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.

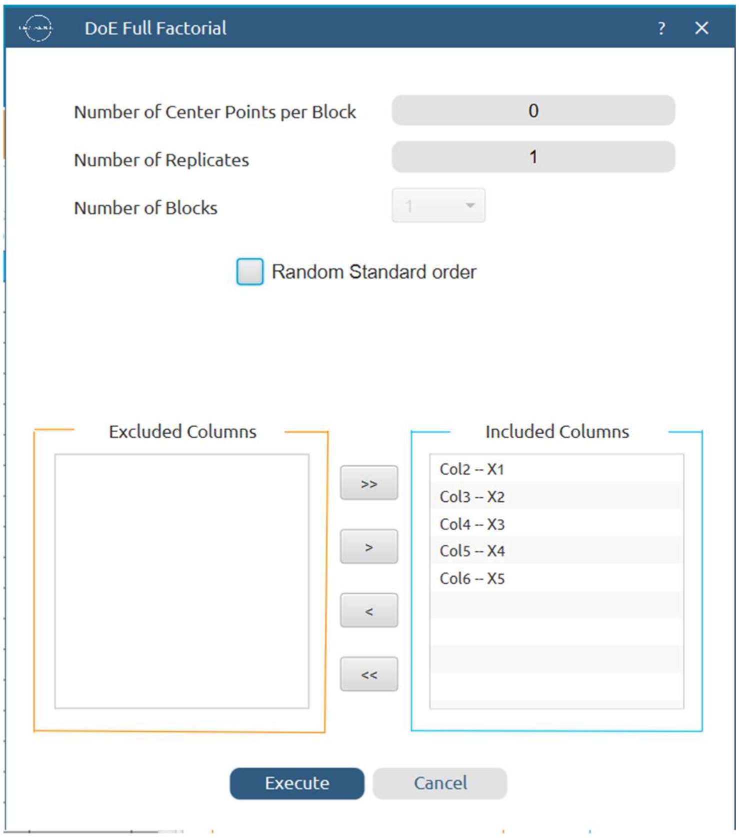
Isalos version used: 2.0.6

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



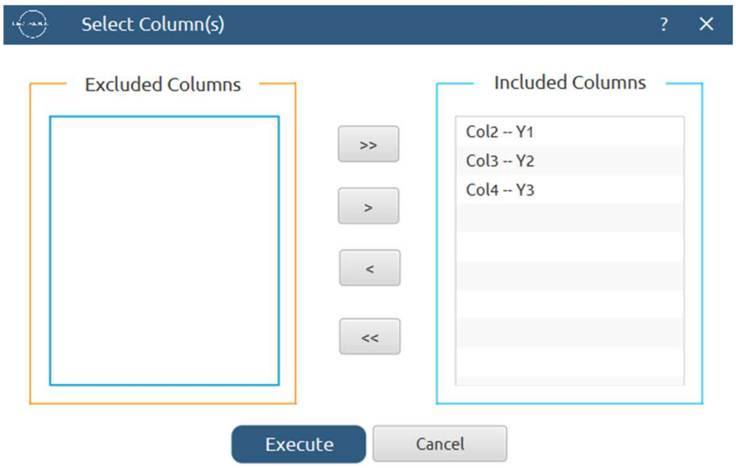
Results (right spreadsheet):

	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	500.0	5.0	70.0	1000.0	15.0
2		2	Block: 1	Replicate: 1	Design Point	1500.0	5.0	70.0	1000.0	15.0
3		3	Block: 1	Replicate: 1	Design Point	500.0	10.0	70.0	1000.0	15.0
4		4	Block: 1	Replicate: 1	Design Point	1500.0	10.0	70.0	1000.0	15.0
5		5	Block: 1	Replicate: 1	Design Point	500.0	5.0	120.0	1000.0	15.0
6		6	Block: 1	Replicate: 1	Design Point	1500.0	5.0	120.0	1000.0	15.0
7		7	Block: 1	Replicate: 1	Design Point	500.0	10.0	120.0	1000.0	15.0
8		8	Block: 1	Replicate: 1	Design Point	1500.0	10.0	120.0	1000.0	15.0
9		9	Block: 1	Replicate: 1	Design Point	500.0	5.0	70.0	2000.0	15.0
10		10	Block: 1	Replicate: 1	Design Point	1500.0	5.0	70.0	2000.0	15.0
11		11	Block: 1	Replicate: 1	Design Point	500.0	10.0	70.0	2000.0	15.0
12		12	Block: 1	Replicate: 1	Design Point	1500.0	10.0	70.0	2000.0	15.0
13		13	Block: 1	Replicate: 1	Design Point	500.0	5.0	120.0	2000.0	15.0
14		14	Block: 1	Replicate: 1	Design Point	1500.0	5.0	120.0	2000.0	15.0
15		15	Block: 1	Replicate: 1	Design Point	500.0	10.0	120.0	2000.0	15.0
16		16	Block: 1	Replicate: 1	Design Point	1500.0	10.0	120.0	2000.0	15.0
17		17	Block: 1	Replicate: 1	Design Point	500.0	5.0	70.0	1000.0	30.0
18		18	Block: 1	Replicate: 1	Design Point	1500.0	5.0	70.0	1000.0	30.0
19		19	Block: 1	Replicate: 1	Design Point	500.0	10.0	70.0	1000.0	30.0
20		20	Block: 1	Replicate: 1	Design Point	1500.0	10.0	70.0	1000.0	30.0
21		21	Block: 1	Replicate: 1	Design Point	500.0	5.0	120.0	1000.0	30.0

Step 2: Definition of response variables

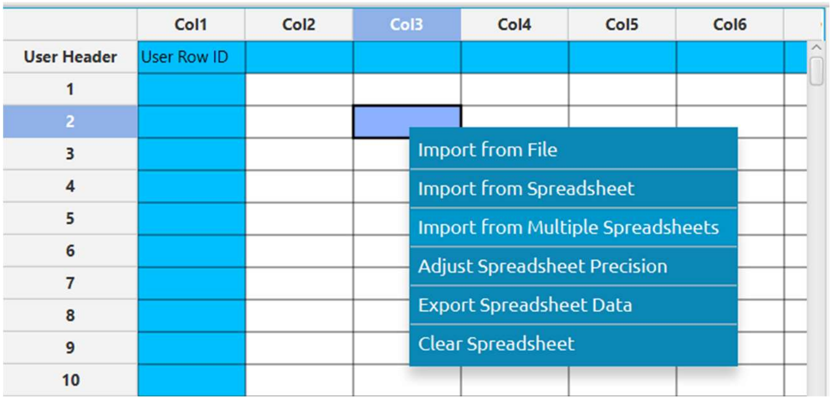
Create a new tab named "Responses" and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the full factorial method. Then, select all columns to be transferred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

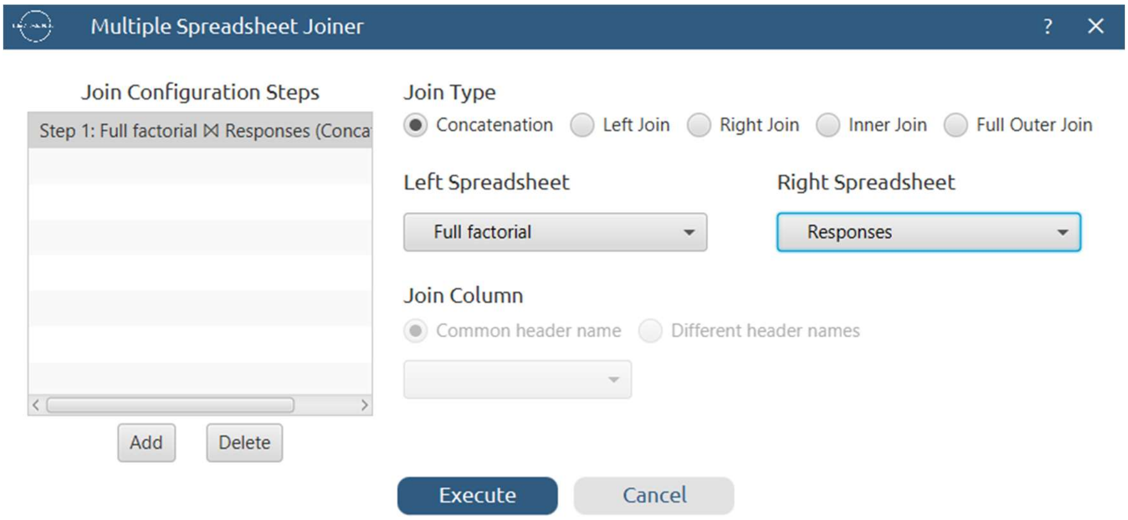
	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	Y1	Y2	Y3
1		68.39378	9.18	1.27
2		69.43005	4.99	1.37
3		96.89119	2.42	1.3
4		70.46632	5.29	1.4
5		71.50259	5.94	1.2
6		79.79275	4.45	1.39
7		84.45596	5.71	1.34
8		67.87565	8.87	1.38
9		70.46632	6.39	1.16
10		75.12953	3.87	1.27
11		71.50259	3.01	1.22
12		83.93782	5.36	1.23
13		77.72021	3.86	1.17
14		81.34715	3.48	1.28
15		65.80311	3.14	1.34
16		47.15026	6.57	1.27
17		72.53886	6.39	1.25
18		62.6943	4.79	1.4
19		86.5285	2.55	1.2
20		56.47668	7.71	1.39
21		67.87565	6.82	1.22



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)_



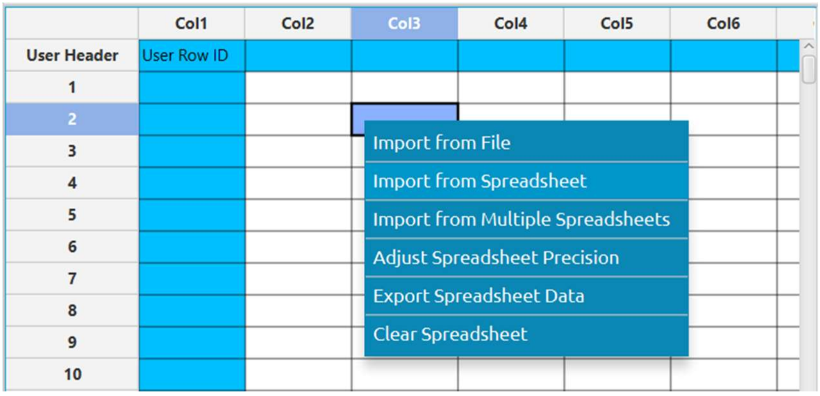


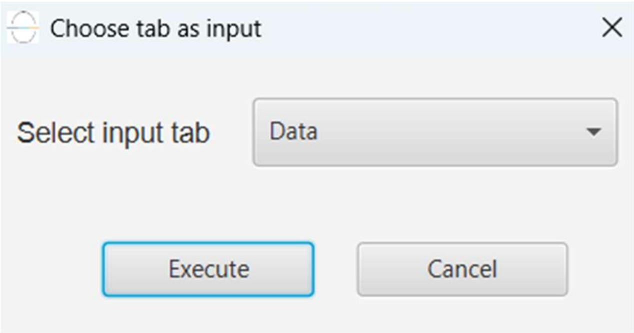
Results:

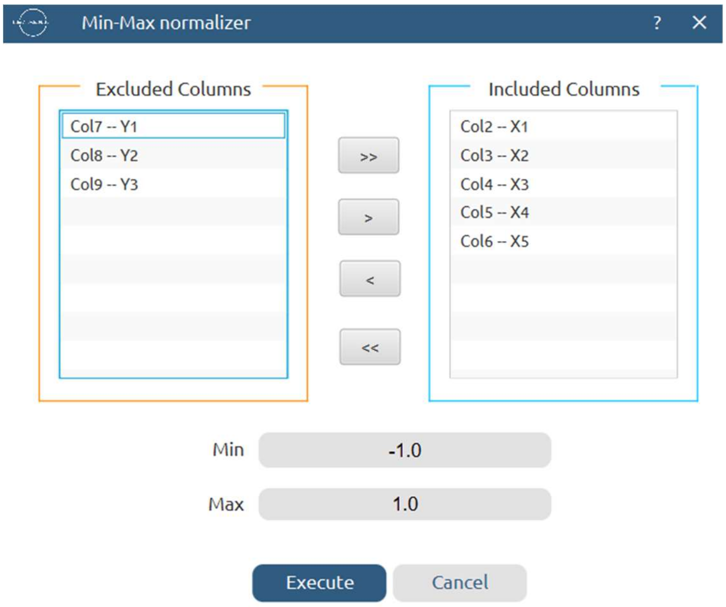
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	X4	X5	Y1	Y2	Y3
1		500.0	5.0	70.0	1000.0	15.0	68.39378	9.18	1.27
2		1500.0	5.0	70.0	1000.0	15.0	69.43005	4.99	1.37
3		500.0	10.0	70.0	1000.0	15.0	96.89119	2.42	1.3
4		1500.0	10.0	70.0	1000.0	15.0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

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:

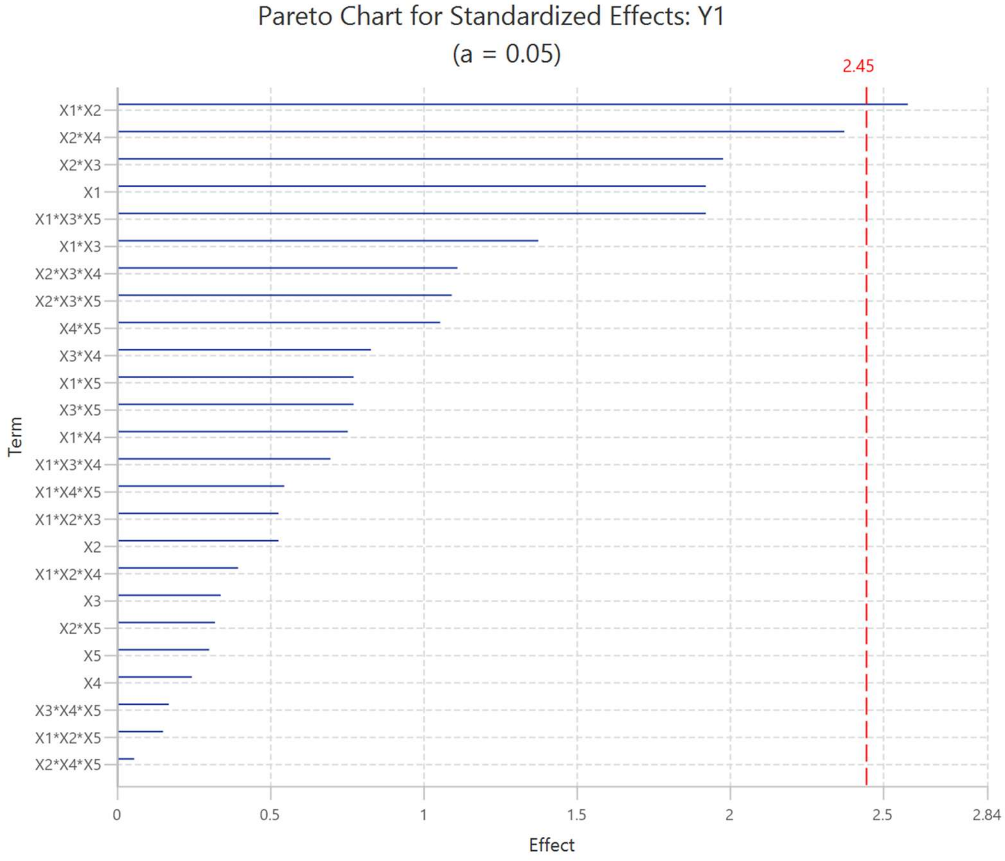
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	X4	X5	Y1	Y2	Y3
1		-1.0	-1.0	-1.0	-1.0	-1.0	68.39378	9.18	1.27
2		1.0	-1.0	-1.0	-1.0	-1.0	69.43005	4.99	1.37
3		-1.0	1.0	-1.0	-1.0	-1.0	96.89119	2.42	1.3
4		1.0	1.0	-1.0	-1.0	-1.0	70.46632	5.29	1.4
5		-1.0	-1.0	1.0	-1.0	-1.0	71.50259	5.94	1.2
6		1.0	-1.0	1.0	-1.0	-1.0	79.79275	4.45	1.39
7		-1.0	1.0	1.0	-1.0	-1.0	84.45596	5.71	1.34
8		1.0	1.0	1.0	-1.0	-1.0	67.87565	8.87	1.38
9		-1.0	-1.0	-1.0	1.0	-1.0	70.46632	6.39	1.16
10		1.0	-1.0	-1.0	1.0	-1.0	75.12953	3.87	1.27
11		-1.0	1.0	-1.0	1.0	-1.0	71.50259	3.01	1.22
12		1.0	1.0	-1.0	1.0	-1.0	83.93782	5.36	1.23
13		-1.0	-1.0	1.0	1.0	-1.0	77.72021	3.86	1.17
14		1.0	-1.0	1.0	1.0	-1.0	81.34715	3.48	1.28
15		-1.0	1.0	1.0	1.0	-1.0	65.80311	3.14	1.34
16		1.0	1.0	1.0	1.0	-1.0	47.15026	6.57	1.27
17		-1.0	-1.0	-1.0	-1.0	1.0	72.53886	6.39	1.25
18		1.0	-1.0	-1.0	-1.0	1.0	62.6943	4.79	1.4
19		-1.0	1.0	-1.0	-1.0	1.0	86.5285	2.55	1.2
20		1.0	1.0	-1.0	-1.0	1.0	56.47668	7.71	1.39
21		-1.0	-1.0	1.0	-1.0	1.0	67.87565	6.82	1.22
22		1.0	-1.0	1.0	-1.0	1.0	72.53886	3.48	1.42
23		-1.0	1.0	1.0	-1.0	1.0	78.75648	2.42	1.29
24		1.0	1.0	1.0	-1.0	1.0	74.09326	4.9	1.4
25		-1.0	-1.0	-1.0	1.0	1.0	75.64767	6.48	1.14
26		1.0	-1.0	-1.0	1.0	1.0	77.20207	3.27	1.29
27		-1.0	1.0	-1.0	1.0	1.0	94.81865	3.16	1.27
28		1.0	1.0	-1.0	1.0	1.0	50.7772	6.0	1.29
29		-1.0	-1.0	1.0	1.0	1.0	80.82902	4.24	1.31
30		1.0	-1.0	1.0	1.0	1.0	84.97409	5.41	1.39
31		-1.0	1.0	1.0	1.0	1.0	62.6943	3.78	1.32
32		1.0	1.0	1.0	1.0	1.0	66.83938	7.18	1.35

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*

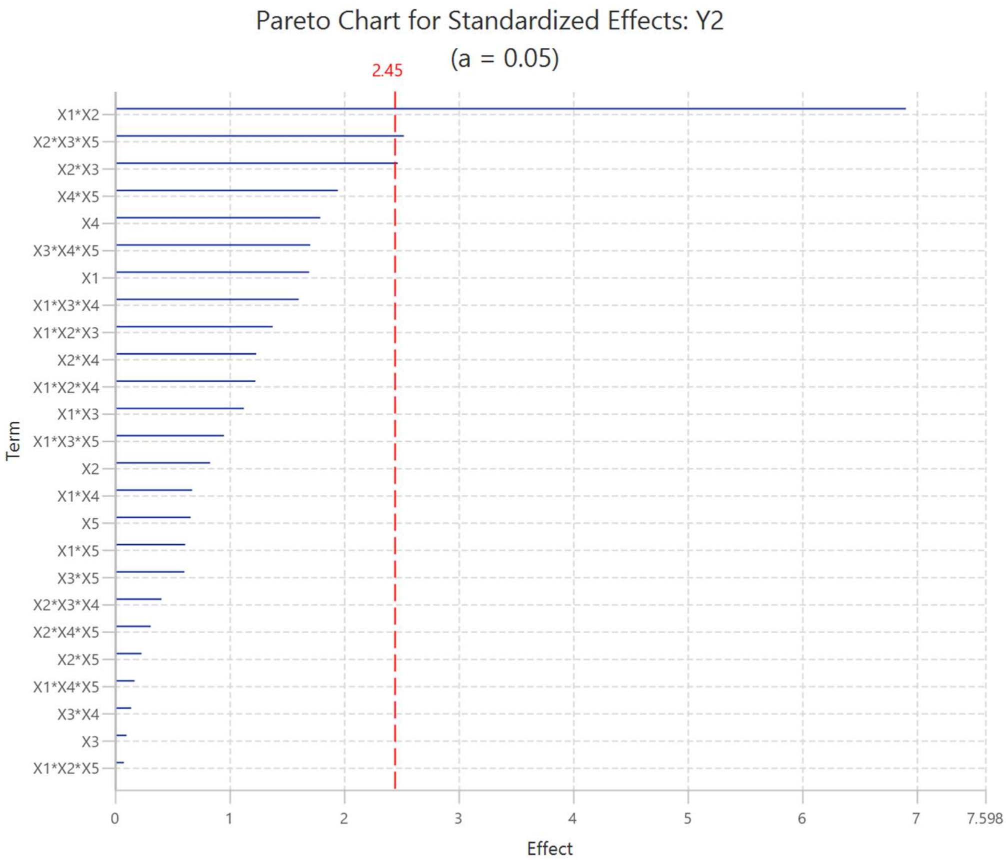
Results:

	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



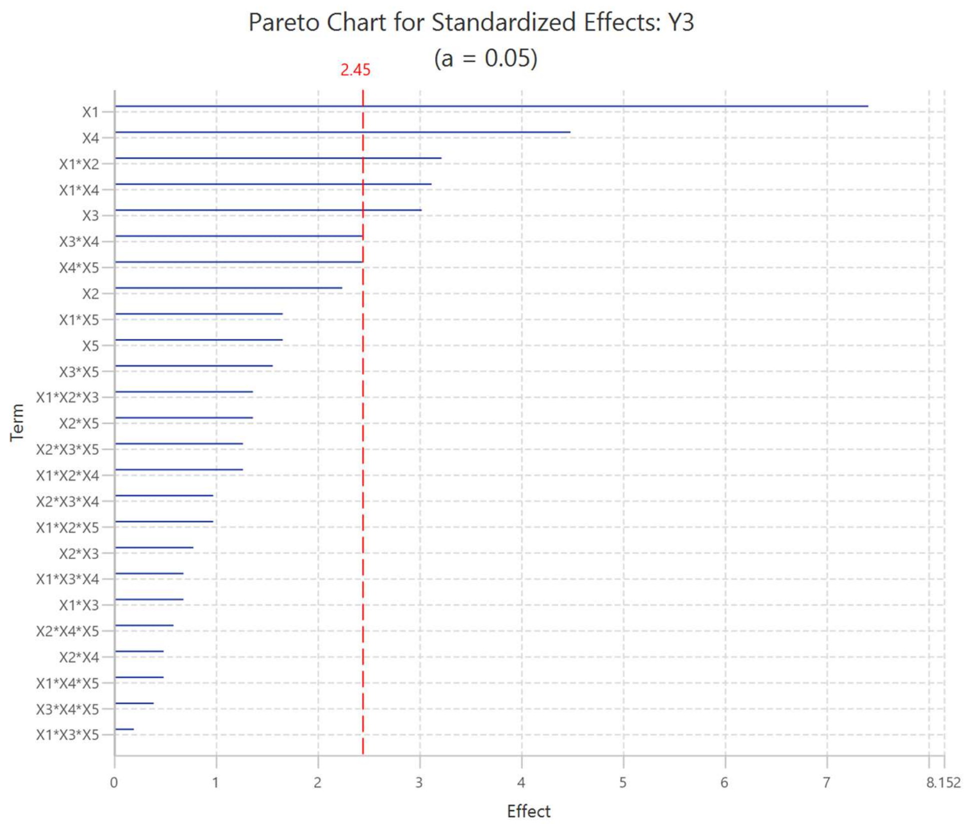
Repeat this step for the rest of the response variables. Results, Y_2:

	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

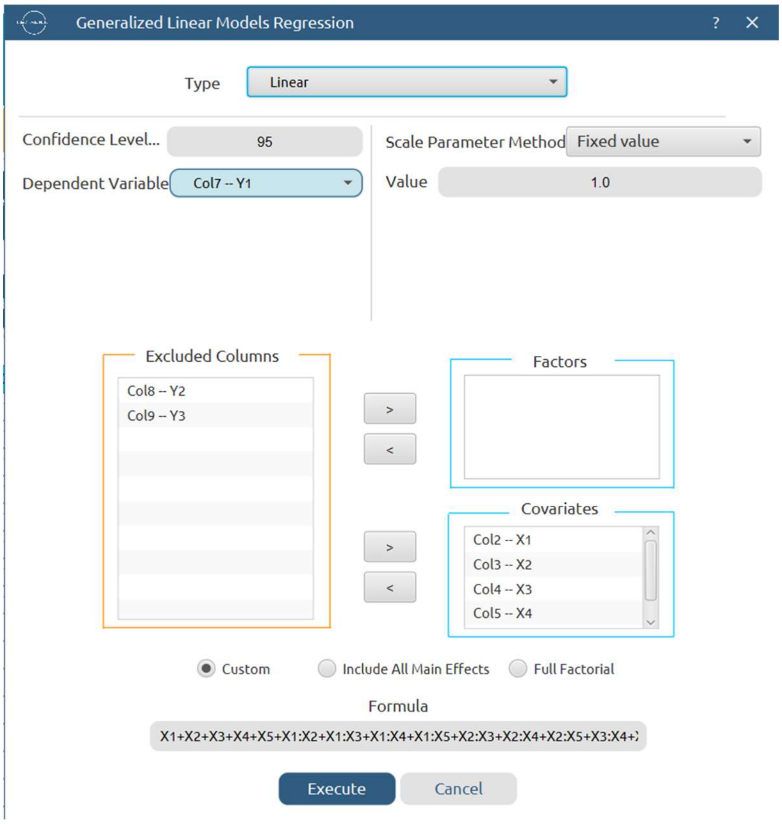


Step 6: Regression

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		68.39378	67.8108769
2		69.43005	70.4015537
3		96.89119	93.7176162
4		70.46632	73.2512931
5		71.50259	75.4533675
6		79.79275	75.4533719
7		84.45596	84.2616594
8		67.87565	68.4585512
9		70.46632	65.8031087
10		75.12953	79.4041406
11		71.50259	79.9222781
12		83.93782	75.9067325
13		77.72021	79.0155469
14		81.34715	80.4404137
15		65.80311	60.7512962
16		47.15026	51.8134731
17		72.53886	72.9922288
18		62.6943	61.8523306
19		86.5285	89.8316081
20		56.47668	53.5621725
21		67.87565	64.0544069
22		72.53886	76.7487037
23		78.75648	78.8212462
24		74.09326	73.6398931
25		75.64767	80.4404156
26		77.20207	72.797925
27		94.81865	86.2694275
28		50.7772	58.9378219
29		80.82902	79.4041488
30		84.97409	86.0103606
31		62.6943	67.8756481
32		66.83938	62.0466325

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*X5	-1.3277209	0.1767767	-1.6741969	-0.9812450	56.4109724	1	0E-7
X1*X4	1.2953366	0.1767767	0.9488606	1.6418125	53.6926979	1	0E-7
X2*X5	-0.5505184	0.1767767	-0.8969944	-0.2040425	9.6982576	1	0.0018444
X1*X3	2.3639897	0.1767767	2.0175137	2.7104656	178.8303118	1	0.0
X2*X4	-4.0803109	0.1767767	-4.4267869	-3.7338350	532.7659951	1	0.0
X3*X5	1.3277197	0.1767767	0.9812437	1.6741956	56.4108662	1	0E-7
X1*X2	-4.4365284	0.1767767	-4.7830044	-4.0900525	629.8491065	1	0.0
X2*X3	-3.4002591	0.1767767	-3.7467350	-3.0537831	369.9763741	1	0.0
X3*X4	-1.4248703	0.1767767	-1.7713463	-1.0783944	64.9681730	1	0E-7
X4*X5	1.8134716	0.1767767	1.4669956	2.1599475	105.2377315	1	0.0
X2*X3*X4	-1.9106216	0.1767767	-2.2570975	-1.5641456	116.8151922	1	0.0
X2*X4*X5	0.0971503	0.1767767	-0.2493256	0.4436263	0.3020219	1	0.5826177
X2*X3*X5	1.8782384	0.1767767	1.5317625	2.2247144	112.8889481	1	0.0
X1*X2*X4	0.6800528	0.1767767	0.3335769	1.0265288	14.7990985	1	0.0001196
X1*X2*X3	0.9067359	0.1767767	0.5602600	1.2532119	26.3094419	1	3E-7
X1*X3*X4	-1.1981866	0.1767767	-1.5446625	-0.8517106	45.9408332	1	0E-7
X1*X4*X5	-0.9391184	0.1767767	-1.2855944	-0.5926425	28.2221901	1	1E-7
X1*X2*X5	-0.2590672	0.1767767	-0.6055431	0.0874088	2.1477058	1	0.1427831
X1*X3*X5	3.3031084	0.1767767	2.9566325	3.6495844	349.1368112	1	0.0
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		9.18	8.5668750
2		4.99	4.9562500
3		2.42	2.4625000
4		5.29	5.8943750
5		5.94	6.6325000
6		4.45	4.404375
7		5.71	5.5881250
8		8.87	8.3450000
9		6.39	7.0837500
10		3.87	3.8231250
11		3.01	2.8868750
12		5.36	4.8362500
13		3.86	3.0868750
14		3.48	3.6062500
15		3.14	3.3425000
16		6.57	7.0143750
17		6.39	7.0987500
18		4.79	4.7281250
19		2.55	2.4118750
20		7.71	7.2012500
21		6.82	6.0318750
22		3.48	3.6212500
23		2.42	2.6375000
24		4.9	5.3293750
25		6.48	5.6906250
26		3.27	3.4125000
27		3.16	3.3787500
28		6.0	6.4281250
29		4.24	5.1087500
30		5.41	5.1881250
31		3.78	3.4818750
32		7.18	6.8312500

Goodness of Fit	
	Value
Deviance	6.6871937
Scaled Deviance	6.6871937
Pearson Chi-Square	6.6871937
Scaled Pearson Chi-Square	6.6871937
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*X5	0.1146875	0.1767767	-0.2317885	0.4611635	0.4209031	1	0.5164867
X1*X4	0.1259375	0.1767767	-0.2205385	0.4724135	0.5075281	1	0.4762109
X2*X5	-0.0434375	0.1767767	-0.3899135	0.3030385	0.0603781	1	0.8058993
X1*X3	0.2103125	0.1767767	-0.1361635	0.5567885	1.4154031	1	0.2341615
X2*X4	0.2303125	0.1767767	-0.1161635	0.5767885	1.6974031	1	0.1926279
X3*X5	-0.1134375	0.1767767	-0.4599135	0.2330385	0.4117781	1	0.5210684
X1*X2	1.2890625	0.1767767	0.9425865	1.6355385	53.1738281	1	0E-7
X2*X3	0.4609375	0.1767767	0.1144615	0.8074135	6.7988281	1	0.0091218
X3*X4	0.0265625	0.1767767	-0.3199135	0.3730385	0.0225781	1	0.8805593
X4*X5	0.3634375	0.1767767	0.0169615	0.7099135	4.2267781	1	0.0397909
X2*X3*X4	-0.0759375	0.1767767	-0.4224135	0.2705385	0.1845281	1	0.6675104
X2*X4*X5	0.0584375	0.1767767	-0.2880385	0.4049135	0.1092781	1	0.7409675
X2*X3*X5	-0.4709375	0.1767767	-0.8174135	-0.1244615	7.0970281	1	0.0077212
X1*X2*X4	-0.2290625	0.1767767	-0.5755385	0.1174135	1.6790281	1	0.1950536
X1*X2*X3	-0.2571875	0.1767767	-0.6036635	0.0892885	2.1166531	1	0.1457046
X1*X3*X4	0.2996875	0.1767767	-0.0467885	0.6461635	2.8740031	1	0.0900207
X1*X4*X5	-0.0321875	0.1767767	-0.3786635	0.3142885	0.0331531	1	0.8555199
X1*X2*X5	0.0146875	0.1767767	-0.3317885	0.3611635	0.0069031	1	0.9337839
X1*X3*X5	-0.1778125	0.1767767	-0.5242885	0.1686635	1.0117531	1	0.3144832
X3*X4*X5	0.3184375	0.1767767	-0.0280385	0.6649135	3.2448781	1	0.0716470

Results, Y_3:

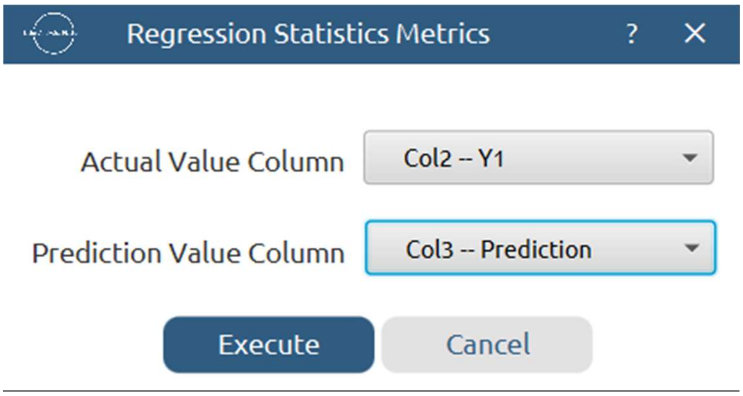
	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y3	Prediction
1		1.27	1.2681250
2		1.37	1.3931250
3		1.3	1.2968750
4		1.4	1.3818750
5		1.2	1.2018750
6		1.39	1.366875
7		1.34	1.3431250
8		1.38	1.398125
9		1.16	1.1506250
10		1.27	1.258125
11		1.22	1.2343750
12		1.23	1.2368750
13		1.17	1.1793750
14		1.28	1.2918750
15		1.34	1.3256250
16		1.27	1.2631250
17		1.25	1.2343750
18		1.4	1.3943750
19		1.2	1.2206250
20		1.39	1.3906250
21		1.22	1.235625
22		1.42	1.425625
23		1.29	1.2693750
24		1.4	1.3993750
25		1.14	1.1668750
26		1.29	1.284375
27		1.27	1.238125
28		1.29	1.3006250
29		1.31	1.2831250
30		1.39	1.3956250
31		1.32	1.351875
32		1.35	1.3393750

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*X5	0.0106250	0.1767767	-0.3358510	0.3571010	0.0036125	1	0.9520727
X1*X4	-0.0200000	0.1767767	-0.3664760	0.3264760	0.0128000	1	0.9099219
X2*X5	-0.0087500	0.1767767	-0.3552260	0.3377260	0.0024500	1	0.9605228
X1*X3	-0.0043750	0.1767767	-0.3508510	0.3421010	0.0006125	1	0.9802554
X2*X4	0.0031250	0.1767767	-0.3433510	0.3496010	0.0003125	1	0.9858960
X3*X5	0.0100000	0.1767767	-0.3364760	0.3564760	0.0032000	1	0.9548889
X1*X2	-0.0206250	0.1767767	-0.3671010	0.3258510	0.0136125	1	0.9071195
X2*X3	0.0050000	0.1767767	-0.3414760	0.3514760	0.0008000	1	0.9774354
X3*X4	0.0156250	0.1767767	-0.3308510	0.3621010	0.0078125	1	0.9295680
X4*X5	0.0156250	0.1767767	-0.3308510	0.3621010	0.0078125	1	0.9295680
X2*X3*X4	-0.0062500	0.1767767	-0.3527260	0.3402260	0.0012500	1	0.9717964
X2*X4*X5	0.0037500	0.1767767	-0.3427260	0.3502260	0.0004500	1	0.9830756
X2*X3*X5	-0.0081250	0.1767767	-0.3546010	0.3383510	0.0021125	1	0.9633406
X1*X2*X4	-0.0081250	0.1767767	-0.3546010	0.3383510	0.0021125	1	0.9633406
X1*X2*X3	-0.0087500	0.1767767	-0.3552260	0.3377260	0.0024500	1	0.9605228
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
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
X3*X4*X5	0.0025000	0.1767767	-0.3439760	0.3489760	0.0002000	1	0.9887166

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		17.7164979	4.2090970	3.3840673	0.8477895

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

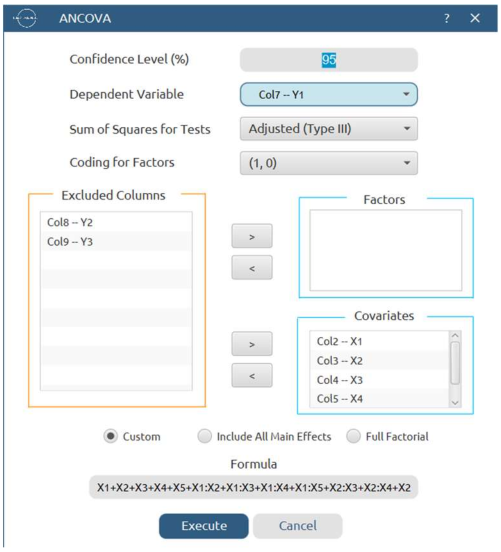
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.2089748	0.4571376	0.3725781	0.9343377

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 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: *<u>Statistics → Analysis of (Co)Variance → ANCOVA</u>*



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	349.1370754	349.1370754	3.6950419	0.1029446
2		X2	1	26.3094419	26.3094419	0.2784422	0.6166594
3		X3	1	10.8727288	10.8727288	0.1150700	0.7460022
4		X4	1	5.6712994	5.6712994	0.0600214	0.8146261
5		X5	1	8.5908337	8.5908337	0.0909198	0.7731884
6		X1*X2	1	629.8491065	629.8491065	6.6659172	0.0416661
7		X1*X3	1	178.8303118	178.8303118	1.8926248	0.2180538
8		X1*X4	1	53.6926979	53.6926979	0.5682489	0.4794826
9		X1*X5	1	56.4109724	56.4109724	0.5970174	0.4690523
10		X2*X3	1	369.9763741	369.9763741	3.9155916	0.0951840
11		X2*X4	1	532.7659951	532.7659951	5.6384521	0.0551775
12		X2*X5	1	9.6982576	9.6982576	0.1026401	0.7595478
13		X3*X4	1	64.9681730	64.9681730	0.6875813	0.4387244
14		X3*X5	1	56.4108662	56.4108662	0.5970163	0.4690527
15		X4*X5	1	105.2377315	105.2377315	1.1137684	0.3318946
16		X1*X2*X3	1	26.3094419	26.3094419	0.2784422	0.6166594
17		X1*X2*X4	1	14.7990985	14.7990985	0.1566241	0.7059727
18		X1*X2*X5	1	2.1477058	2.1477058	0.0227299	0.8851024
19		X1*X3*X4	1	45.9408332	45.9408332	0.4862082	0.5117232
20		X1*X3*X5	1	349.1368112	349.1368112	3.6950391	0.1029447
21		X1*X4*X5	1	28.2221901	28.2221901	0.2986855	0.6044205
22		X2*X3*X4	1	116.8151922	116.8151922	1.2362967	0.3087421
23		X2*X3*X5	1	112.8889481	112.8889481	1.1947439	0.3163060
24		X2*X4*X5	1	0.3020219	0.3020219	0.0031964	0.9567499
25		X3*X4*X5	1	2.7181968	2.7181968	0.0287676	0.8708907
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:

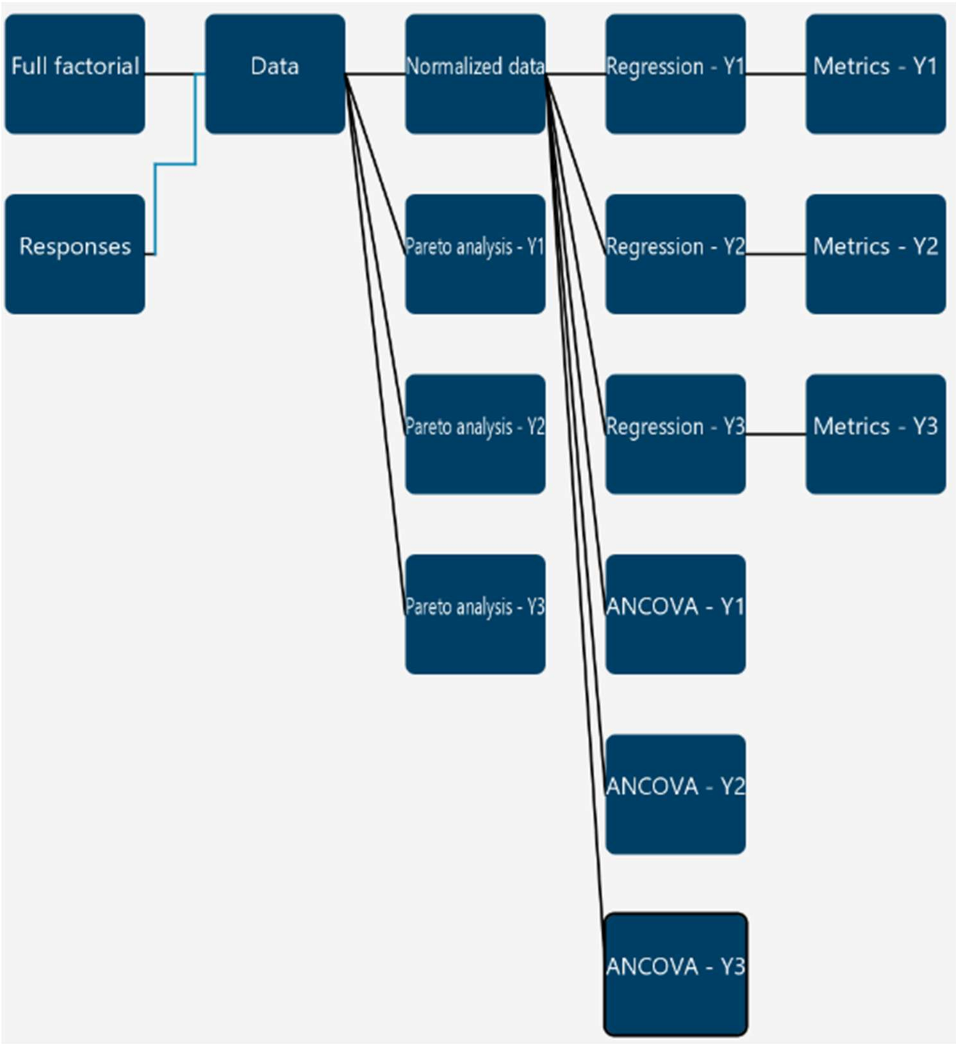
	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	3.2067781	3.2067781	2.8772411	0.1407678
2		X2	1	0.7719031	0.7719031	0.6925803	0.4371510
3		X3	1	0.0116281	0.0116281	0.0104332	0.9219713
4		X4	1	3.5845031	3.5845031	3.2161501	0.1230785
5		X5	1	0.4875781	0.4875781	0.4374733	0.5329063
6		X1*X2	1	53.1738281	53.1738281	47.7095446	0.0004552
7		X1*X3	1	1.4154031	1.4154031	1.2699525	0.3028173
8		X1*X4	1	0.5075281	0.5075281	0.4553732	0.5249297
9		X1*X5	1	0.4209031	0.4209031	0.3776500	0.5614282
10		X2*X3	1	6.7988281	6.7988281	6.1001625	0.0484675
11		X2*X4	1	1.6974031	1.6974031	1.5229735	0.2633120
12		X2*X5	1	0.0603781	0.0603781	0.0541735	0.8236905
13		X3*X4	1	0.0225781	0.0225781	0.0202579	0.8914782
14		X3*X5	1	0.4117781	0.4117781	0.3694627	0.5655783
15		X4*X5	1	4.2267781	4.2267781	3.7924232	0.0994147
16		X1*X2*X3	1	2.1166531	2.1166531	1.8991402	0.2173611
17		X1*X2*X4	1	1.6790281	1.6790281	1.5064867	0.2656438
18		X1*X2*X5	1	0.0069031	0.0069031	0.0061937	0.9398301
19		X1*X3*X4	1	2.8740031	2.8740031	2.5786629	0.1594361
20		X1*X3*X5	1	1.0117531	1.0117531	0.9077827	0.3774926
21		X1*X4*X5	1	0.0331531	0.0331531	0.0297462	0.8687380
22		X2*X3*X4	1	0.1845281	0.1845281	0.1655655	0.6982007
23		X2*X3*X5	1	7.0970281	7.0970281	6.3677187	0.0450775
24		X2*X4*X5	1	0.1092781	0.1092781	0.0980484	0.7647831
25		X3*X4*X5	1	3.2448781	3.2448781	2.9114259	0.1388287
26		Error	6	6.6871937	1.1145323		
27		Total	31	101.8421969			

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	0.0722000	0.0722000	54.9223455	0.0003102
2		X2	1	0.0066125	0.0066125	5.0301109	0.0660930
3		X3	1	0.0120125	0.0120125	9.1378764	0.0233102
4		X4	1	0.0264500	0.0264500	20.1204437	0.0041675
5		X5	1	0.0036125	0.0036125	2.7480190	0.1484497
6		X1*X2	1	0.0136125	0.0136125	10.3549921	0.0181848
7		X1*X3	1	0.0006125	0.0006125	0.4659271	0.5203357
8		X1*X4	1	0.0128000	0.0128000	9.7369255	0.0205743
9		X1*X5	1	0.0036125	0.0036125	2.7480190	0.1484497
10		X2*X3	1	0.0008000	0.0008000	0.6085578	0.4649829
11		X2*X4	1	0.0003125	0.0003125	0.2377179	0.6431610
12		X2*X5	1	0.0024500	0.0024500	1.8637084	0.2211672
13		X3*X4	1	0.0078125	0.0078125	5.9429477	0.0506217
14		X3*X5	1	0.0032000	0.0032000	2.4342314	0.1697311
15		X4*X5	1	0.0078125	0.0078125	5.9429477	0.0506217
16		X1*X2*X3	1	0.0024500	0.0024500	1.8637084	0.2211672
17		X1*X2*X4	1	0.0021125	0.0021125	1.6069731	0.2518901
18		X1*X2*X5	1	0.0012500	0.0012500	0.9508716	0.3671567
19		X1*X3*X4	1	0.0006125	0.0006125	0.4659271	0.5203357
20		X1*X3*X5	1	0.0000500	0.0000500	0.0380349	0.8518095
21		X1*X4*X5	1	0.0003125	0.0003125	0.2377179	0.6431610
22		X2*X3*X4	1	0.0012500	0.0012500	0.9508716	0.3671567
23		X2*X3*X5	1	0.0021125	0.0021125	1.6069731	0.2518901
24		X2*X4*X5	1	0.0004500	0.0004500	0.3423138	0.5798183
25		X3*X4*X5	1	0.0002000	0.0002000	0.1521395	0.7099696
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.