
Supplementary Materials: Stability-Indicating UPLC-PDA-QDa Methodology for Carvedilol and Felodipine in Fixed-Dose Combinations Using AQbD Principles

Jesús Alberto Afonso Urich, Viktoria Marko, Katharina Boehm, Raymar Andreina Lara Garcia, Anna Fedorko, Sharareh Salar-Behzadi and Dalibor Jeremic.

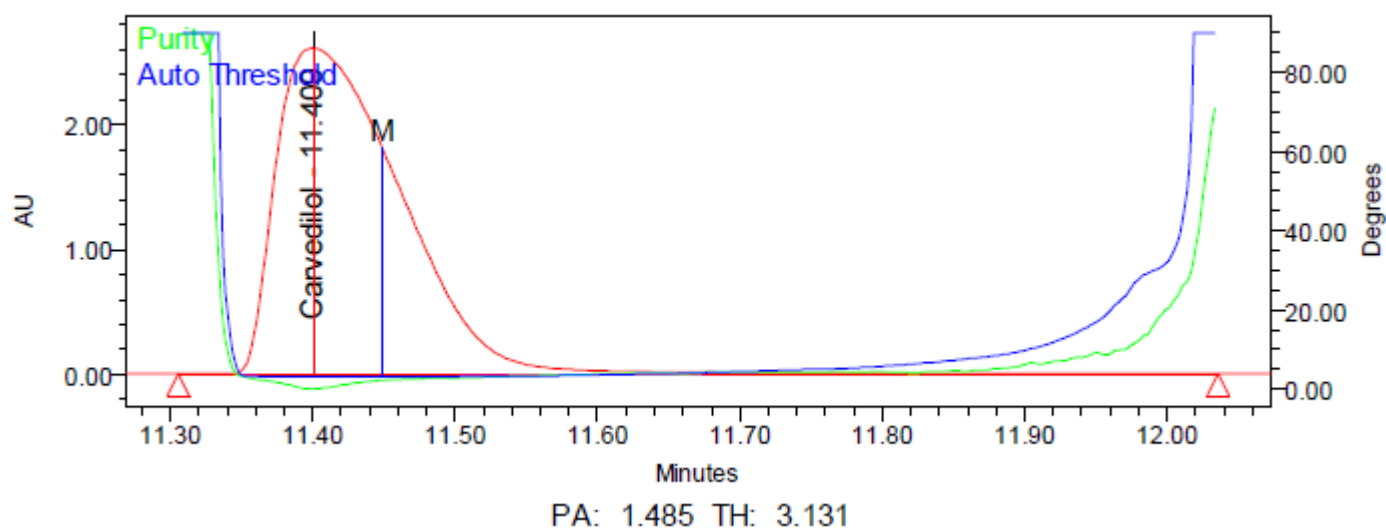


Figure S1. Purity plot of Carvedilol on forced degradation (control)

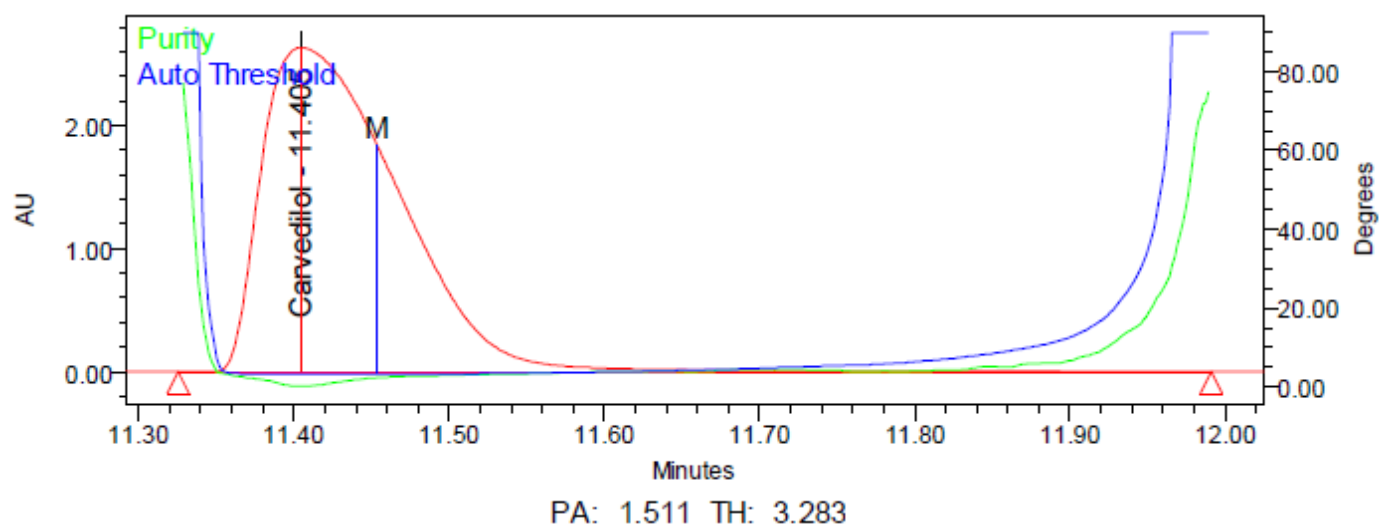


Figure S2. Purity plot of Carvedilol on forced degradation (acid)

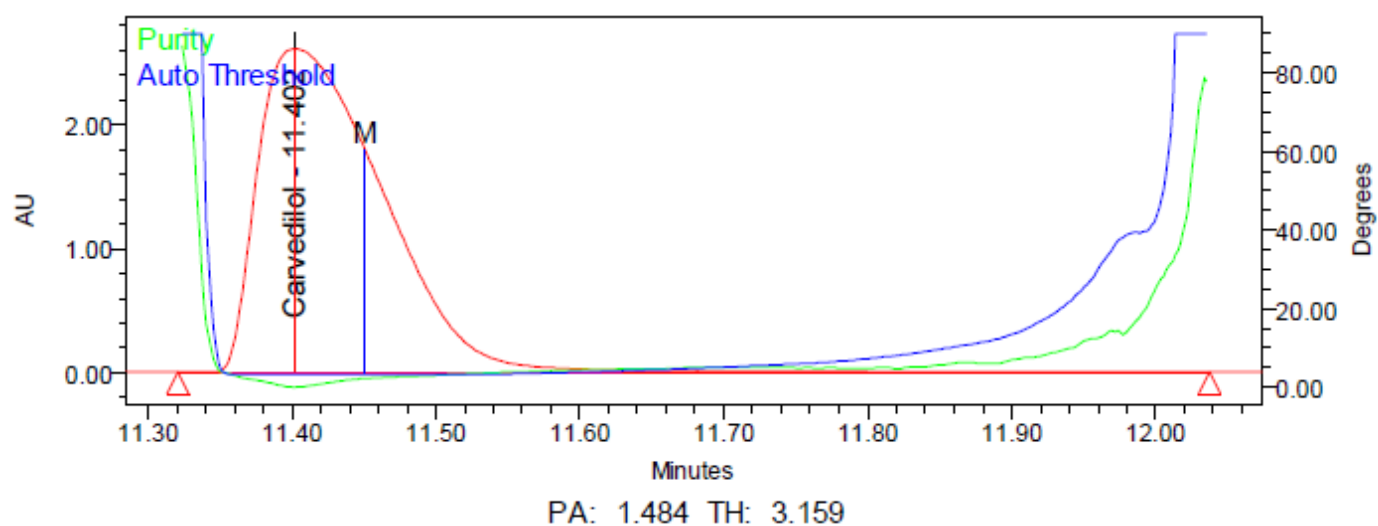


Figure S3. Purity plot of Carvedilol on forced degradation (basic)

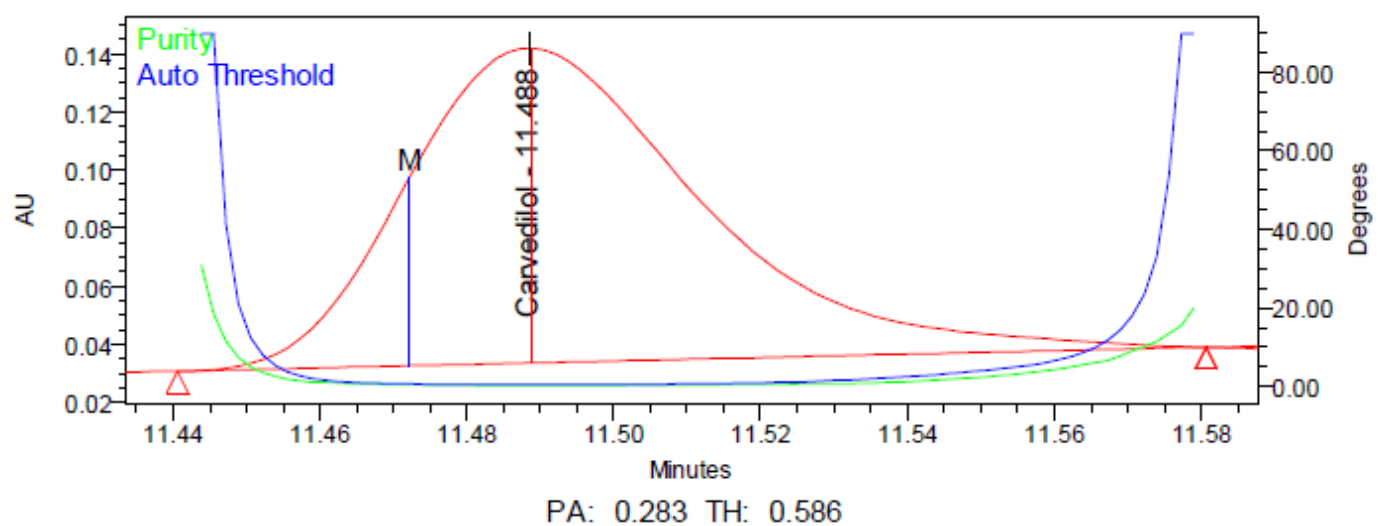


Figure S4. Purity plot of Carvedilol on forced degradation (oxidative)

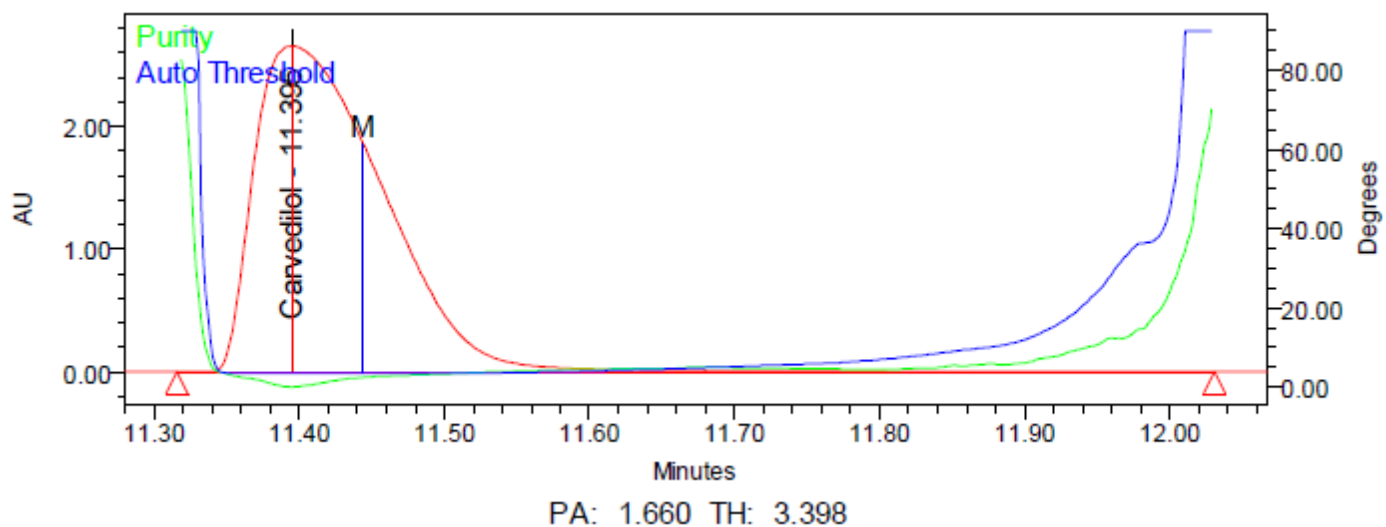


Figure S5. Purity plot of Carvedilol on forced degradation (thermal)

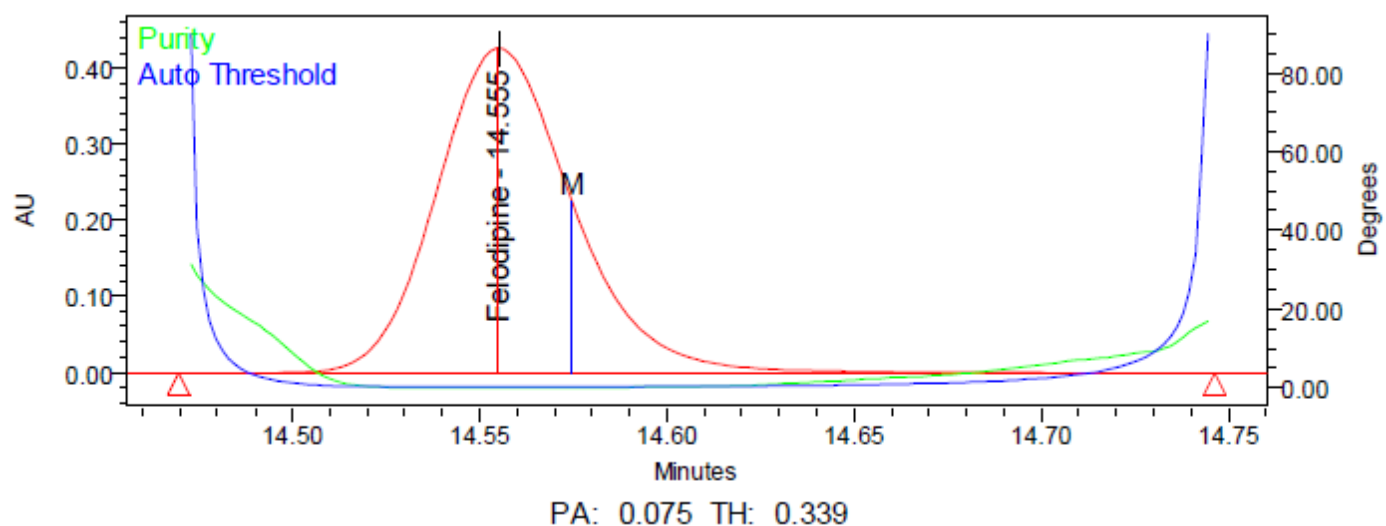


Figure S6. Purity plot of Felodipine on forced degradation (control)

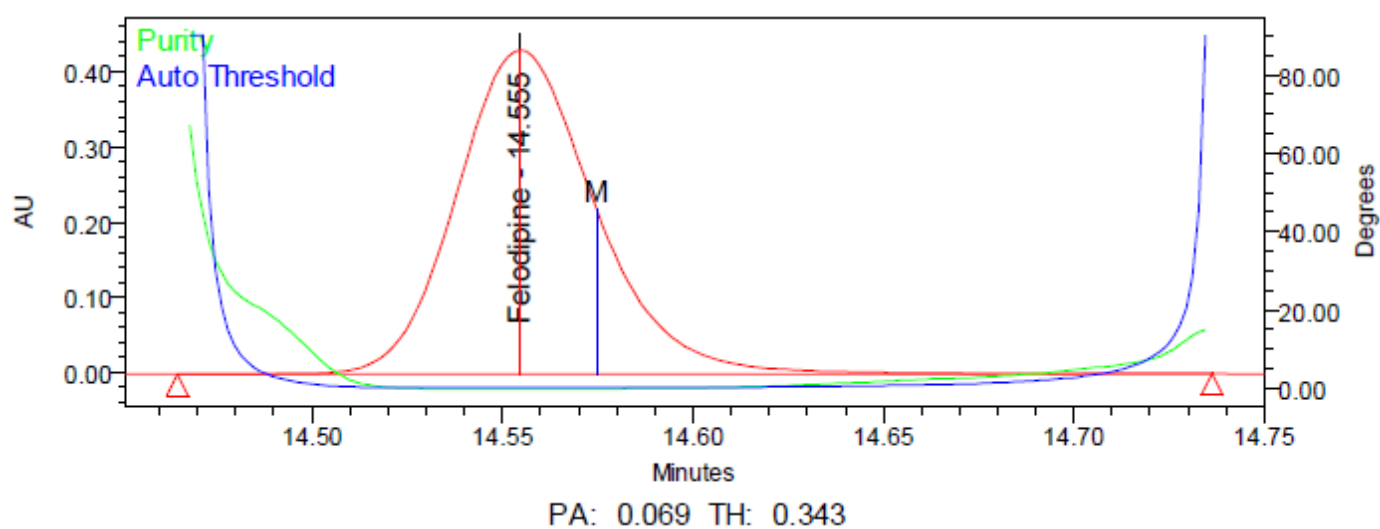


Figure S7. Purity plot of Felodipine on forced degradation (acid)

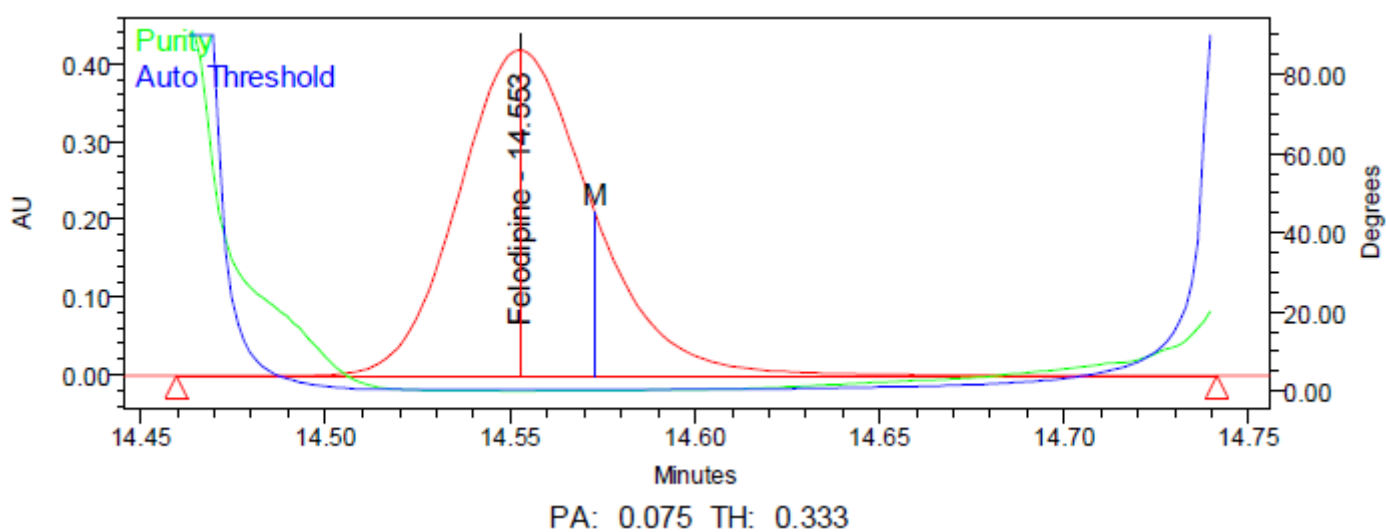


Figure S8. Purity plot of Felodipine on forced degradation (basic)

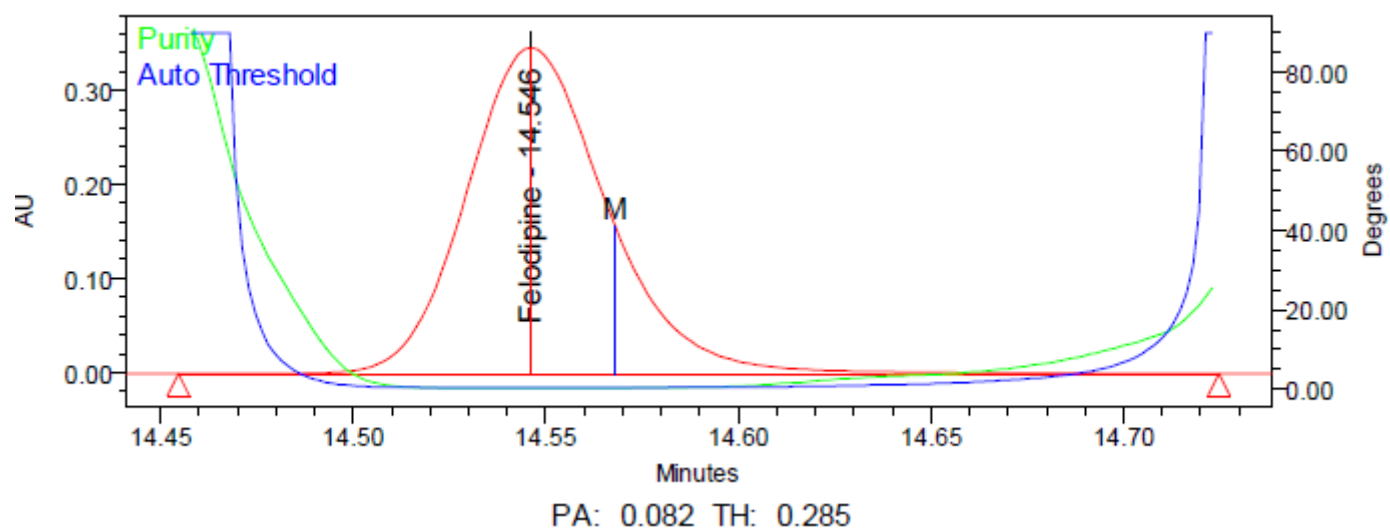


Figure S9. Purity plot of Felodipine on forced degradation (oxidative)

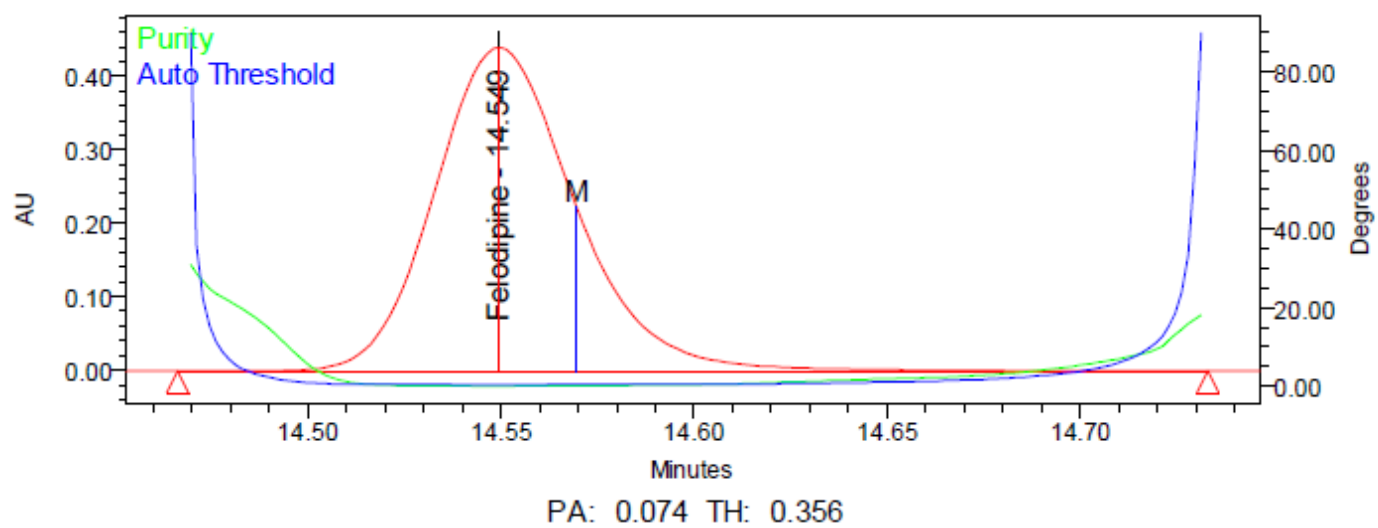


Figure S10. Purity plot of Felodipine on forced degradation (thermal)

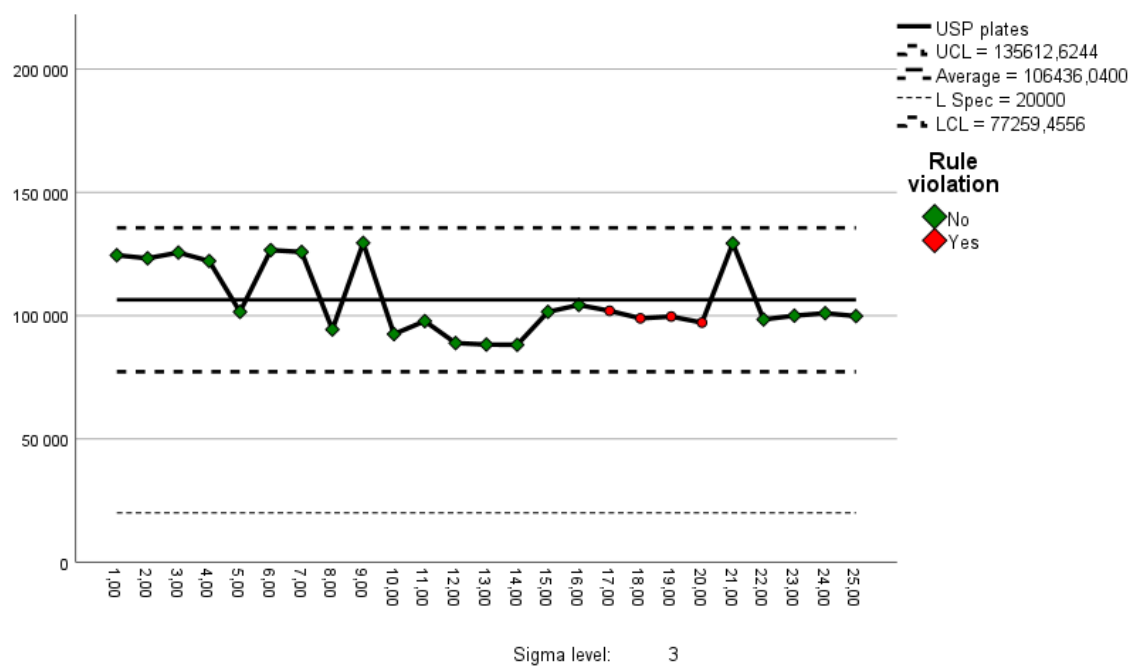


Figure S11 CAV USP plate: X-mR control chart - individual values at sigma level of 3

(Points 17,18,19 and 20: 8 consecutive points below the center line. Cpk: 2,98 (90%CI 2,16-3,80); Ppk: 2,02 (90%CI 1,53-2,51))

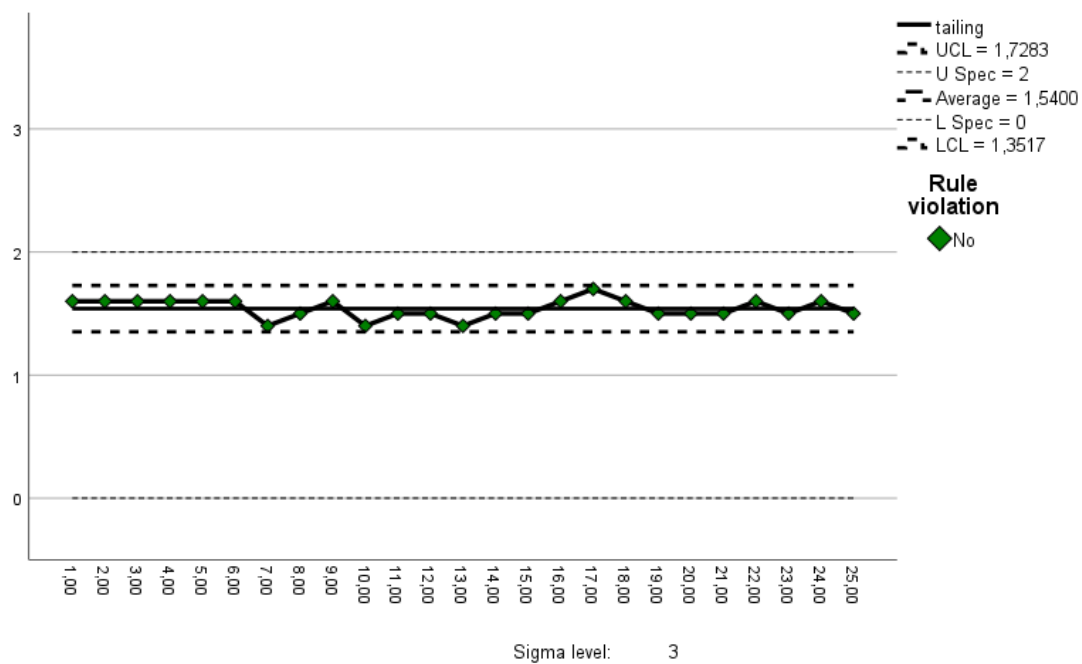


Figure S12 CAV tailing: X-mR control chart - individual values at sigma level of 3.no rules were violated. Cpk: 2,97 (90%CI 2,15-3,79); Ppk: 2,01 (90%CI 1,52-2,50)

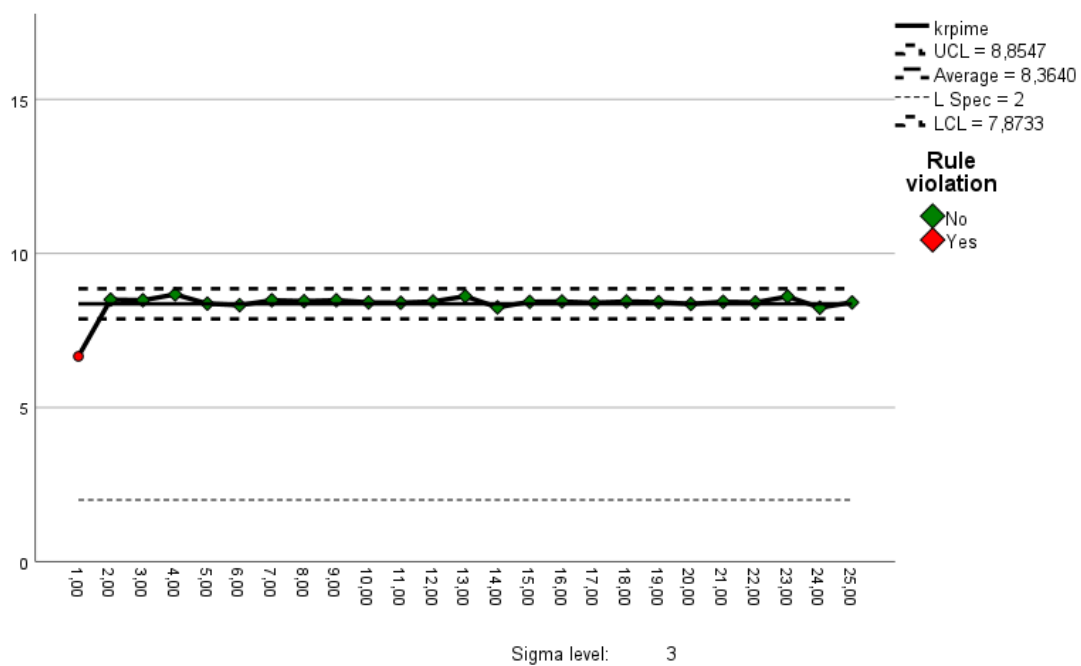


Figure S13 CAV capacity factor: X-mR control chart - individual values at sigma level of 3

(Point 1: less then -3 sigma. Cpk: 8,31 (90%CI 6,06-10,59); PpK: 5,77 (90%CI 4,39-7,14))

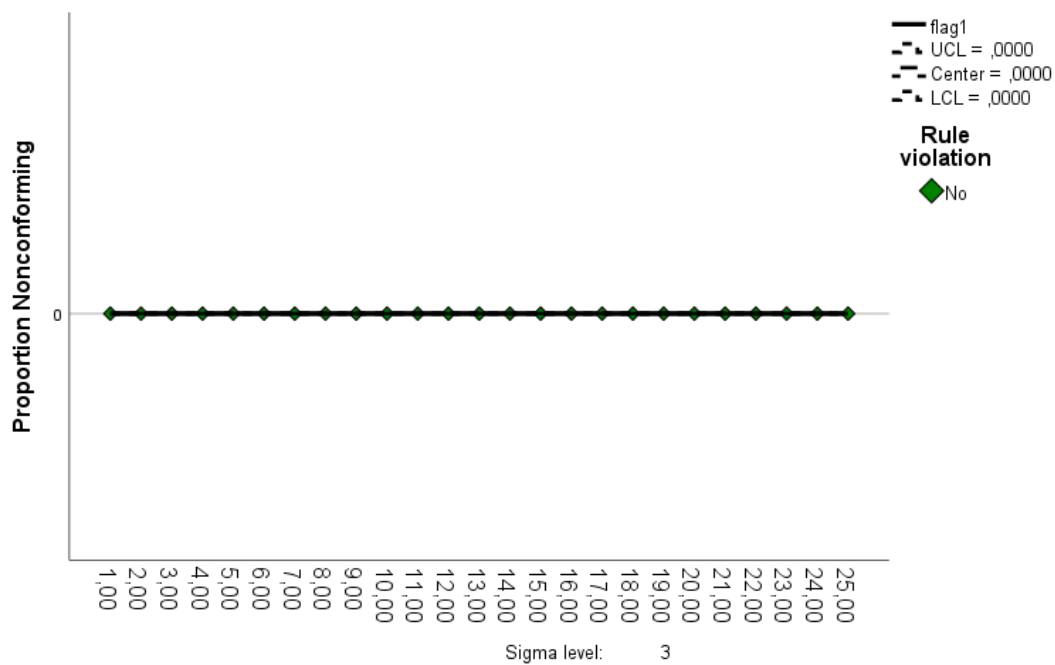


Figure S14 CAV flag: SPchart - at sigma level of 3

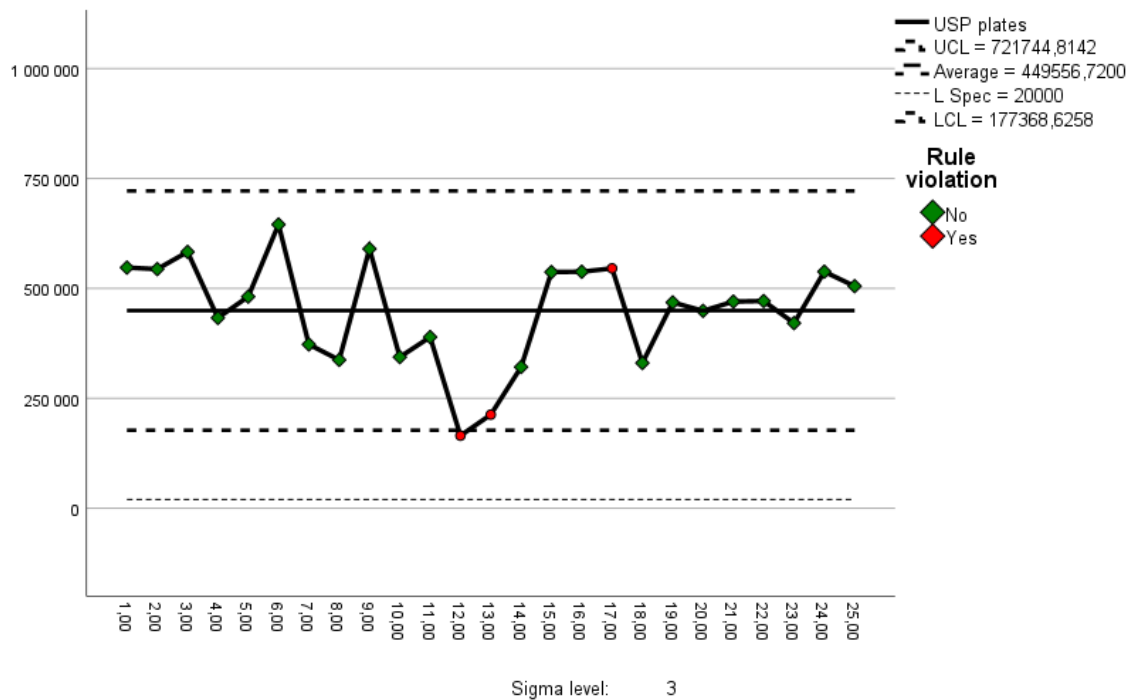


Figure S15 FLP USP plate: X-mR control chart - individual values at sigma level of 3

(Point 12: less than -3 sigma; Point 13: 2 points out of the last 3 below -2sigma; Point 17: 6 points in a row trending up; Cpk: 1,43 (90%CI 1,02-1,84); PpK: 1,21 (90%CI 0,91-1,52))

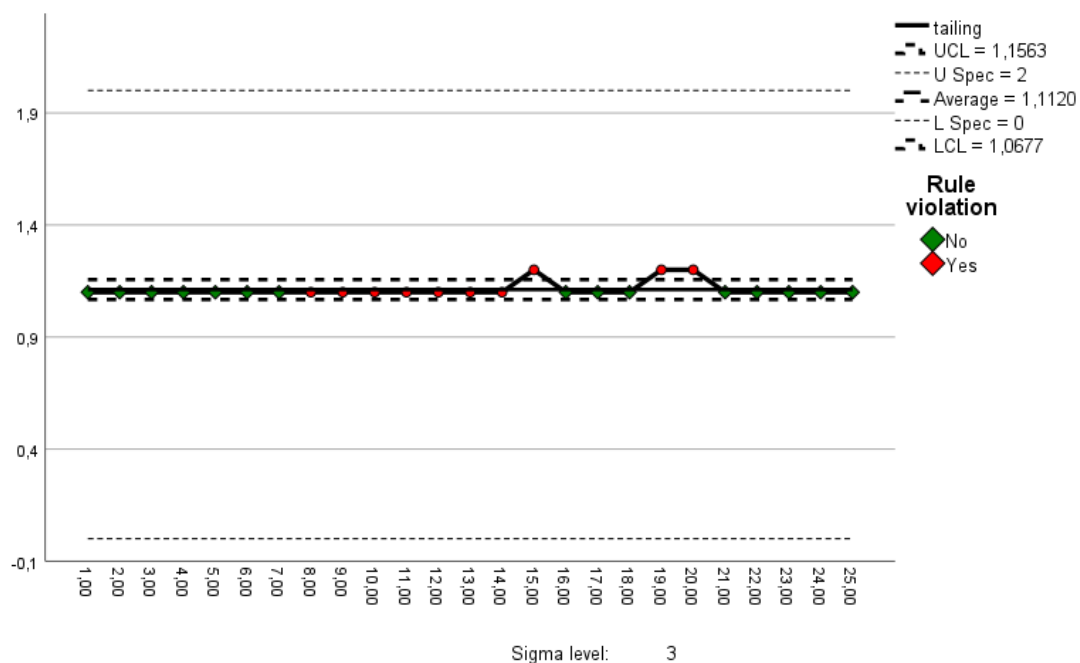


Figure S16 FLP tailing: X-mR control chart - individual values at sigma level of 3

(Points 8,9,10,11,12,13,14: 8 consecutive points below the center line; Points 15,19,20: greater than +3 sigma; Cpk: 17,21 (90%CI 12,49-21,93); PpK: 8,92 (90%CI 6,80-11,05))

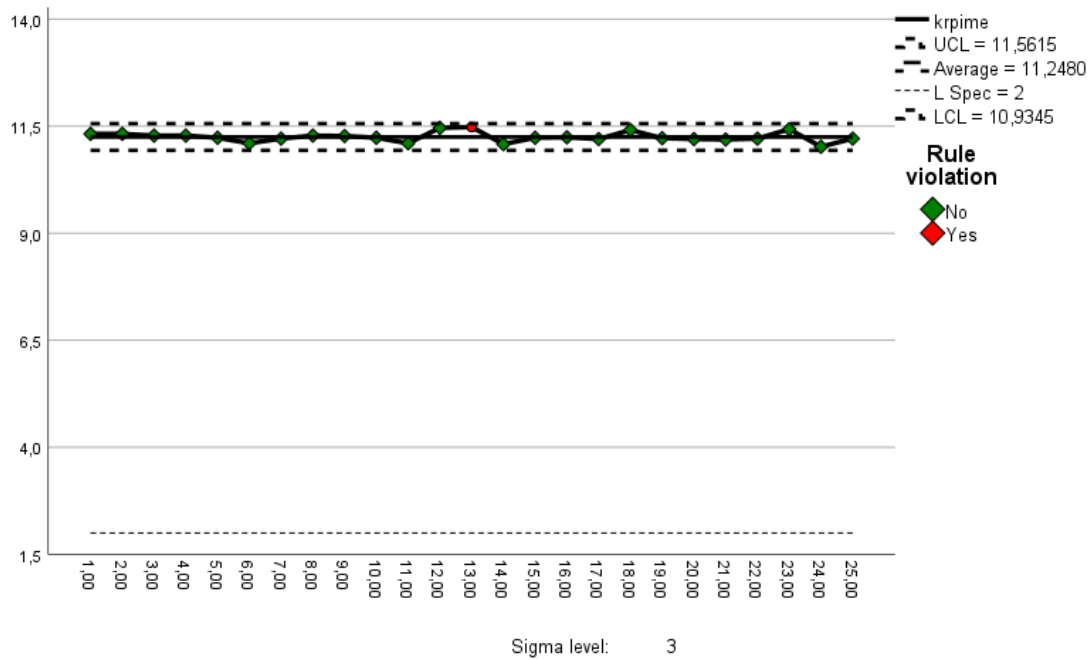


Figure S17 FLP capacity factor: X-mR control chart - individual values at sigma level of 3

(Point 13: 2 points out of the last 3 above +2 sigma; Cpk: 27,79 (90%CI 20,17-35,41); PpK: 27,12 (90%CI 20,68-33,55))

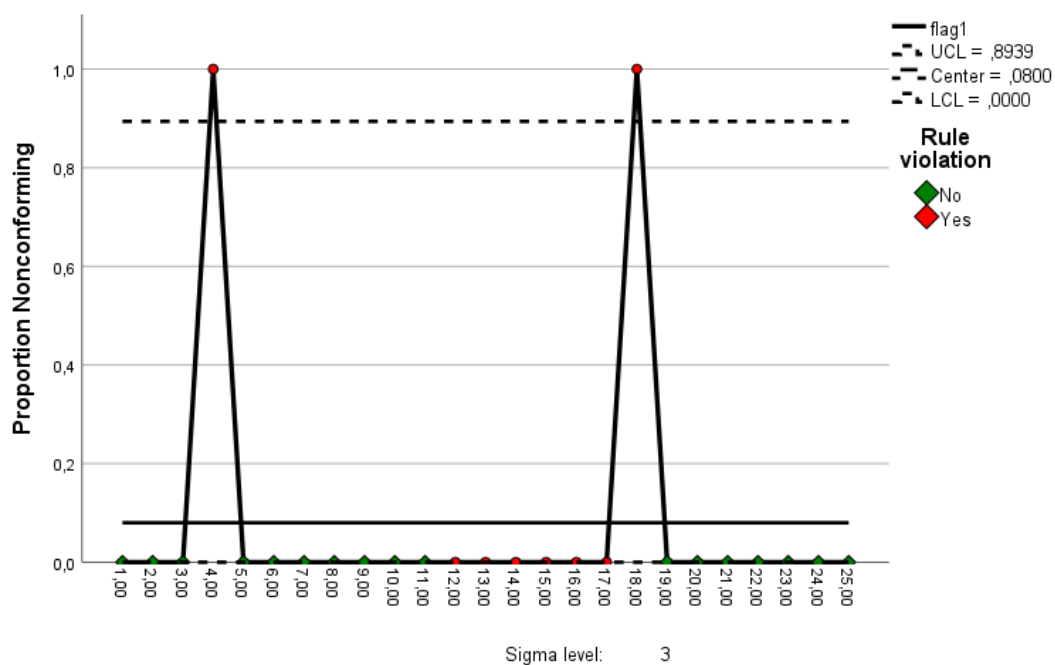


Figure S18 FLP flag: SPchart at sigma level of 3

Table S1. Chromatographic feature results from robustness test from method validation for CAV.

Stability Solution (h)		Column Temperature (°C)		Flow (mL/min)		Mobile phase buffer pH		Gradient %	
0	72	37	39	0.19	0.21	4.3	4.7	88	92
Assay Concentration (ppm)									
236.05	238.98	239.25	238.19	241.29	239.33	239.45	241.69	238.22	235.21
242.9	247.73	246.97	244.73	247.99	248.57	245.5	249.45	245.63	242.47
249.49	251.72	251.39	248.51	252.93	253.36	249.99	252.31	251.37	247
240.6	243.71	246.21	244.37	245.75	243.82	241.94	245.55	244.06	239.77
255.11	257.65	257.39	256.97	258.81	258.4	258.47	257.56	257.3	255.65
253.71	256.25	255.17	254.32	256.66	255.8	253.69	255.35	254.53	251.69
Tailing									
1.60	1.6	1.6	1.6	1.6	1.5	1.5	1.3	1.6	1.5
1.60	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.6	1.5
1.50	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.6	1.6
1.60	1.5	1.6	1.6	1.6	1.6	1.5	1.5	1.6	1.5
1.60	1.5	1.7	1.7	1.5	1.6	1.4	1.4	1.7	1.6
1.60	1.5	1.7	1.6	1.5	1.6	1.5	1.4	1.6	1.6
USP Plates									
101392	99936	103394	102033	99690	101041	98674	129234	99500	104755
101358	100240	104337	102133	100134	101387	96690	130652	98815	105818
101527	99936	103697	101980	99755	100741	99170	129408	98494	105326
101537	99913	104409	101898	100338	101427	97169	131430	98727	106151
101466	100511	104081	101949	99682	101660	100038	131895	98875	106317
101633	99929	103362	102019	99975	101695	97569	131249	98974	106064
Capacity (k')									
8.5	8.5	6.6	6.6	8.7	8.3	8.4	8.5	6.6	8.5
8.5	8.5	6.6	6.6	8.7	8.3	8.4	8.5	6.6	8.5

Table S2. Chromatographic feature results from robustness test from method validation for FLP.

Stability Solution (h)		Column Temperature (°C)		Flow (mL/min)		Mobile phase buffer pH		Gradient %	
0	72	37	39	0.19	0.21	4.3	4.7	88	92
Assay Concentration (ppm)									
138.69	138.71	139.34	139.85	140.82	139.23	141.94	139.12	139.41	141.35
97.12	96.69	97.53	97.5	97.34	97.4	97.63	97.2	97.82	97.9
88.73	88.5	88.77	88.28	89.28	88.99	90.06	88.95	89.25	90.03
100.13	100	100.79	101.63	101.52	100.6	101.71	100.47	100.89	102.1
101.03	100.86	101.29	101.83	102.11	101.26	103.26	101.4	101.9	103.75
88.52	88.09	88.5	89.23	88.93	88.09	89.16	88.37	88.33	90.05
Tailing									
1.2	1.1	1.2	1.2	1.1	1.1	1.2	1.1	1.1	1.2
1.2	1.1	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.2
1.2	1.1	1.1	1.2	1.1	1.1	1.2	1.1	1.1	1.2
1.2	1.1	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.2
1.2	1.1	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.2
1.2	1.1	1.2	1.1	1.1	1.1	1.2	1.1	1.1	1.2
USP Plates									
526589	492859	515845	516702	413724	531609	444308	451876	336970	532904
526440	492437	519919	542128	419448	530371	438997	456465	321442	538868
529413	493469	524943	528913	417311	531169	442629	461743	334318	523805
530696	495240	530179	538232	418233	532636	442129	465655	320281	530434
529700	495485	535267	537286	415922	530107	450984	468589	321807	530312
528338	496410	537445	533949	414228	531042	450998	455902	325000	535845
Capacity (k')									
8.8	11.1	8.8	8.8	11.3	10.9	11.1	11.1	11.3	11
8.8	11.1	8.8	8.8	11.3	10.9	11.1	11.1	11.3	11

Table S3. The coefficient table for the final reduced model for each substance and response. A, being the mobile pH and the hard-to-change-factor, B the column type and C the organic modifier. The 2-Factor interactions A[1]B[1], A[2]B[1], A[3]B[1], A[1]B[2], A[2]B[2], A[3]B[2], A[1]B[3], A[2]B[3] and A[3]B[3] were non-significant for neither of the cases and were left out in the table. P-value shading: p<0,05 0,05≤p<0,1 p≥0,1.

API	Intercept	A[1]	A[2]	A[3]	B[1]	B[2]	B[3]	C	A[1]C	A[2]C	A[3]C	B[1]C	B[2]C	B[3]C
CAV	Log10(Tailing -1.00)	-0.1610	-0.0513	-0.1639	0.0871	-0.1011	0.1948	-0.0162						
	p-values		0.0053	0.0053	0.0053	0.0006	0.0006	0.0006						
	Log10(Resolution -0.50)	0.4780	-0.2447	0.6651	-0.1467			0.0568	-	-	0.1672			
	p-values		< 0.0001	< 0.0001	< 0.0001			0.2125	0.0086	0.0086	0.0086			
	Capacity Factor	10.3790	-0.5203	1.5512	-0.2440	0.3057	-0.0139	0.2432	1.3757	-	-	-	-	0.3090
	p-values		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0631	0.1234	0.0766	0.0161	0.0648	
	Log10(USP Plate Count -0.50)	4.6580	-0.0708	0.4932	-0.0993	0.2387	-0.0055	-0.0842	0.1035	0.3016	0.3016	0.3016	0.0001	0.0001
	p-values		0.0921	0.0921	0.0921	0.0012	0.0012	0.0012	0.0019					
	1/Sqrt(Purity Flag 0/1 + 0.00)	23.6860				8.1297	-0.2403	-0.8718	-			3.8718	6.2797	0.5444
	p-values					0.0941	0.0941	0.0941	4.2492			0.0368	0.0368	0.0368
	Log10(Purity flag forces degr. + 0.00)	-1.7490	0.2817	-0.5327	-0.2457	-0.3579	0.6328	-0.0919	0.3245					
	p-values		0.9342	0.9342	0.9342	0.3862	0.3862	0.3862	0.1548					
	(Tailing)^-2.3	0.5970				0.1002	0.0508	0.0386						
	p-values					0.0096	0.0096	0.0096						
FLP	Resolution	84.1070	9.1396	-40.9265	15.8935									
	p-values		0.0003	0.0003	0.0003									
	Capacity Factor	12.1560				0.7369	0.0528	-0.4450	1.0928			-	-	0.2848
	p-values					< 0.0001	< 0.0001	< 0.0001	< 0.0001			< 0.0001	< 0.0001	< 0.0001
	Log10(USP Plate Count)	5.2360				0.3355	0.4739	-0.0590	0.0470					
	p-values					< 0.0001	< 0.0001	< 0.0001	0.0471					

1/Sqrt(Purity Flag with 0/1 + 0.00)	22.4360				9.1870	9.1870	3.0623	- 1.5312		1.5312	1.5312	- 4.5935
p-values					< 0.0001	< 0.0001	< 0.0001	0.1489		0.0794	0.0794	0.0794
1/(Felo: Prutiy flag forced degradation + 0.00)	805.2690	135.5870	- 348.8530	58.6228	77.2172	127.7060	- 280.6130					
p-values		0.0280	0.0280	0.0280	0.0130	0.0130	0.0130					

Table S4. The coefficient table for the final reduced model for each substance and response. A, being methanol concentration, b the column temperature and C the flow rate. The 2-Factor interaction AB and BC were non-significant for neither of the cases and were left out in the table. P-value shading: p<0,05 0,05≤p<0,1 p≥0,1.

4
5

API	Response	Intercept	Block [1]	A	B	C	AC	A ²	B ²	C ²
CAV	Tailing	1.9157	-0.1681		0.0962				0.0662	
	p-values				0.2493				0.0090	
	1/Sqrt(Resolution)	0.6694	0.0369			-0.0007				-0.1214
	p-values					0.9862				0.0624
	Capacity F.	11.2496	-0.0684	-0.0165	-0.2016	-1.2423		-0.1775		0.61393
	p-values			0.7356	< 0.0001	< 0.0001		0.0457		< 0.0001
	USP PC	78471.5000	-5364.1100	11116.0000						
	p-values			0.0013						
	Logit[Prob(purity flag=1)]	-43.2126	15.1923	-0.5493	0.5493	29.1189				
	p-values	< 0.0001	0.1044	0.4620	0.4620	0.0001				
FLP	Logit[Prob(purity flag (degradation)=1)]	-43.4098	14.3378			29.0719				
	p-values	< 0.0001	0.1185			0.0001				
	(Tailing)^-2.81	0.7003	-0.0376	-0.0838		-0.0687	0.1481			
	p-values			0.09570		0.1698	0.0129			
	(Resolution)^2.81	8806.7100	-424.2790	-529.408				-2349.2100		
	p-values			0.3646				0.0151		
	(Capacity F.)^-1.5	0.019762	0.000504341	9.19885E-05	0.00058748	0.00179306	0.00104276	0.000798013		
	p-values			0.5251	0.0001	< 0.0001	< 0.0001	0.0013		
	Sqrt(USP PC)	691.047	-223.584	142.654	-70.858	83.0146				
	p-values			0.0007	0.0607	0.0383				
	Logit[Prob(purity flag=1)]	-15.178	12.6821	-1.09436	2.65331E-15	-1.09436				
	p-values	0.0003	0.2687	0.1504		0.1504				

Logit[Prob(purity flag (degradation)=1)]	-3.25931	-0.250235	-1.43193	-1.07072	-0.273009
p-values	0.0002	0.7404	0.0119	0.0535	0.6368