SUPPORTING INFORMATION.

Development of a Stability-Indicating Analytical Method for Determination of Venetoclax Using AQbD Principles

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List of supporting information:

Table S1. Solvent testing [a]

Solvent composition	Mass (m) of venetoclax [mg]	Visual evaluation	HPLC analysis		Visual evaluation of vials in autosampler at 5 °C after 3 days	Visual evaluation of vials in autosampler at 5 °C after 2 months
			Area under curve (AUC)	AUC/m		
ACN-DMSO-buffer (7:2:1, v/v/v)	4.937	Clear solution	175,195	35,607	Clear solution	Bigger crystals have precipitated
ACN-DMSO-buffer (6:3:1, v/v/v)	5.033	Clear solution	175,966	34,962	Clear solution	Clear solution
ACN-DMSO-buffer (5:4:1, v/v/v)	4.861	Clear solution	169,722	34,915	Clear solution	Clear solution
ACN-DMSO-buffer (4:5:1, v/v/v)	4.856	Clear solution	170,514	35,114	Clear solution	Clear solution
ACN-buffer (8:2, v/v)	5.291	Cloudy, partially dissolved	148,618	28,089	Clear solution	Bigger crystals have precipitated
MeOH-buffer (8:2, v/v)	5.321	Cloudy, partially dissolved	7,738	1,454	Clear solution	Clear solution
MeOH-DMSO-buffer (7:2:1, v/v/v)	5.512	Cloudy, partially dissolved	98,241	17,823	Clear solution	Smaller crystals have precipitated
MeOH-DMSO-buffer (4:5:1, v/v/v)	7.583	Clear solution	213,965	28,216	Clear solution	Smaller crystals have precipitated

[a] About 5 mg of venetoclax was weighed into a flask and 5 mL of solvent was added. Flasks were then put in an ultrasound bath for 5 min, left at room temperature for 2 h, visually evaluated and then filtered through 0.22 µm polyvinylidene fluoride (PVDF) filters into HPLC vials for analysis.

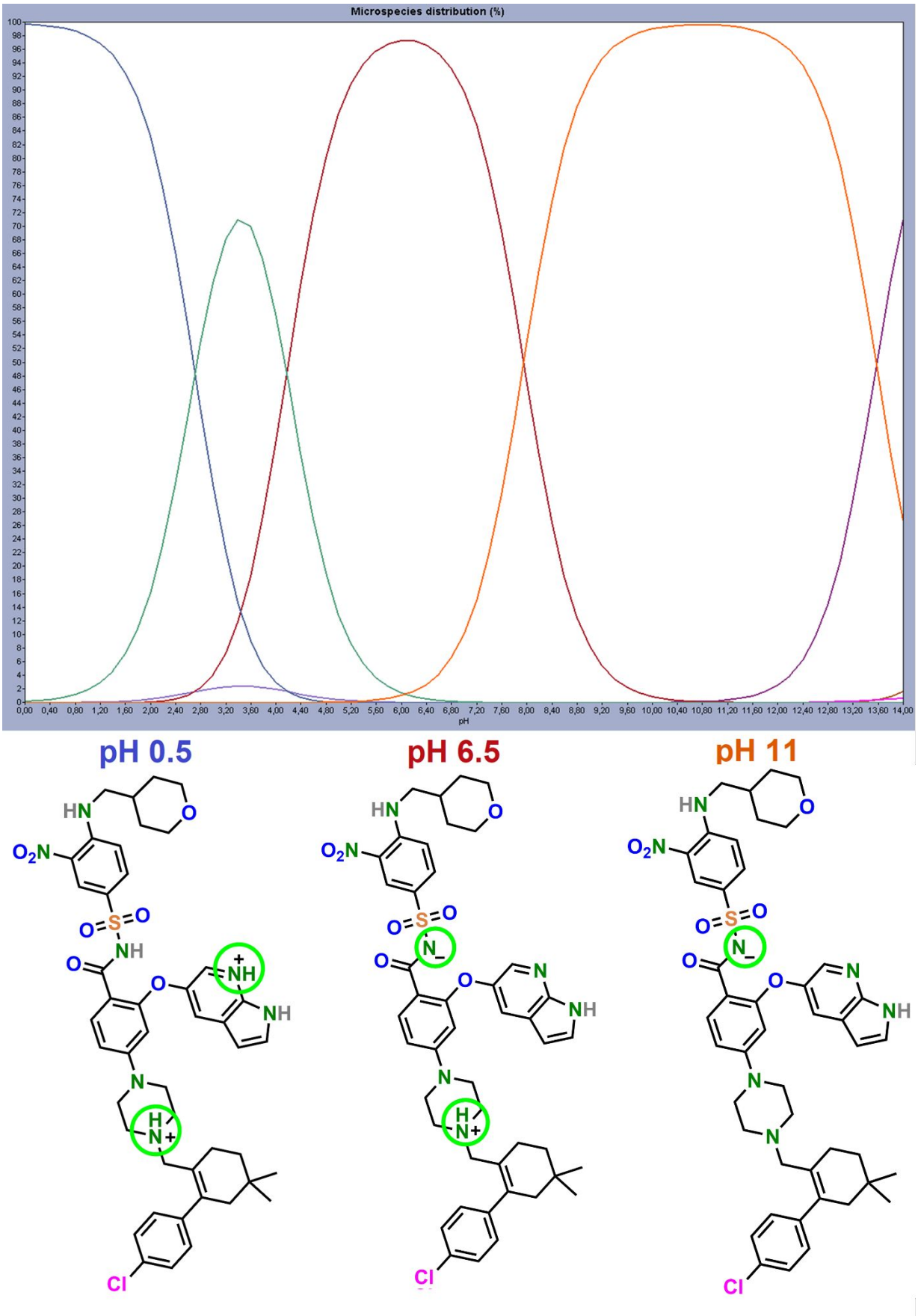


Figure S1. Predicted pH curves of venetoclax made by MarvinSketch. The three most prevalent microspecies are presented under the graph. Ionized parts of the molecule are marked with a green circle.

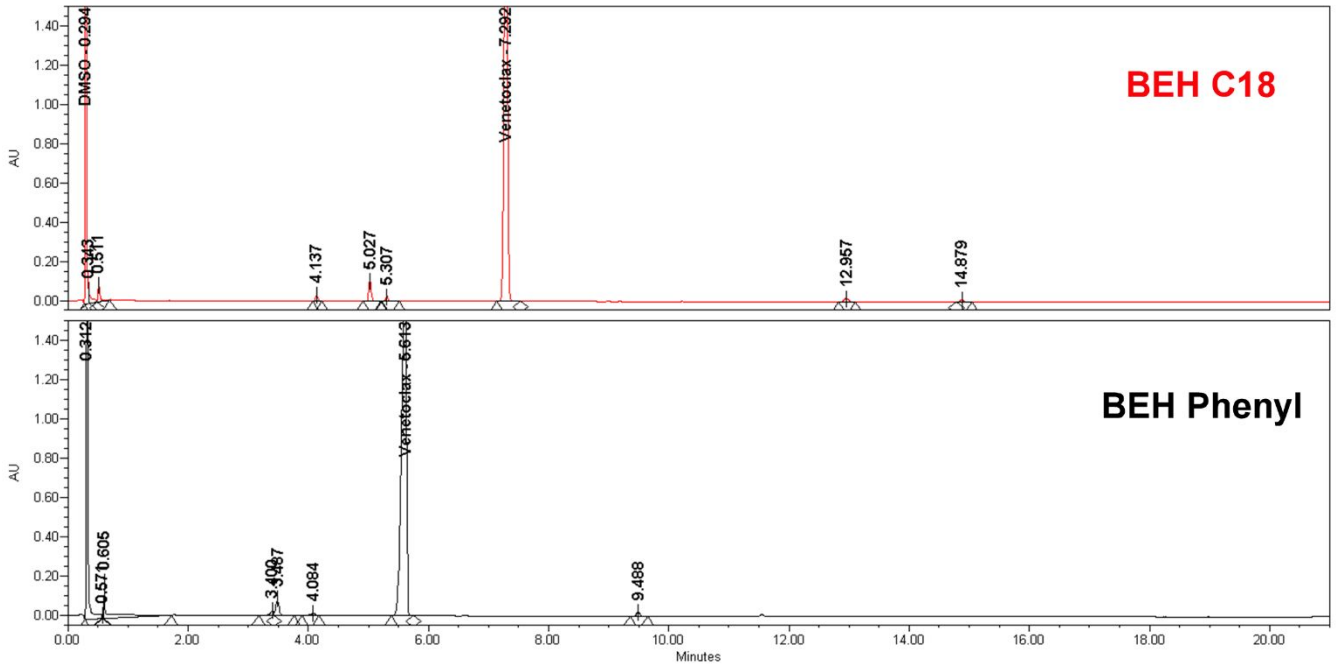


Figure S2. Chromatograms of venetoclax sample degraded with 1 M HCl at 50 °C for 3 days, using a BEH C18 column (top) and BEH Phenyl column (bottom) of the same particle size (1.7 µm), column length and width (100 mm × 2.1 mm), and chromatographic conditions (mobile phase A: A = NH4HCO3 (pH 7.0 adjusted with acetic acid, 10 mM); mobile phase B: B = ACN-MTBE (850:80, v/v); column temperature 70 °C; autosampler temperature 5 °C; flow rate 0.75 mL/min, gradient: t = 0 min, 30% B; t = 1 min, 40% B; t = 15 min, 70% B; t = 20 min, 70% B; t = 21 min, 30% B).

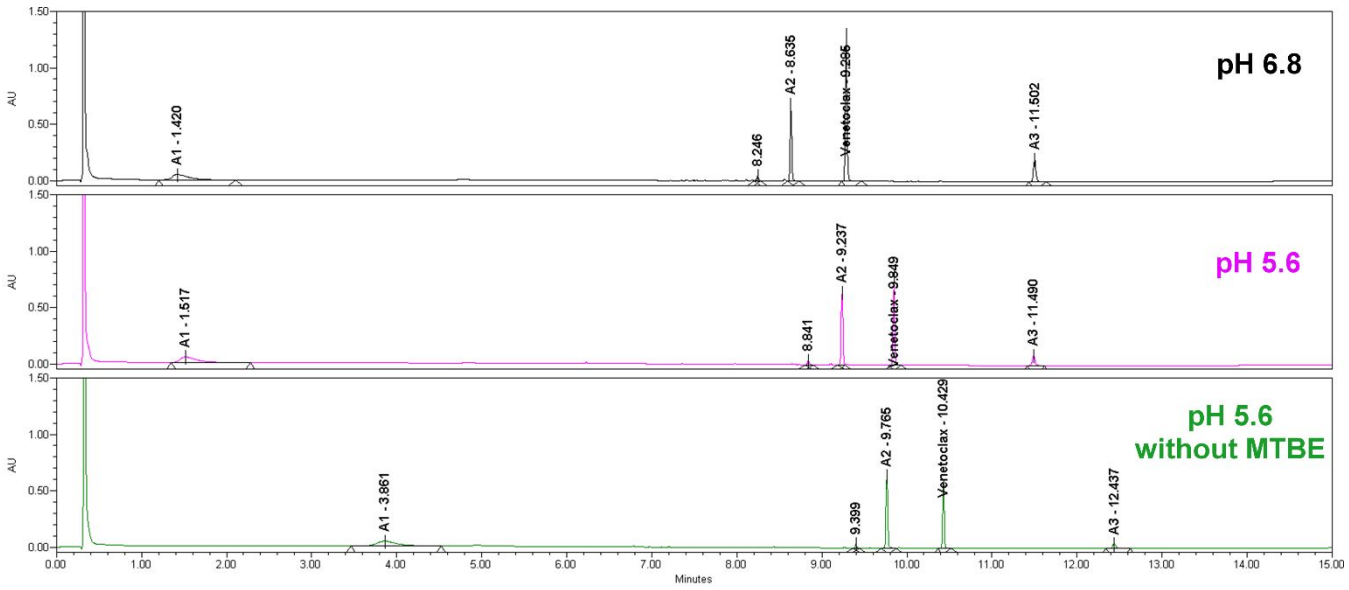
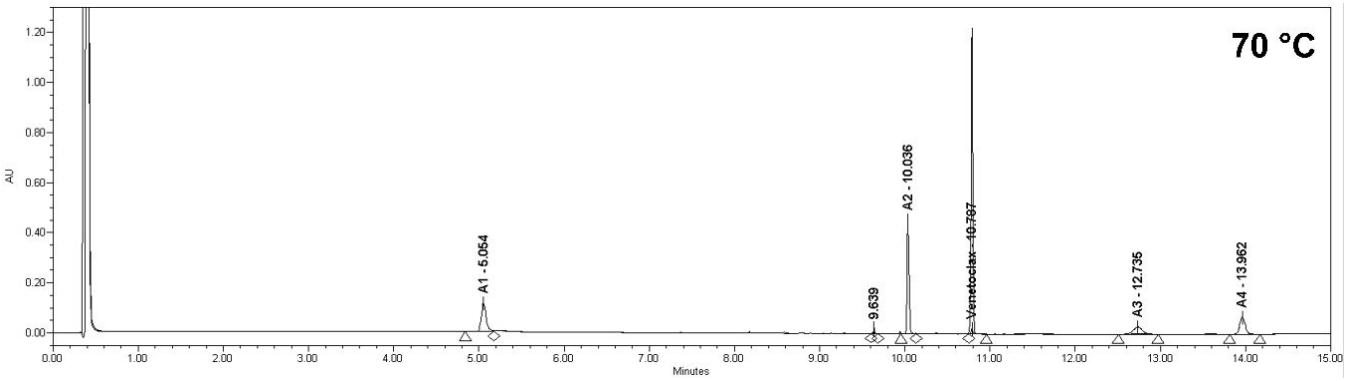
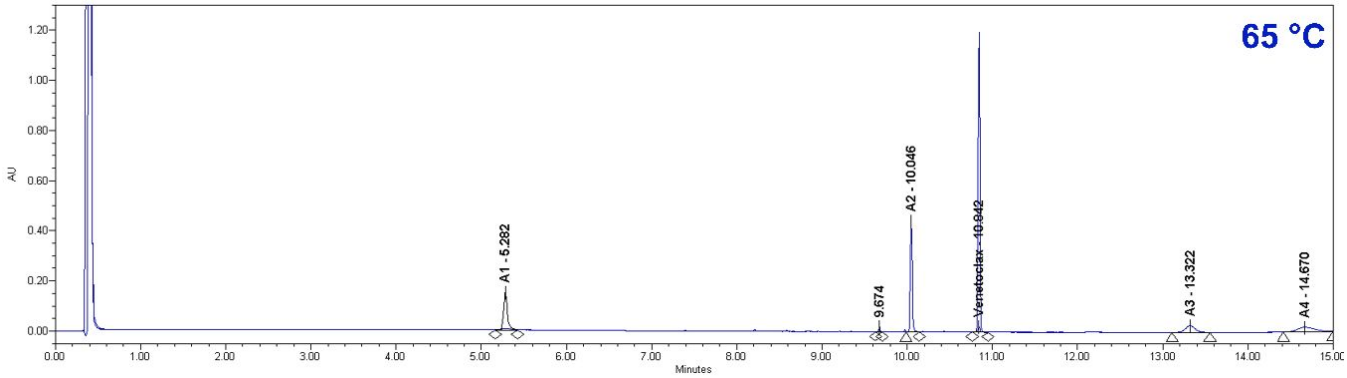


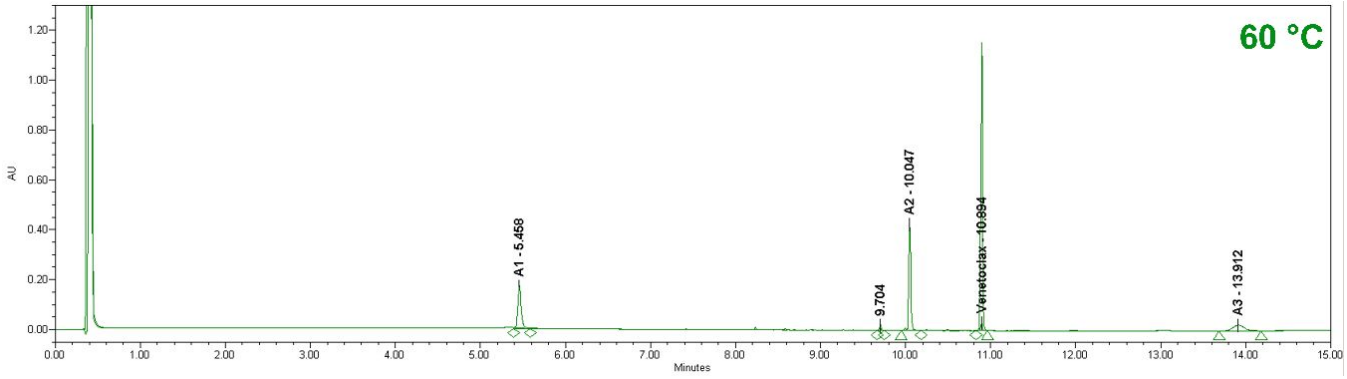
Figure S3. Chromatograms of venetoclax sample degraded with 1 M HCl at 50 °C for 3 days – the influence of small pH changes can be seen (top and middle) as well as the omission of MTBE in mobile phase B (bottom). Chromatographic conditions were: UPLC BEH C18 (1.7 µm, 100 mm × 2.1 mm) column; mobile phase A: A = NH_4HCO_3 (pH adjusted with acetic acid, 10 mM)-ACN (9:1, v/v); mobile phase B: B = ACN-MTBE (850:80, v/v) (top and middle) or 100% ACN (bottom); column temperature 70 °C; autosampler temperature 5 °C; flow rate 0.75 mL/min, gradient: t = 0 min, 0% B; t = 3 min, 0% B; t = 6 min, 30% B; t = 10 min, 70% B; t = 13 min, 70% B; t = 15 min, 30% B. The peak eluting at approximately 0.3 min is a solvent peak of DMSO.



(**a**)



(**b**)



(**c**)

Figure S4. The effects of column temperature change. Presented are chromatogram overlays of venetoclax drug substance sample and venetoclax sample degraded with 1 M HCl at 50 °C for 6 days. The chromatograms are presented as overlays due to the significant decrease of the venetoclax concentration in the degraded sample. (**a**) Column temperature was 70 °C. (**b**) Column temperature was 65 °C. (**c**) Column temperature was 60 °C. Other chromatographic conditions were: UPLC CSH C18 (1.7 µm, 100 mm × 2.1 mm) column; mobile phase A: A = NH_4HCO_3 (pH 6.0, 10 mM)-ACN (9:1, v/v); mobile phase B: B = ACN; pump flow 0.6 mL/min; autosampler temperature 5 °C; gradient: t = 0 min, 0% B; t = 3 min, 0% B; t = 10 min, 70% B; t = 13 min, 70% B; t = 15 min, 30% B. The peak eluting at approximately 0.4 min is a solvent peak of DMSO.

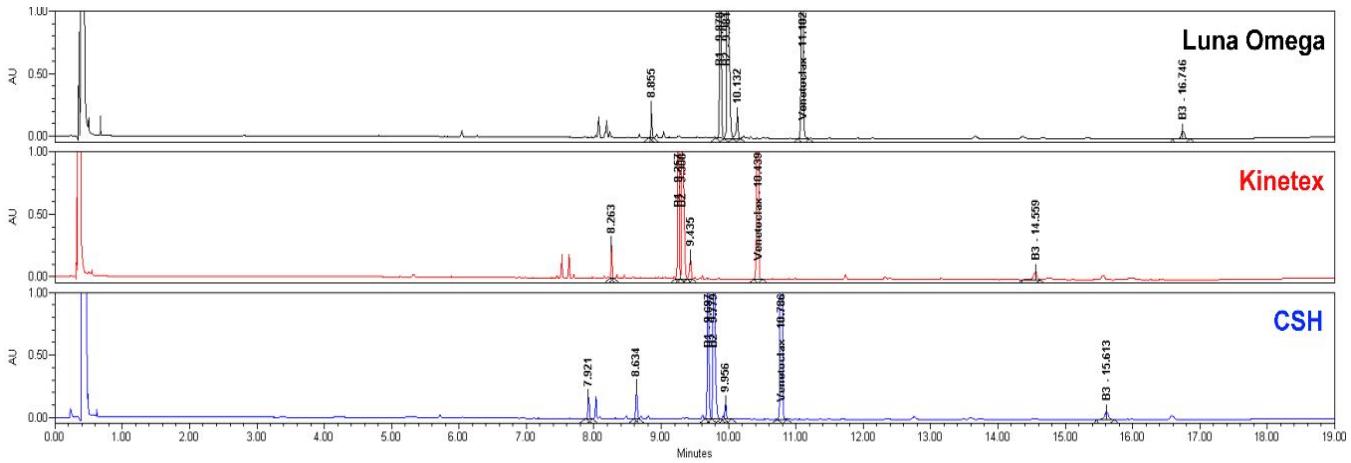


Figure S5. Testing of different C18 chromatographic columns: Luna Omega C18 (top), Kinetex C18 (middle), and CSH C18 (bottom) column. Presented are chromatograms of venetoclax sample degraded with 1 M NaOH at 50 °C for 14 days. Other chromatographic conditions were: mobile phase A: A = NH_4HCO_3 (pH 6.0, 10 mM)-ACN (9:1, v/v); mobile phase B: B = ACN; pump flow 0.6 mL/min; column temperature 60 °C; autosampler temperature 5 °C; gradient: t = 0 min, 0% B; t = 3 min, 0% B; t = 10 min, 70% B; t = 12 min, 70% B; t = 16 min, 80% B; t = 18 min, 80% B; t = 19 min, 0% B. The peak eluting at approximately 0.4 min is a solvent peak of DMSO.

Table S2. DoE from method scouting experiment.

Run No.	Strong Solvent Type (*)	Gradient Time (min)	Column Type (*)
Condition Column - 1	Acetonitrile	10,0	Column 1
Condition Column - 2	Acetonitrile	10,0	Column 2
1	Acetonitrile	15,0	Column 1
2	Acetonitrile	30,0	Column 1
3	Acetonitrile	15,0	Column 2
4	Acetonitrile	30,0	Column 2
5	Acetonitrile	22,5	Column 1
6	Acetonitrile	22,5	Column 2
7	Acetonitrile	22,5	Column 1
8	Acetonitrile	22,5	Column 2
Condition Column - 3	Methanol	10,0	Column 1
Condition Column - 4	Methanol	10,0	Column 2
9	Methanol	15,0	Column 1
10	Methanol	30,0	Column 1
11	Methanol	15,0	Column 2
12	Methanol	30,0	Column 2
13	Methanol	22,5	Column 1
14	Methanol	22,5	Column 2
15	Methanol	22,5	Column 1
16	Methanol	22,5	Column 2
Condition Column - 5	Methanol	10,0	Column 1
Condition Column - 6	Methanol	10,0	Column 2

Table S3. First DoE from method screening.

Run No.	Pump Flow Rate (mL/min)	Final % Organic (%)	pH (*)
Condition Column - 1	0,300	95,0	6,00
1	0,300	95,0	6,00
2	0,300	80,0	6,00
3	0,300	87,5	6,00
4	0,400	95,0	6,00
5	0,400	80,0	6,00
6	0,400	87,5	6,00
Condition Column - 2	0,300	91,3	6,50
7	0,300	91,3	6,50
8	0,300	83,8	6,50
9	0,400	91,3	6,50
10	0,400	83,8	6,50
Condition Column - 3	0,300	87,5	7,00
11	0,300	87,5	7,00
12	0,300	95,0	7,00
13	0,300	80,0	7,00
14	0,400	95,0	7,00
15	0,400	80,0	7,00
16	0,400	87,5	7,00
17	0,300	87,5	7,00
Condition Column - 4	0,300	91,3	7,50
18	0,300	91,3	7,50
19	0,400	83,8	7,50
Condition Column - 5	0,300	95,0	8,00
20	0,300	95,0	8,00
21	0,300	80,0	8,00
22	0,300	87,5	8,00
23	0,400	95,0	8,00
24	0,400	80,0	8,00
25	0,400	87,5	8,00
26	0,300	80,0	8,00
27	0,400	95,0	8,00
Condition Column - 6	0,300	95,0	8,00

Table S4. Second DoE from method screening.

Run No.	Gradient Time (min)	Oven Temperature (°C)
Condition Column - 1	2,0	40,0
1	18,0	40,0
2	6,0	40,0
3	12,0	40,0
4	12,0	45,0
5	15,0	45,0
6	9,0	45,0
7	18,0	45,0
8	12,0	45,0
9	15,0	50,0
10	9,0	50,0
11	18,0	55,0
12	6,0	55,0
13	12,0	55,0
14	18,0	55,0
15	6,0	55,0
Condition Column - 2	2,0	55,0

Table S5. DoE from method optimization.

Run No.	Pump Flow Rate (mL/min)	Final % Organic (%)	Oven Temperature (°C)	pH (*)
Condition Column - 1	0,400	85,0	45,0	6,00
1	0,450	85,0	45,0	6,00
2	0,450	75,0	45,0	6,00
3	0,350	75,0	45,0	6,00
4	0,400	85,0	45,0	6,00
5	0,350	80,0	45,0	6,00
6	0,450	75,0	45,0	6,00
Condition Column - 2	0,400	85,0	45,0	7,00
7	0,450	85,0	45,0	7,00
8	0,350	85,0	45,0	7,00
9	0,450	75,0	45,0	7,00
10	0,400	75,0	45,0	7,00
11	0,400	80,0	45,0	7,00
Condition Column - 3	0,400	85,0	45,0	8,00
12	0,350	85,0	45,0	8,00
13	0,450	75,0	45,0	8,00
14	0,350	75,0	45,0	8,00
15	0,400	85,0	45,0	8,00
16	0,450	80,0	45,0	8,00
17	0,350	75,0	45,0	8,00
Condition Column - 4	0,400	82,5	48,8	6,50
18	0,375	82,5	48,8	6,50
19	0,425	77,5	48,8	6,50
Condition Column - 5	0,400	85,0	52,5	6,00
20	0,450	85,0	52,5	6,00
21	0,350	85,0	52,5	6,00
22	0,400	75,0	52,5	6,00
Condition Column - 6	0,400	80,0	52,5	7,00
23	0,400	80,0	52,5	7,00

24	0,450	75,0	52,5	7,00
25	0,350	75,0	52,5	7,00
26	0,350	80,0	52,5	7,00
27	0,400	80,0	52,5	7,00
Condition Column - 7	0,400	85,0	52,5	8,00
28	0,450	85,0	52,5	8,00
29	0,350	85,0	52,5	8,00
30	0,400	75,0	52,5	8,00
31	0,400	80,0	52,5	8,00
Condition Column - 8	0,400	77,5	56,3	6,50
32	0,375	77,5	56,3	6,50
Condition Column - 9	0,400	82,5	56,3	7,50
33	0,425	82,5	56,3	7,50
Condition Column - 10	0,400	85,0	60,0	6,00
34	0,450	85,0	60,0	6,00
35	0,350	85,0	60,0	6,00
36	0,450	75,0	60,0	6,00
37	0,350	75,0	60,0	6,00
38	0,400	85,0	60,0	6,00
39	0,450	80,0	60,0	6,00
Condition Column - 11	0,400	85,0	60,0	7,00
40	0,450	85,0	60,0	7,00
41	0,350	85,0	60,0	7,00
42	0,350	75,0	60,0	7,00
43	0,400	75,0	60,0	7,00
Condition Column - 12	0,400	85,0	60,0	8,00
44	0,450	85,0	60,0	8,00
45	0,450	75,0	60,0	8,00
46	0,350	75,0	60,0	8,00
47	0,400	85,0	60,0	8,00
48	0,350	80,0	60,0	8,00
49	0,450	75,0	60,0	8,00
Condition Column - 13	0,400	75,0	60,0	8,00