

Supplementary Materials

Table S1. Screening FFD matrix (2³) used for selection of the most important variables in sample preparation. The independent variables (X₁ to X₃), and their levels (not coded) are shown in the matrix. Trial 9 is the central point, which was prepared in 6 replicates.

Trial	X ₁ - Agitation Time (min)	X ₂ – Sonication time (min)	X ₃ - Extraction volume (mL)
1	5	15	2.50
2	15	15	2.50
3	5	45	2.50
4	15	45	2.50
5	5	15	7.50
6	15	15	7.50
7	5	45	7.50
8	15	45	7.50
9 (n = 6)	10	30	5.00

Table S2. BBD matrix of experiments (trials) performed to find the robust working region for the sample preparation protocol. The independent variables (from X₁ to X₃, not coded) and their levels are shown in the matrix. Trial 13 is the central point, which was prepared in 5 replicates.

Trial	X ₁ - Agitation Time (min)	X ₂ - Sonication time (min)	X ₃ - Extraction volume (mL)
1	10	5	7.50
2	20	5	7.50
3	10	25	7.50
4	20	25	7.50
5	10	15	5.00
6	20	15	5.00
7	10	15	10.0
8	20	15	10.0
9 ^a	15	5	5.00
10	15	25	5.00
11	15	5	10.0
12	15	25	10.0
13 (n = 5)	15	15	7.50

Table S3. Results from the screening FFD matrix (2³) used for the selection of the most important variables in sample preparation. Trial 9 is the central point, which was prepared in 5 replicates.

Trial	CBD (Area/IS)	THC (Area/IS)	CBN (Area/IS)
1	0.015	0.042	0.066
2	0.016	0.027	0.041
3	0.013	0.022	0.032
4	0.015	0.022	0.035
5	0.019	0.042	0.064
6	0.013	0.021	0.034
7	0.012	0.021	0.029
8	0.017	0.032	0.045
9 (n = 6)	0.012 ± 0.001	0.019 ± 0.002	0.028 ± 0.003

Table S4. Results from BBD experiments (trials) performed to find the robust working region for the sample preparation protocol. Trial 13 is the central point, which was prepared in 5 replicates.

Trial	CBD (Area/IS)	THC (Area/IS)	CBN (Area/IS)
1	0.009	0.012	0.028
2	0.007	0.012	0.031
3	0.006	0.010	0.032
4	0.012	0.016	0.039
5	0.007	0.009	0.024
6	0.007	0.009	0.021
7	0.008	0.011	0.024
8	0.005	0.011	0.034
9^a	0.007	0.007	0.019
10	0.009	0.014	0.037
11	0.009	0.014	0.034
12	0.007	0.010	0.026
13	0.010	0.015	0.039
(n = 5)	± 0.001	±0.002	± 0.005

Table S5. Analysis of Variance (ANOVA) parameters, p-values of the predictive model, lack-of-fit and calculated not-coded regression coefficients, for CBD, THC and CBN.

	CBD	THC	CBN
R²	0.8554	0.8800	0.8571
R² (Adj.)	0.6695	0.7257	0.6733
Model	0.028	0.016	0.027
Lack-of-fit	0.080	0.601	0.901
<i>b</i>₀	-0.01910	-0.0471	-0.0936
<i>b</i>₁	0.001479	0.00222	0.00383
<i>b</i>₂	-0.000291	0.000679	0.00249
<i>b</i>₃	0.00529	0.01004	0.02052
<i>b</i>_{1,2}	0.000037	0.000029	0.000018
<i>b</i>_{1,3}	-0.000055	0.000006	0.000261
<i>b</i>_{2,3}	-0.000033	-0.000107	-0.000261
<i>b</i>_{1,1}	-0.000054	-0.000084	-0.000188
<i>b</i>_{2,2}	0.000001	-0.000008	-0.000017
<i>b</i>_{3,3}	-0.000266	-0.000546	-0.001316

Table S6. Validation of model predictability for quantification of CBD, THC, and CBN in CHE by UHPLC-HRMS/MS.

Trial	CBD		THC		CBN	
	Exp.	Predicted (% Accuracy)	Exp.	Predicted (% Accuracy)	Exp.	Predicted (% Accuracy)
1	0.009	0.01 (111)	0.012	0.012 (100)	0.028	0.028 (100)
2	0.007	0.006 (86)	0.012	0.011 (92)	0.031	0.031 (100)
3	0.006	0.007 (117)	0.010	0.011 (110)	0.032	0.032 (100)
4	0.012	0.011 (92)	0.016	0.015 (94)	0.039	0.038 (97)
5	0.007	0.006 (86)	0.009	0.008 (89)	0.024	0.025 (104)
6	0.007	0.008 (114)	0.009	0.010 (111)	0.021	0.023 (110)
7	0.008	0.007 (88)	0.011	0.010 (91)	0.024	0.023 (96)
8	0.005	0.006 (120)	0.011	0.011 (100)	0.034	0.033 (97)
9 ^a	0.007	0.007 (100)	0.007	0.007 (100)	0.019	0.018 (95)
10	0.009	0.009 (100)	0.014	0.013 (93)	0.037	0.036 (97)
11	0.009	0.009 (100)	0.014	0.014 (100)	0.034	0.035 (103)
12	0.007	0.008 (114)	0.010	0.010 (100)	0.026	0.027 (104)
13	0.010	0.010 (100)	0.015	0.015 (100)	0.039	0.039 (100)