X3*X4	0.1423304
25		X3	0.1021430
26		X1*X2*X5	0.0787003
27		Significance Value	2.4469119



Results, Y_3:

	Col1	Col2 (S)	Col3 (S)
User Header	User Row ID	Pareto Analysis of :	Standardized Effects
1		Variable	Effect
2		X1	7.4109612
3		X4	4.4855818
4		X1*X2	3.2179174
5		X1*X4	3.1204047
6		X3	3.0228921
7		X3*X4	2.4378162
8		X4*X5	2.4378162
9		X2	2.2427909
10		X1*X5	1.6577150
11		X5	1.6577150
12		X3*X5	1.5602024
13		X1*X2*X3	1.3651771
14		X2*X5	1.3651771
15		X2*X3*X5	1.2676644
16		X1*X2*X4	1.2676644
17		X2*X3*X4	0.9751265
18		X1*X2*X5	0.9751265
19		X2*X3	0.7801012
20		X1*X3*X4	0.6825885
21		X1*X3	0.6825885
22		X2*X4*X5	0.5850759
23		X2*X4	0.4875632
24		X1*X4*X5	0.4875632
25		X3*X4*X5	0.3900506
26		X1*X3*X5	0.1950253
27		Significance Value	2.4469119

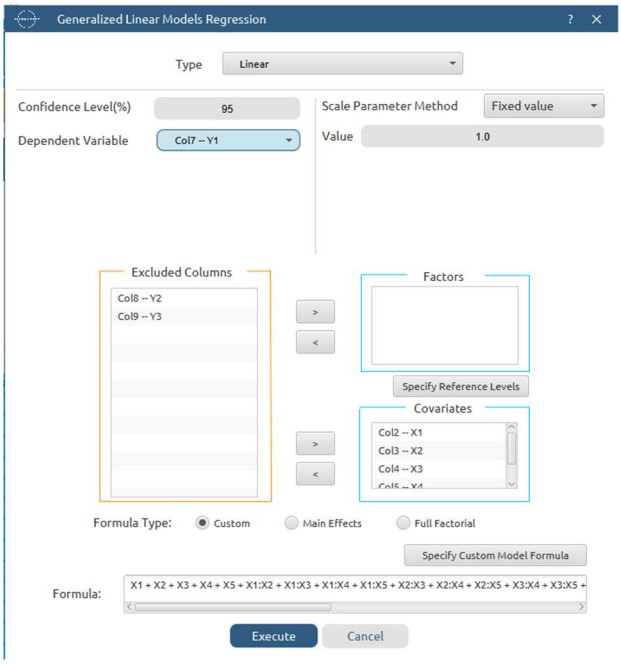


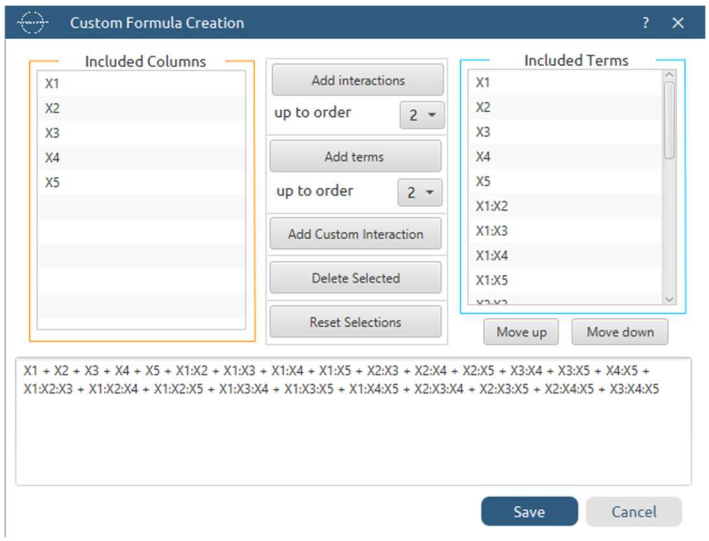
Step 6: Regression

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

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{15}X_1X_5 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{25}X_2X_5 + b_{34}X_3X_4 + b_{35}X_3X_5 + b_{45}X_4X_5 + b_{123}X_1X_2X_3 + b_{124}X_1X_2X_4 + b_{125}X_1X_2X_5 + b_{134}X_1X_3X_4 + b_{135}X_1X_3X_5 + b_{145}X_1X_4X_5 + b_{234}X_2X_3X_4 + b_{235}X_2X_3X_5 + b_{245}X_2X_4X_5 + b_{345}X_3X_4X_5$$

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:

	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y1	Prediction
1		67.87565	68.4585512
2		84.45596	84.2616594
3		79.79275	75.4533719
4		71.50259	75.4533675
5		70.46632	73.2512931
6		96.89119	93.7176162
7		69.43005	70.4015537
8		68.39378	67.8108769
9		47.15026	51.8134731
10		65.80311	60.7512962
11		81.34715	80.4404137
12		77.72021	79.0155469
13		83.93782	75.9067325
14		71.50259	79.9222781
15		75.12953	79.4041406
16		70.46632	65.8031087
17		74.09326	73.6398931
18		78.75648	78.8212463
19		72.53886	76.7487037
20		67.87565	64.0544069
21		56.47668	53.5621725
22		86.5285	89.8316081
23		62.6943	61.8523306
24		72.53886	72.9922287
25		66.83938	62.0466325
26		62.6943	67.8756481
27		84.97409	86.0103606
28		80.82902	79.4041487
29		50.7772	58.9378219
30		94.81865	86.2694275
31		77.20207	72.7979250
32		75.64767	80.4404156

Goodness of Fit	
	Value
Deviance	566.9279329
Scaled Deviance	566.9279329
Pearson Chi-Square	566.9279329
Scaled Pearson Chi-Square	566.9279329
Log Likelihood	-312.8699995
Akaike's Information Criterion (AIC)	677.7399990
Finite Sample Corrected AIC (AICC)	958.5399990
Bayesian Information Criterion (BIC)	715.8491325
Consistent AIC (CAIC)	741.8491325

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	73.3484453	0.1767767	73.0019694	73.6949213	172159.8217523	1	0.0
X1	-3.3031097	0.1767767	-3.6495856	-2.9566337	349.1370754	1	0.0
X2	-0.9067359	0.1767767	-1.2532119	-0.5602600	26.3094419	1	3E-7
X3	-0.5829003	0.1767767	-0.9293763	-0.2364244	10.8727288	1	0.0009759
X4	-0.4209847	0.1767767	-0.7674606	-0.0745087	5.6712994	1	0.0172447
X5	-0.5181347	0.1767767	-0.8646106	-0.1716587	8.5908337	1	0.0033786
X1*X2	-4.4365284	0.1767767	-4.7830044	-4.0900525	629.8491065	1	0.0
X1*X3	2.3639897	0.1767767	2.0175137	2.7104656	178.8303118	1	0.0
X1*X4	1.2953366	0.1767767	0.9488606	1.6418125	53.6926979	1	0E-7
X1*X5	-1.3277209	0.1767767	-1.6741969	-0.9812450	56.4109724	1	0E-7
X2*X3	-3.4002591	0.1767767	-3.7467350	-3.0537831	369.9763741	1	0.0
X2*X4	-4.0803109	0.1767767	-4.4267869	-3.7338350	532.7659951	1	0.0
X2*X5	-0.5505184	0.1767767	-0.8969944	-0.2040425	9.6982576	1	0.0018444
X3*X4	-1.4248703	0.1767767	-1.7713463	-1.0783944	64.9681730	1	0E-7
X3*X5	1.3277197	0.1767767	0.9812437	1.6741956	56.4108662	1	0E-7
X4*X5	1.8134716	0.1767767	1.4669956	2.1599475	105.2377315	1	0.0
X1*X2*X3	0.9067359	0.1767767	0.5602600	1.2532119	26.3094419	1	3E-7
X1*X2*X4	0.6800528	0.1767767	0.3335769	1.0265288	14.7990985	1	0.0001196
X1*X2*X5	-0.2590672	0.1767767	-0.6055431	0.0874088	2.1477058	1	0.1427831
X1*X3*X4	-1.1981866	0.1767767	-1.5446625	-0.8517106	45.9408332	1	0E-7
X1*X3*X5	3.3031084	0.1767767	2.9566325	3.6495844	349.1368112	1	0.0
X1*X4*X5	-0.9391184	0.1767767	-1.2855944	-0.5926425	28.2221901	1	1E-7
X2*X3*X4	-1.9106216	0.1767767	-2.2570975	-1.5641456	116.8151922	1	0.0
X2*X3*X5	1.8782384	0.1767767	1.5317625	2.2247144	112.8889481	1	0.0
X2*X4*X5	0.0971503	0.1767767	-0.2493256	0.4436263	0.3020219	1	0.5826177
X3*X4*X5	0.2914509	0.1767767	-0.0550250	0.6379269	2.7181968	1	0.0992100

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

	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y2	Prediction
1		8.87	8.3450000
2		5.71	5.5881250
3		4.45	4.4043750
4		5.94	6.6325
5		5.29	5.8943750
6		2.42	2.4625000
7		4.99	4.9562500
8		9.18	8.566875
9		6.57	7.0143750
10		3.14	3.3425000
11		3.48	3.6062500
12		3.86	3.0868750
13		5.36	4.8362500
14		3.01	2.8868750
15		3.87	3.8231250
16		6.39	7.08375
17		4.9	5.3293750
18		2.42	2.6375000
19		3.48	3.6212500
20		6.82	6.0318750
21		7.71	7.20125
22		2.55	2.4118750
23		4.79	4.728125
24		6.39	7.0987500
25		7.18	6.8312500
26		3.78	3.4818750
27		5.41	5.1881250
28		4.24	5.1087500
29		6	6.4281250
30		3.16	3.3787500
31		3.27	3.4125000
32		6.48	5.6906250

Goodness of Fit	
	Value
Deviance	6.6871938
Scaled Deviance	6.6871938
Pearson Chi-Square	6.6871938
Scaled Pearson Chi-Square	6.6871938
Log Likelihood	-32.7496299
Akaike's Information Criterion (AIC)	117.4992599
Finite Sample Corrected AIC (AICC)	398.2992599
Bayesian Information Criterion (BIC)	155.6083933
Consistent AIC (CAIC)	181.6083933

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	5.0346875	0.1767767	4.6882115	5.3811635	811.1385031	1	0.0
X1	0.3165625	0.1767767	-0.0299135	0.6630385	3.2067781	1	0.0733338
X2	-0.1553125	0.1767767	-0.5017885	0.1911635	0.7719031	1	0.3796289
X3	-0.0190625	0.1767767	-0.3655385	0.3274135	0.0116281	1	0.9141275
X4	-0.3346875	0.1767767	-0.6811635	0.0117885	3.5845031	1	0.0583209
X5	-0.1234375	0.1767767	-0.4699135	0.2230385	0.4875781	1	0.4850096
X1*X2	1.2890625	0.1767767	0.9425865	1.6355385	53.1738281	1	0E-7
X1*X3	0.2103125	0.1767767	-0.1361635	0.5567885	1.4154031	1	0.2341615
X1*X4	0.1259375	0.1767767	-0.2205385	0.4724135	0.5075281	1	0.4762109
X1*X5	0.1146875	0.1767767	-0.2317885	0.4611635	0.4209031	1	0.5164867
X2*X3	0.4609375	0.1767767	0.1144615	0.8074135	6.7988281	1	0.0091218
X2*X4	0.2303125	0.1767767	-0.1161635	0.5767885	1.6974031	1	0.1926279
X2*X5	-0.0434375	0.1767767	-0.3899135	0.3030385	0.0603781	1	0.8058993
X3*X4	0.0265625	0.1767767	-0.3199135	0.3730385	0.0225781	1	0.8805593
X3*X5	-0.1134375	0.1767767	-0.4599135	0.2330385	0.4117781	1	0.5210684
X4*X5	0.3634375	0.1767767	0.0169615	0.7099135	4.2267781	1	0.0397909
X1*X2*X3	-0.2571875	0.1767767	-0.6036635	0.0892885	2.1166531	1	0.1457046
X1*X2*X4	-0.2290625	0.1767767	-0.5755385	0.1174135	1.6790281	1	0.1950536
X1*X2*X5	0.0146875	0.1767767	-0.3317885	0.3611635	0.0069031	1	0.9337839
X1*X3*X4	0.2996875	0.1767767	-0.0467885	0.6461635	2.8740031	1	0.0900207
X1*X3*X5	-0.1778125	0.1767767	-0.5242885	0.1686635	1.0117531	1	0.3144832
X1*X4*X5	-0.0321875	0.1767767	-0.3786635	0.3142885	0.0331531	1	0.8555199
X2*X3*X4	-0.0759375	0.1767767	-0.4224135	0.2705385	0.1845281	1	0.6675104
X2*X3*X5	-0.4709375	0.1767767	-0.8174135	-0.1244615	7.0970281	1	0.0077212
X2*X4*X5	0.0584375	0.1767767	-0.2880385	0.4049135	0.1092781	1	0.7409675
X3*X4*X5	0.3184375	0.1767767	-0.0280385	0.6649135	3.2448781	1	0.0716470

Results, Y_3:

	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y3	Prediction
1		1.38	1.3981250
2		1.34	1.3431250
3		1.39	1.3668750
4		1.2	1.2018750
5		1.4	1.3818750
6		1.3	1.296875
7		1.37	1.3931250
8		1.27	1.2681250
9		1.27	1.2631250
10		1.34	1.3256250
11		1.28	1.2918750
12		1.17	1.1793750
13		1.23	1.2368750
14		1.22	1.2343750
15		1.27	1.2581250
16		1.16	1.1506250
17		1.4	1.3993750
18		1.29	1.2693750
19		1.42	1.4256250
20		1.22	1.2356250
21		1.39	1.3906250
22		1.2	1.220625
23		1.4	1.3943750
24		1.25	1.2343750
25		1.35	1.339375
26		1.32	1.3518750
27		1.39	1.3956250
28		1.31	1.2831250
29		1.29	1.3006250
30		1.27	1.2381250
31		1.29	1.2843750
32		1.14	1.1668750

Goodness of Fit	
	Value
Deviance	0.0078875
Scaled Deviance	0.0078875
Pearson Chi-Square	0.0078875
Scaled Pearson Chi-Square	0.0078875
Log Likelihood	-29.4099768
Akaike's Information Criterion (AIC)	110.8199536
Finite Sample Corrected AIC (AICC)	391.6199536
Bayesian Information Criterion (BIC)	148.9290871
Consistent AIC (CAIC)	174.9290871

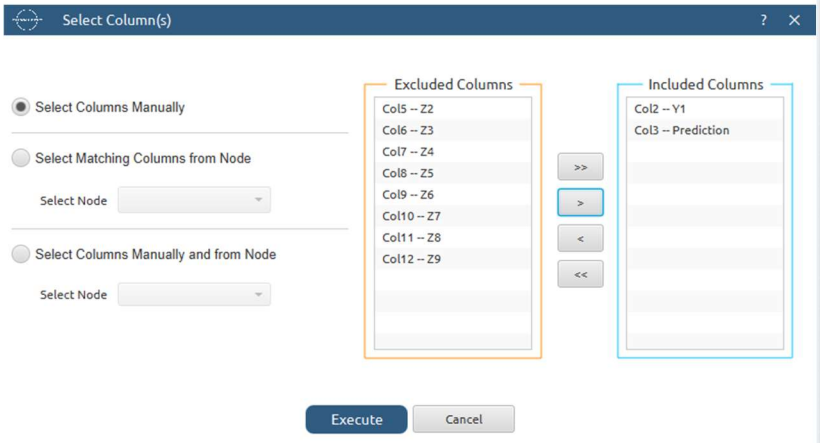
Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	1.2975000	0.1767767	0.9510240	1.6439760	53.8722000	1	0E-7
X1	0.0475000	0.1767767	-0.2989760	0.3939760	0.0722000	1	0.7881601
X2	0.0143750	0.1767767	-0.3321010	0.3608510	0.0066125	1	0.9351896
X3	0.0193750	0.1767767	-0.3271010	0.3658510	0.0120125	1	0.9127254
X4	-0.0287500	0.1767767	-0.3752260	0.3177260	0.0264500	1	0.8708062
X5	0.0106250	0.1767767	-0.3358510	0.3571010	0.0036125	1	0.9520727
X1*X2	-0.0206250	0.1767767	-0.3671010	0.3258510	0.0136125	1	0.9071195
X1*X3	-0.0043750	0.1767767	-0.3508510	0.3421010	0.0006125	1	0.9802554
X1*X4	-0.0200000	0.1767767	-0.3664760	0.3264760	0.0128000	1	0.9099219
X1*X5	0.0106250	0.1767767	-0.3358510	0.3571010	0.0036125	1	0.9520727
X2*X3	0.0050000	0.1767767	-0.3414760	0.3514760	0.0008000	1	0.9774354
X2*X4	0.0031250	0.1767767	-0.3433510	0.3496010	0.0003125	1	0.9858960
X2*X5	-0.0087500	0.1767767	-0.3552260	0.3377260	0.0024500	1	0.9605228
X3*X4	0.0156250	0.1767767	-0.3308510	0.3621010	0.0078125	1	0.9295680
X3*X5	0.0100000	0.1767767	-0.3364760	0.3564760	0.0032000	1	0.9548889
X4*X5	0.0156250	0.1767767	-0.3308510	0.3621010	0.0078125	1	0.9295680
X1*X2*X3	-0.0087500	0.1767767	-0.3552260	0.3377260	0.0024500	1	0.9605228
X1*X2*X4	-0.0081250	0.1767767	-0.3546010	0.3383510	0.0021125	1	0.9633406
X1*X2*X5	0.0062500	0.1767767	-0.3402260	0.3527260	0.0012500	1	0.9717964
X1*X3*X5	-0.0012500	0.1767767	-0.3477260	0.3452260	0.0000500	1	0.9943582
X2*X3*X4	-0.0062500	0.1767767	-0.3527260	0.3402260	0.0012500	1	0.9717964
X2*X3*X5	-0.0081250	0.1767767	-0.3546010	0.3383510	0.0021125	1	0.9633406
X2*X4*X5	0.0037500	0.1767767	-0.3427260	0.3502260	0.0004500	1	0.9830756
X3*X4*X5	0.0025000	0.1767767	-0.3439760	0.3489760	0.0002000	1	0.9887166
X1*X3*X4	-0.0043750	0.1767767	-0.3508510	0.3421010	0.0006125	1	0.9802554
X1*X4*X5	-0.0031250	0.1767767	-0.3496010	0.3433510	0.0003125	1	0.9858960

Step 7: Data isolation 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		67.87565	68.4585512		Goodness of Fit							
2		84.45596	84.2616594			Value						
3		79.79275	75.4533719		Deviance	566.9279329						
4		71.50259	75.4533675		Scaled Deviance	566.9279329						
5		70.46632	73.2512931		Pearson Chi-Square	566.9279329						
6		96.89119	93.7176162		Scaled Pearson Chi-Square	566.9279329						
7		69.43005	70.4015537		Log Likelihood	-312.8699995						
8		68.39378	67.8108769		Akaike's Information Criterion (AIC)	677.7399990						
9		47.15026	51.8134731		Finite Sample Corrected AIC (AICC)	958.5399990						
10		65.80311	60.7512962		Bayesian Information Criterion (BIC)	715.8491325						
11		81.34715	80.4404137		Consistent AIC (CAIC)	741.8491325						
12		77.72021	79.0155469									
13		83.93782	75.9067325									
14		71.50259	79.9222781		Parameter Estimates							
15		75.12953	79.4041406		Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
16		70.46632	65.8031087		intercept	73.3484453	0.1767767	73.0019694	73.6949213	172159.8217523	1	0.0
17		74.09326	73.6398931		X1	-3.3031097	0.1767767	-3.6495856	-2.9566337	349.1370754	1	0.0
18		78.75648	78.8212463		X2	-0.9067359	0.1767767	-1.2532119	-0.5602600	26.3094419	1	3E-7

Results:

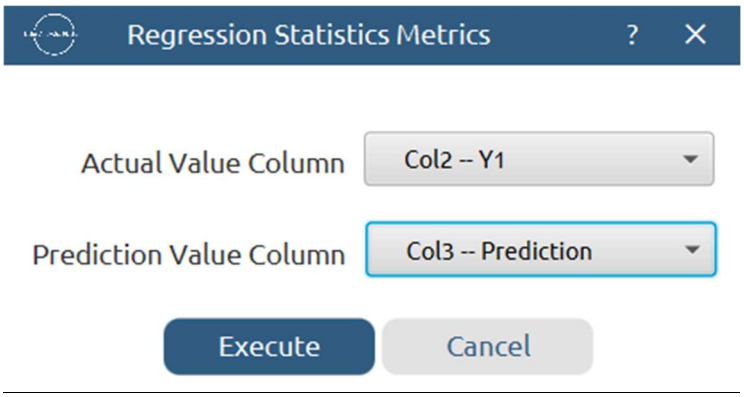


	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y1	Prediction
1		67.87565	68.4585512
2		84.45596	84.2616594
3		79.79275	75.4533719
4		71.50259	75.4533675
5		70.46632	73.2512931
6		96.89119	93.7176162
7		69.43005	70.4015537
8		68.39378	67.8108769
9		47.15026	51.8134731
10		65.80311	60.7512962
11		81.34715	80.4404137
12		77.72021	79.0155469
13		83.93782	75.9067325
14		71.50259	79.9222781
15		75.12953	79.4041406
16		70.46632	65.8031087
17		74.09326	73.6398931
18		78.75648	78.8212463

Repeat this step for the rest of the response variables.

Step 8: 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		17.7164979	4.2090970	3.3840673	0.8477895

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

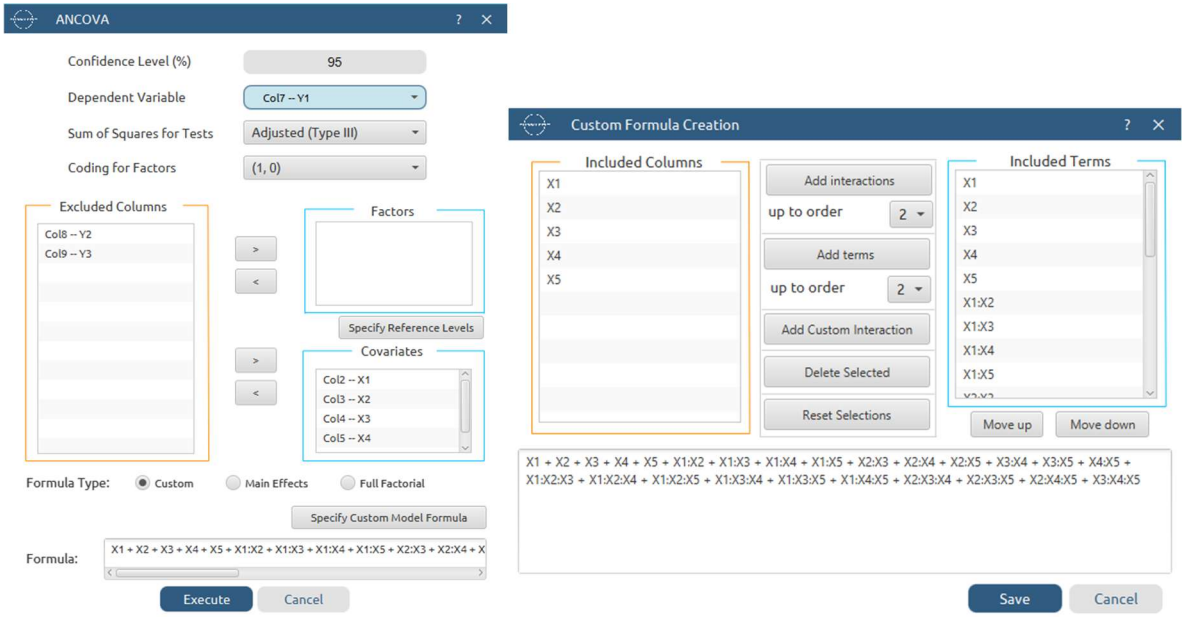
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.2089748	0.4571376	0.3725781	0.9343377

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		0.0002465	0.0156998	0.0128906	0.9590472

Step 9: 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)	Col8 (S)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value	Significant
1		X1	1	349.1370754	349.1370754	3.6950419	0.1029446	No
2		X2	1	26.3094419	26.3094419	0.2784422	0.6166594	No
3		X3	1	10.8727288	10.8727288	0.1150700	0.7460022	No
4		X4	1	5.6712994	5.6712994	0.0600214	0.8146261	No
5		X5	1	8.5908337	8.5908337	0.0909198	0.7731884	No
6		X1*X2	1	629.8491065	629.8491065	6.6659172	0.0416661	Yes
7		X1*X3	1	178.8303118	178.8303118	1.8926248	0.2180538	No
8		X1*X4	1	53.6926979	53.6926979	0.5682489	0.4794826	No
9		X1*X5	1	56.4109724	56.4109724	0.5970174	0.4690523	No
10		X2*X3	1	369.9763741	369.9763741	3.9155916	0.0951840	No
11		X2*X4	1	532.7659951	532.7659951	5.6384521	0.0551775	No
12		X2*X5	1	9.6982576	9.6982576	0.1026401	0.7595478	No
13		X3*X4	1	64.9681730	64.9681730	0.6875813	0.4387244	No
14		X3*X5	1	56.4108662	56.4108662	0.5970163	0.4690527	No
15		X4*X5	1	105.2377315	105.2377315	1.1137684	0.3318946	No
16		X1*X2*X3	1	26.3094419	26.3094419	0.2784422	0.6166594	No
17		X1*X2*X4	1	14.7990985	14.7990985	0.1566241	0.7059727	No
18		X1*X2*X5	1	2.1477058	2.1477058	0.0227299	0.8851024	No
19		X1*X3*X4	1	45.9408332	45.9408332	0.4862082	0.5117232	No
20		X1*X3*X5	1	349.1368112	349.1368112	3.6950391	0.1029447	No
21		X1*X4*X5	1	28.2221901	28.2221901	0.2986855	0.6044205	No
22		X2*X3*X4	1	116.8151922	116.8151922	1.2362967	0.3087421	No
23		X2*X3*X5	1	112.8889481	112.8889481	1.1947439	0.3163060	No
24		X2*X4*X5	1	0.3020219	0.3020219	0.0031964	0.9567499	No
25		X3*X4*X5	1	2.7181968	2.7181968	0.0287676	0.8708907	No
26		Error	6	566.9279329	94.4879888			
27		Total	31	3724.6302380				

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

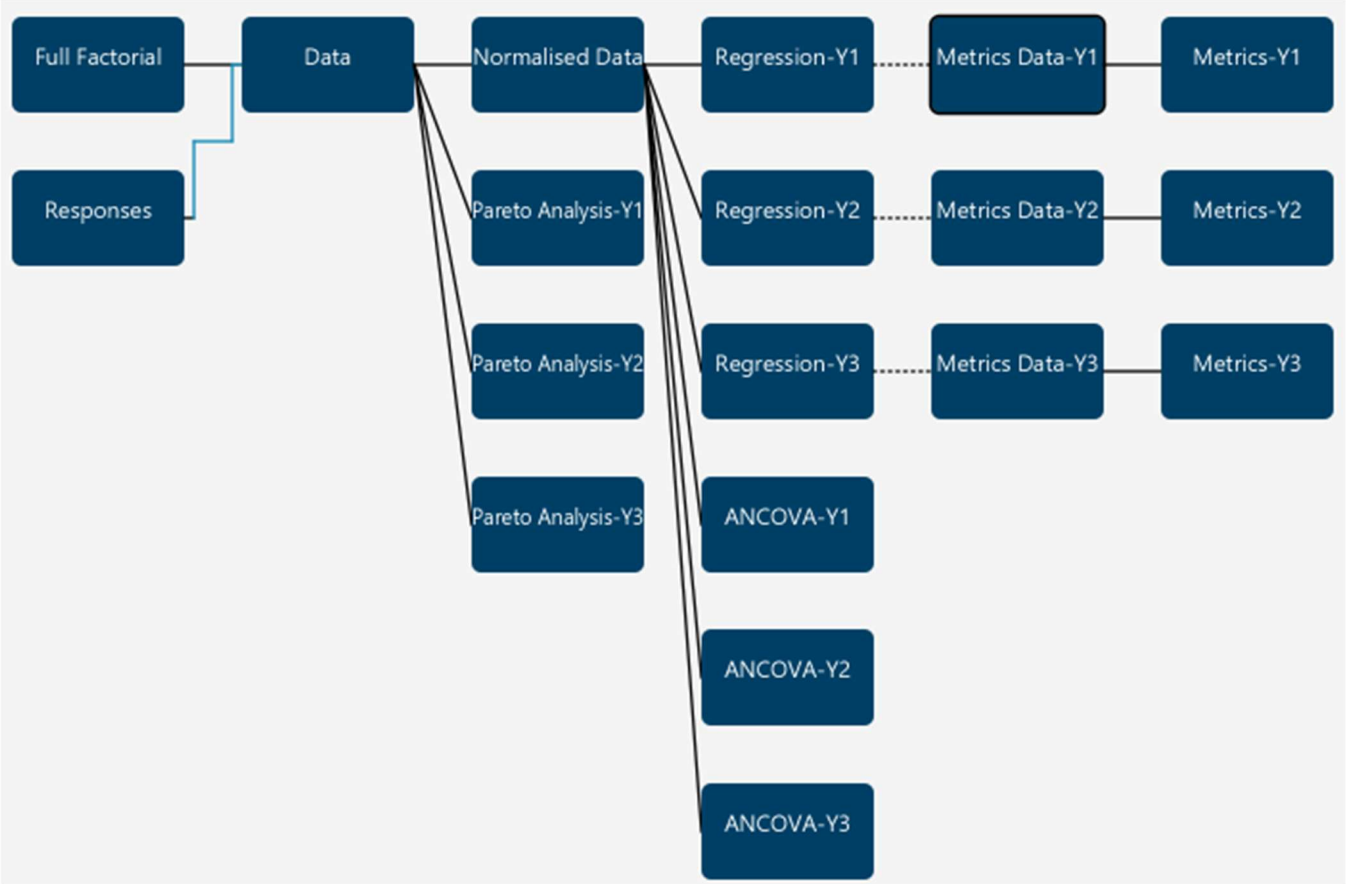
	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (S)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value	Significant
1		X1	1	3.2067781	3.2067781	2.8772411	0.1407678	No
2		X2	1	0.7719031	0.7719031	0.6925803	0.4371510	No
3		X3	1	0.0116281	0.0116281	0.0104332	0.9219713	No
4		X4	1	3.5845031	3.5845031	3.2161501	0.1230785	No
5		X5	1	0.4875781	0.4875781	0.4374733	0.5329063	No
6		X1*X2	1	53.1738281	53.1738281	47.7095446	0.0004552	Yes
7		X1*X3	1	1.4154031	1.4154031	1.2699525	0.3028173	No
8		X1*X4	1	0.5075281	0.5075281	0.4553732	0.5249297	No
9		X1*X5	1	0.4209031	0.4209031	0.3776500	0.5614282	No
10		X2*X3	1	6.7988281	6.7988281	6.1001625	0.0484675	Yes
11		X2*X4	1	1.6974031	1.6974031	1.5229735	0.2633120	No
12		X2*X5	1	0.0603781	0.0603781	0.0541735	0.8236905	No
13		X3*X4	1	0.0225781	0.0225781	0.0202579	0.8914782	No
14		X3*X5	1	0.4117781	0.4117781	0.3694627	0.5655783	No
15		X4*X5	1	4.2267781	4.2267781	3.7924232	0.0994147	No
16		X1*X2*X3	1	2.1166531	2.1166531	1.8991402	0.2173611	No
17		X1*X2*X4	1	1.6790281	1.6790281	1.5064867	0.2656438	No
18		X1*X2*X5	1	0.0069031	0.0069031	0.0061937	0.9398301	No
19		X1*X3*X4	1	2.8740031	2.8740031	2.5786629	0.1594361	No
20		X1*X3*X5	1	1.0117531	1.0117531	0.9077827	0.3774926	No
21		X1*X4*X5	1	0.0331531	0.0331531	0.0297462	0.8687380	No
22		X2*X3*X4	1	0.1845281	0.1845281	0.1655655	0.6982007	No
23		X2*X3*X5	1	7.0970281	7.0970281	6.3677187	0.0450775	Yes
24		X2*X4*X5	1	0.1092781	0.1092781	0.0980484	0.7647831	No
25		X3*X4*X5	1	3.2448781	3.2448781	2.9114259	0.1388287	No
26		Error	6	6.6871938	1.1145323			
27		Total	31	101.8421969				

Results, Y_3:

	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	0.0722000	0.0722000	54.9223455	0.0003102	Yes
2		X2	1	0.0066125	0.0066125	5.0301109	0.0660930	No
3		X3	1	0.0120125	0.0120125	9.1378764	0.0233102	Yes
4		X4	1	0.0264500	0.0264500	20.1204437	0.0041675	Yes
5		X5	1	0.0036125	0.0036125	2.7480190	0.1484497	No
6		X1*X2	1	0.0136125	0.0136125	10.3549921	0.0181848	Yes
7		X1*X3	1	0.0006125	0.0006125	0.4659271	0.5203357	No
8		X1*X4	1	0.0128000	0.0128000	9.7369255	0.0205743	Yes
9		X1*X5	1	0.0036125	0.0036125	2.7480190	0.1484497	No
10		X2*X3	1	0.0008000	0.0008000	0.6085578	0.4649829	No
11		X2*X4	1	0.0003125	0.0003125	0.2377179	0.6431610	No
12		X2*X5	1	0.0024500	0.0024500	1.8637084	0.2211672	No
13		X3*X4	1	0.0078125	0.0078125	5.9429477	0.0506217	No
14		X3*X5	1	0.0032000	0.0032000	2.4342314	0.1697311	No
15		X4*X5	1	0.0078125	0.0078125	5.9429477	0.0506217	No
16		X1*X2*X3	1	0.0024500	0.0024500	1.8637084	0.2211672	No
17		X1*X2*X4	1	0.0021125	0.0021125	1.6069731	0.2518901	No
18		X1*X2*X5	1	0.0012500	0.0012500	0.9508716	0.3671567	No
19		X1*X3*X4	1	0.0006125	0.0006125	0.4659271	0.5203357	No
20		X1*X3*X5	1	0.0000500	0.0000500	0.0380349	0.8518095	No
21		X1*X4*X5	1	0.0003125	0.0003125	0.2377179	0.6431610	No
22		X2*X3*X4	1	0.0012500	0.0012500	0.9508716	0.3671567	No
23		X2*X3*X5	1	0.0021125	0.0021125	1.6069731	0.2518901	No
24		X2*X4*X5	1	0.0004500	0.0004500	0.3423138	0.5798183	No
25		X3*X4*X5	1	0.0002000	0.0002000	0.1521395	0.7099696	No
26		Error	6	0.0078875	0.0013146			
27		Total	31	0.1926000				

Final Isalos Workflow

The final workflow is presented below:



References

(1) Sovány, T.; Csordás, K.; Kelemen, A.; Regdon, G.; Pintye-Hódi, K. Development of Pellets for Oral Lysozyme Delivery by Using a Quality by Design Approach. *Chemical Engineering Research and Design* **2016**, *106*, 92–100. https://doi.org/10.1016/j.cherd.2015.11.022.