A liquid chromatography electrospray ionization tandem mass spectrometry method for quantification of up to eighteen cannabinoids in hemp-derived products

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Fig. 1. LC-ESI/TOFMS extracted ion chromatograms of nineteen cannabinoids using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ions of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish) at 1 μg/mL except for ACBD at 0.5 μg/mL.

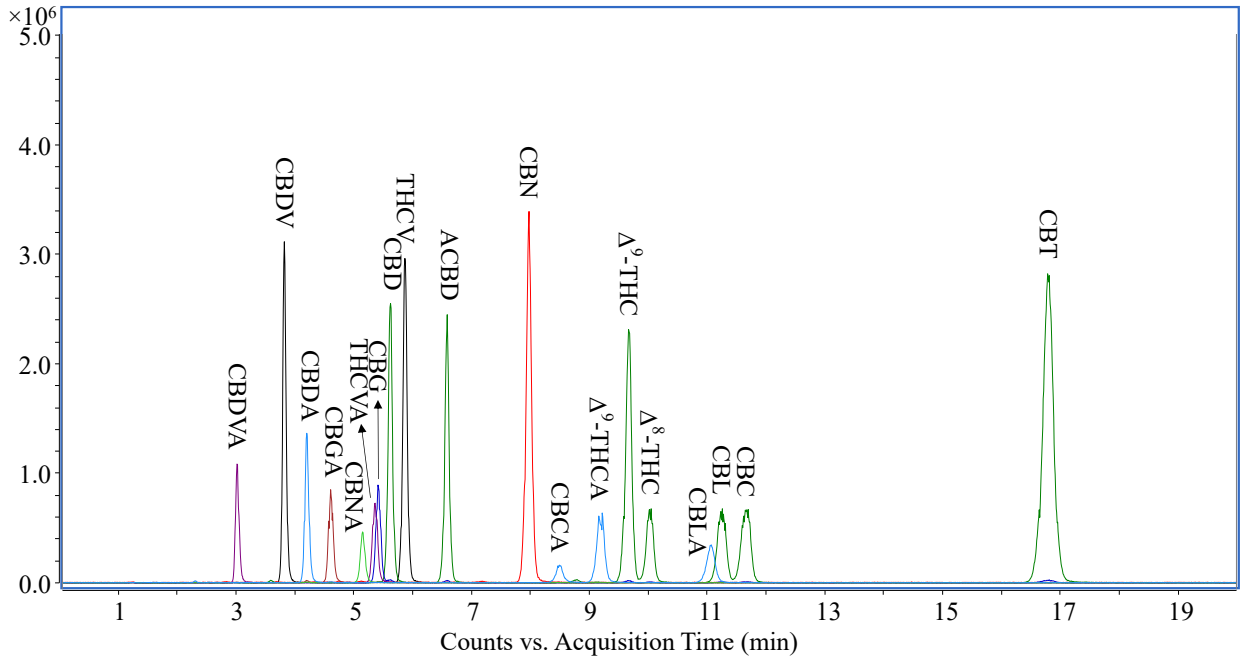
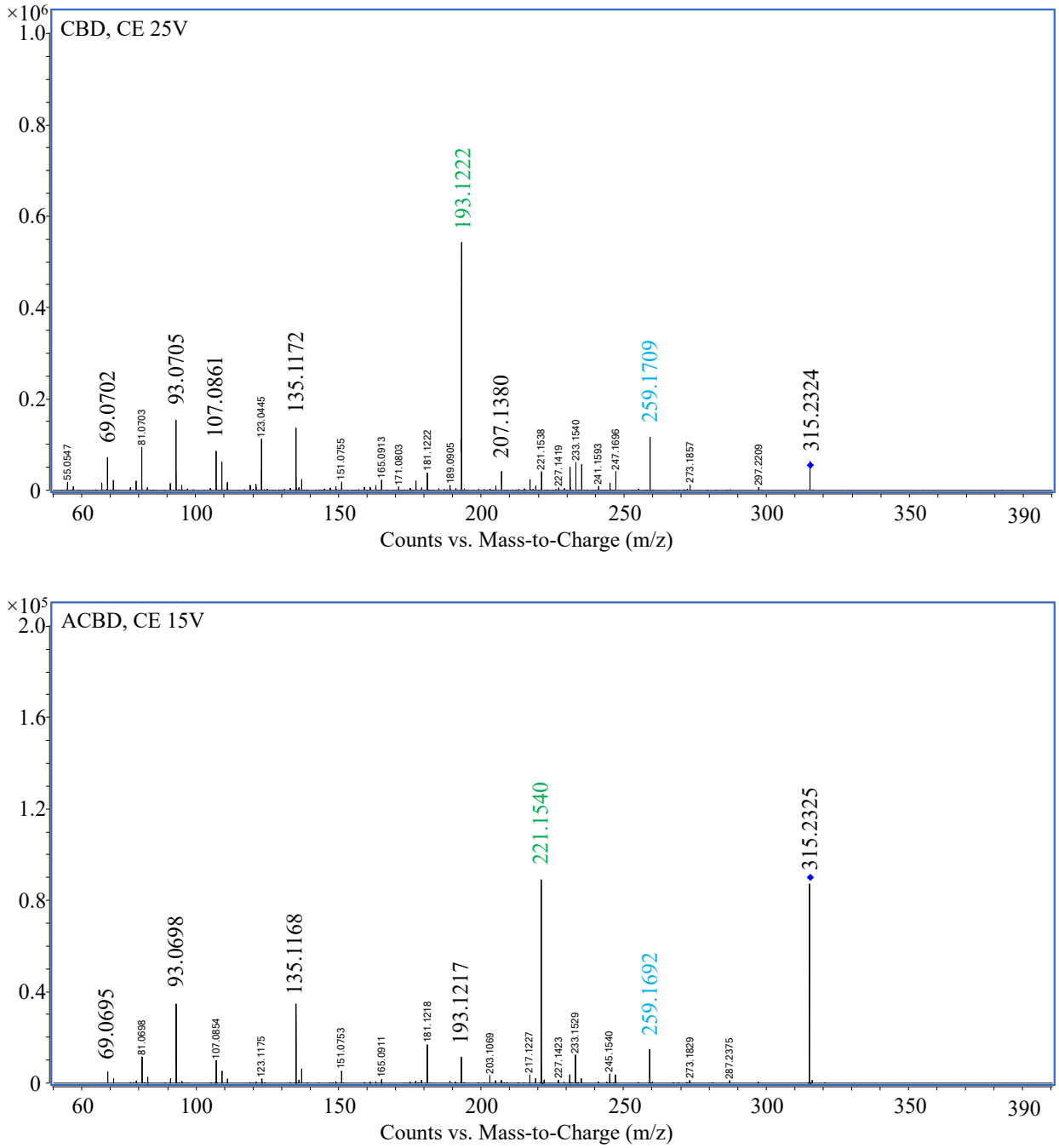
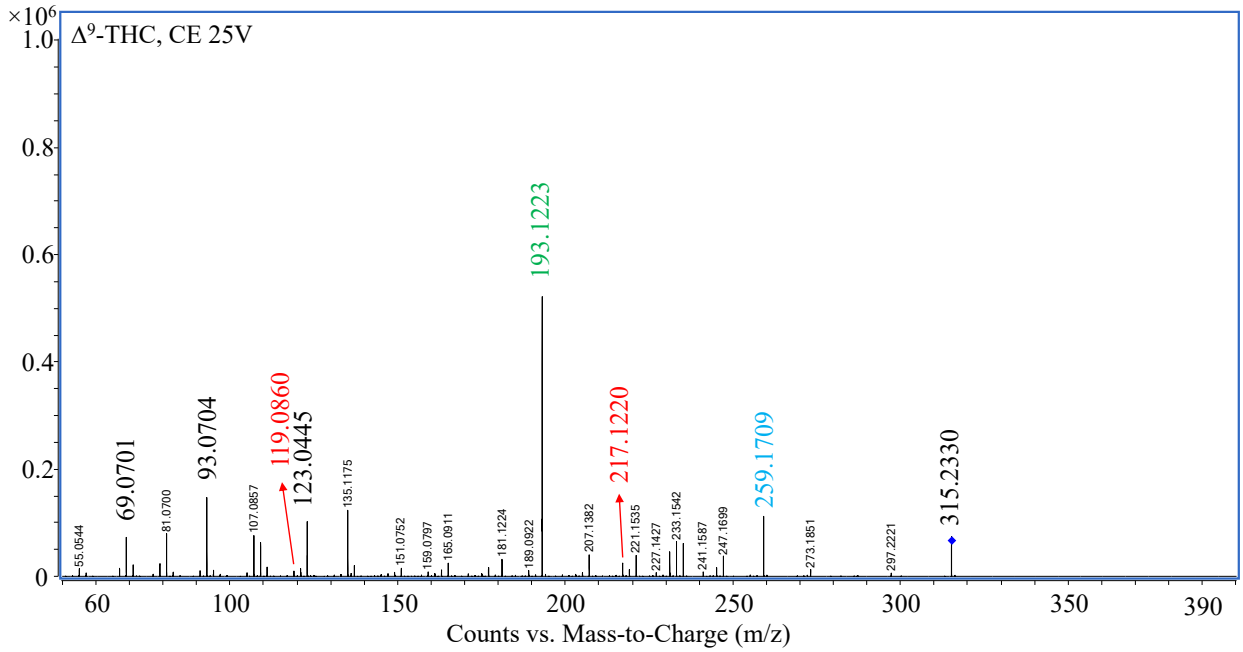


Fig. 2. MS/MS spectra of CBD, ACBD, Δ^9-THC, Δ^8-THC, CBL, CBC and CBT at 1 µg/mL except for ACBD at 0.5 µg/mL. Precursor ions are marked with a blue diamond symbol, while quantifier and qualifier ions are in green and cyan, respectively. Ions marked with red numbers and arrows are discussed in the Discussion section.

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0.6
0.4
0.2
0
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81.0700
93.0704
107.0857
119.0860
123.0445
135.1175
151.0752
159.0797
165.0911
181.1224
189.0922
193.1223
207.1382
217.1220
221.1535
227.1427
233.1542
241.1587
247.1699
259.1709
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300
350
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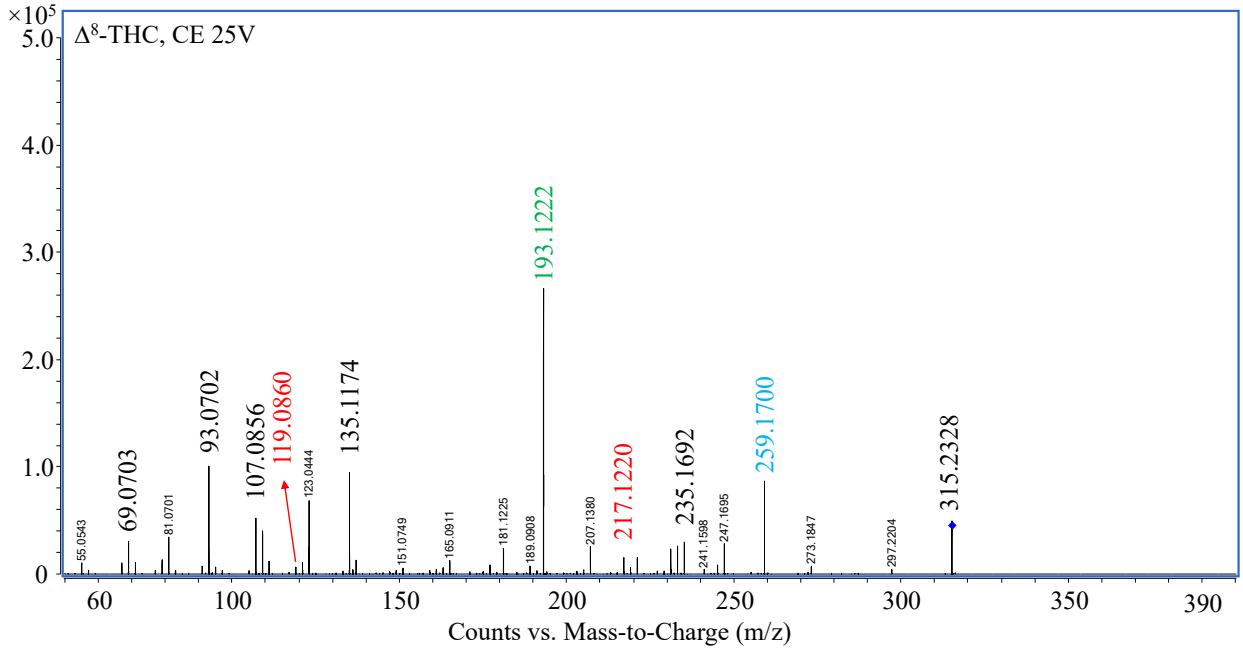
Counts vs. Mass-to-Charge (m/z)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151.0749
165.0911
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193.1222
207.1380
217.1220
235.1692
241.1598
247.1695
259.1700
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350
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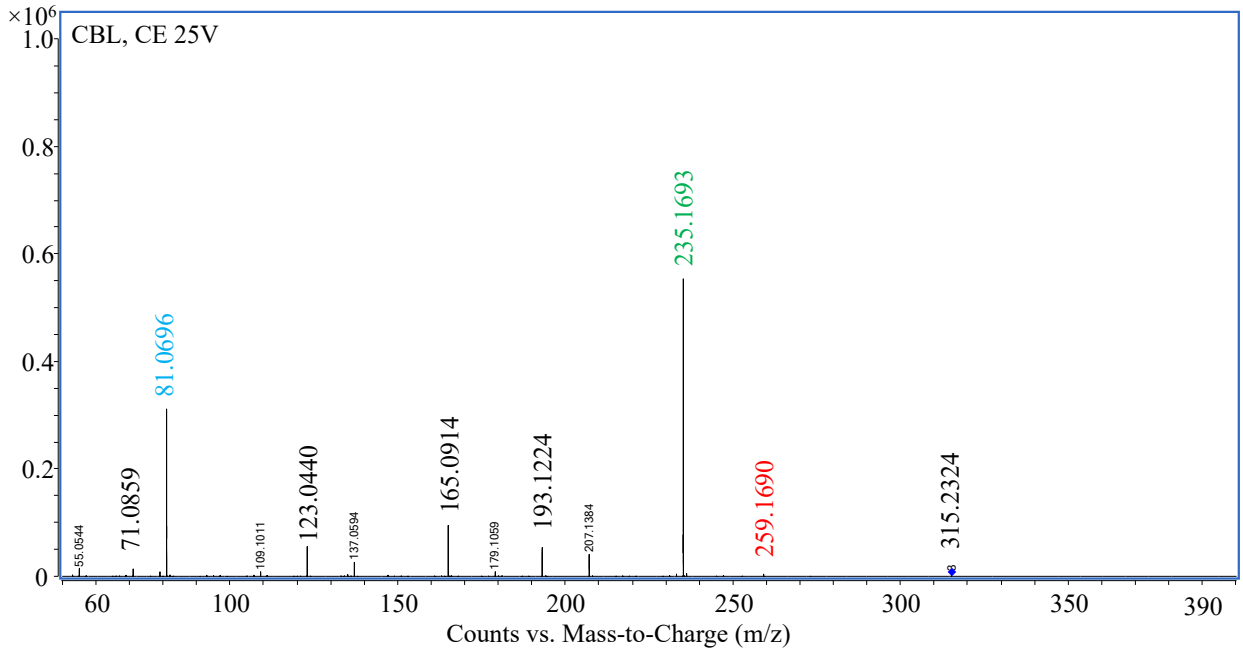
Counts vs. Mass-to-Charge (m/z)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137.0594
165.0914
179.1059
193.1224
207.1384
235.1693
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100
150
200
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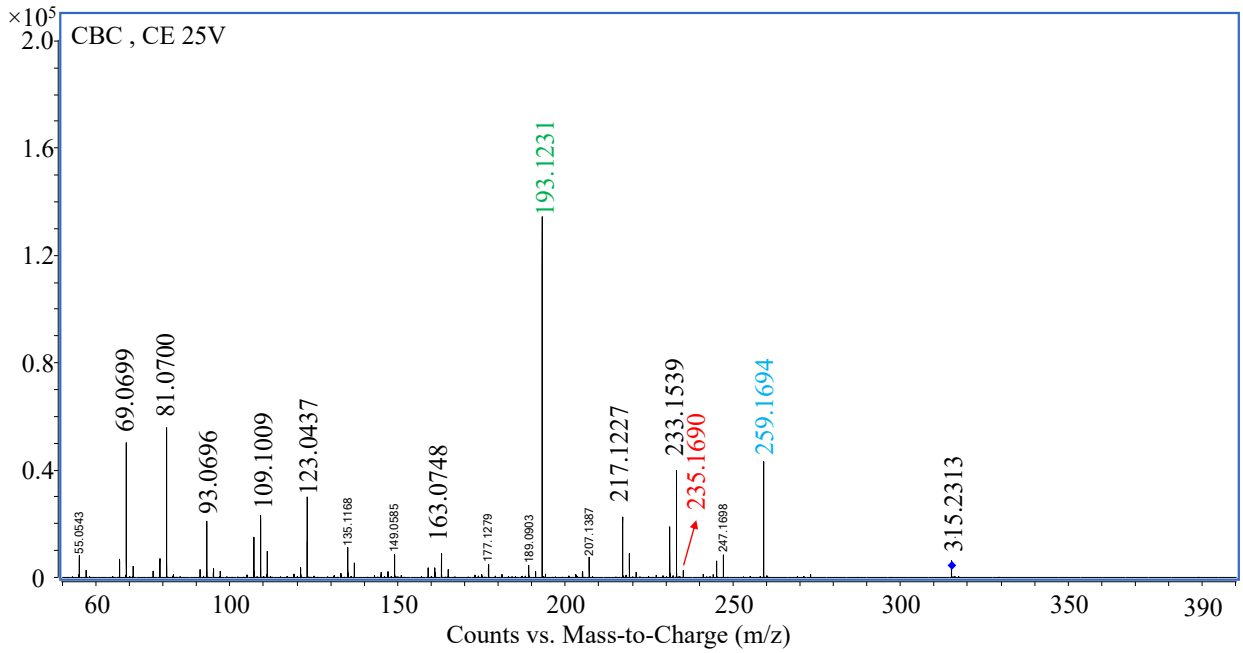
300
350
390
Counts vs. Mass-to-Charge (m/z)


CBC , CE 25V
×10^5
2.0
1.6
1.2
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193.1231
207.1387
217.1227
233.1539
235.1690
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200
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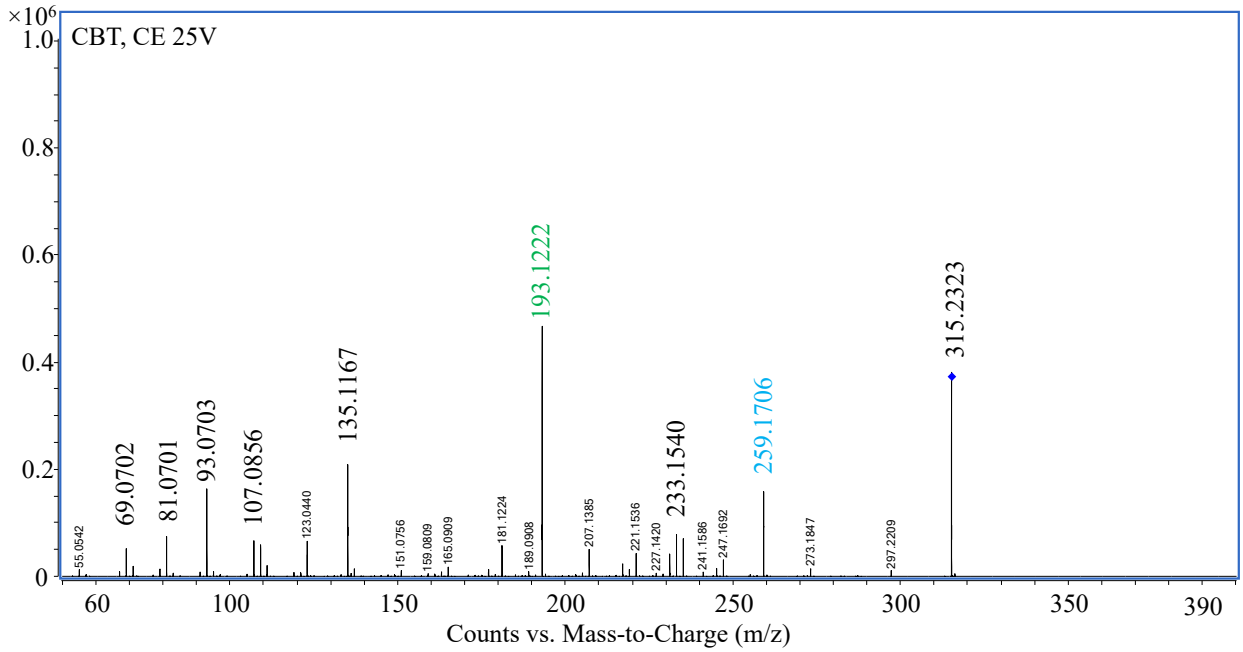
300
350
390
Counts vs. Mass-to-Charge (m/z)


CBT, CE 25V
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107.0856
123.0440
135.1167
151.0756
159.0809
165.0909
181.1224
189.0908
193.1222
207.1385
221.1536
227.1420
233.1540
241.1586
247.1692
259.1706
273.1847
297.2209
315.2323
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

Fig. 3. LC-ESI/MS/MS extracted ion chromatograms of twelve cannabinoids at 0.25 µg/mL (A) and seven isotopically labeled cannabinoids at 0.5 µg/mL (B). Precursor to quantifier ion transition of each cannabinoid is listed in **Table 1** with ±20 ppm mass error (peaks were both color coded and labelled for easy distinguish).

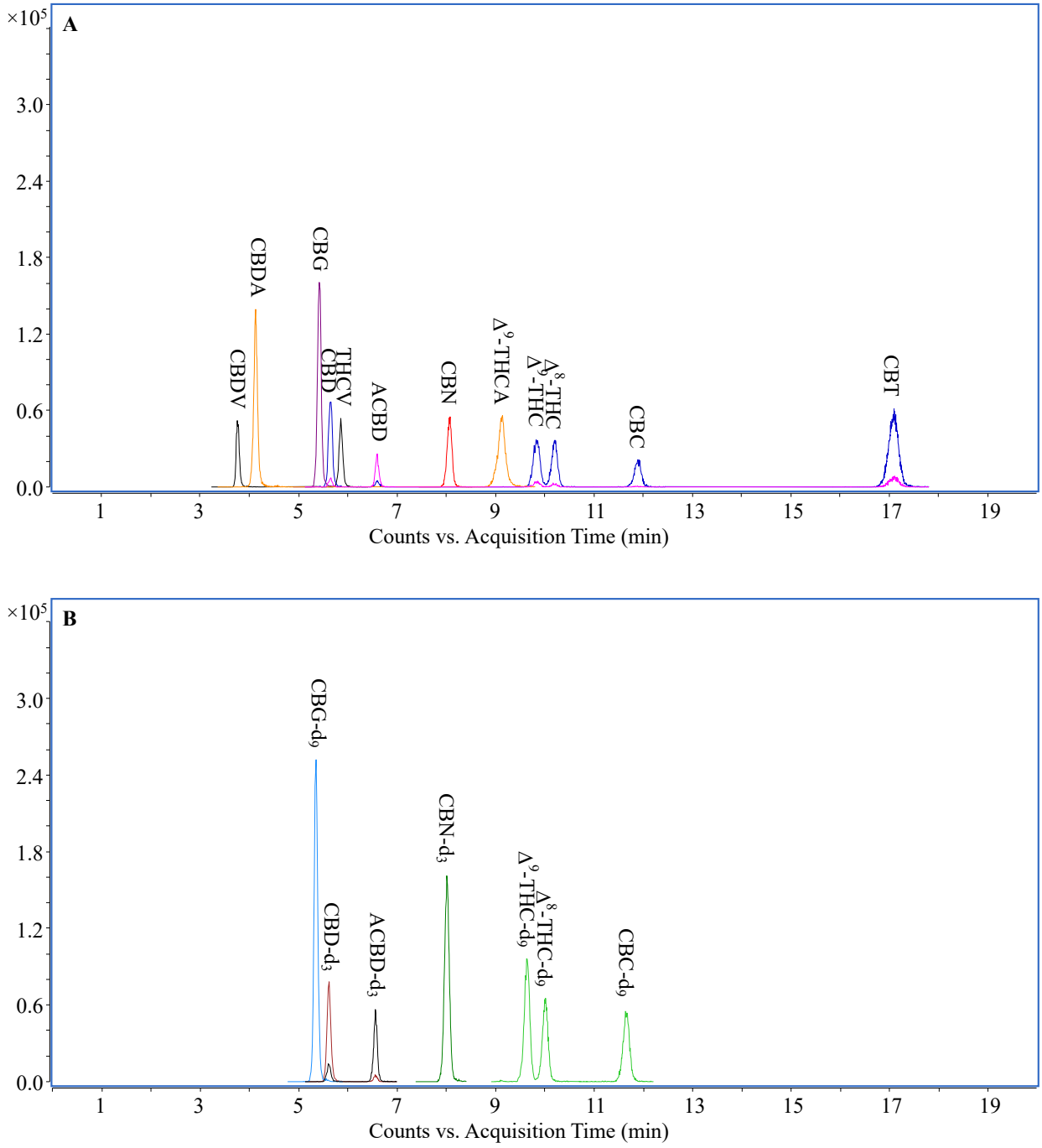


Table 1

Optimized MS/MS parameters specific to individual cannabinoid.

Cannabinoid	Precursor	CE	Quantifier	Qualifier
CBDV	287.2006	25	165.089	231.133
THCV	287.2006	25	165.087	231.133
CBN	311.2006	25	223.111	293.187
CBD	315.2319	25	193.120	259.163
ACBD	315.2319	15	221.152	259.164
Δ^9-THC	315.2319	25	193.120	259.169
Δ^8-THC	315.2319	25	193.120	259.169
CBL	315.2319	25	235.170	81.070
CBC	315.2319	25	193.120	259.169
CBT	315.2319	25	193.120	259.169
CBG	317.2475	15	193.120	207.136
CBDVA	331.1904	15	313.181	191.070
THCVA	331.1904	15	313.181	191.070
CBGA	343.2268	15	219.100	261.146
CBNA	355.1904	15	337.176	311.202
CBDA	359.2217	15	341.211	219.102
CBCA	359.2217	15	341.211	219.102
Δ^9-THCA	359.2217	15	341.211	219.102
CBLA	359.2217	15	341.211	261.149
CBN-d_3	314.2194	25	223.111	296.207
CBD-d_3	318.2507	25	196.142	262.184
ACBD-d_3	318.2507	15	224.172	262.184
Δ^9-THC-d_9	324.2883	25	202.177	268.220
Δ^8-THC-d_9	324.2883	25	202.177	268.220
CBC-d_9	324.2883	25	202.120	268.220
CBG-d_9	326.3034	15	202.177	216.194

Table 2

Average recovery and matrix effect of ACBD spiked in eighteen samples in triplicates.

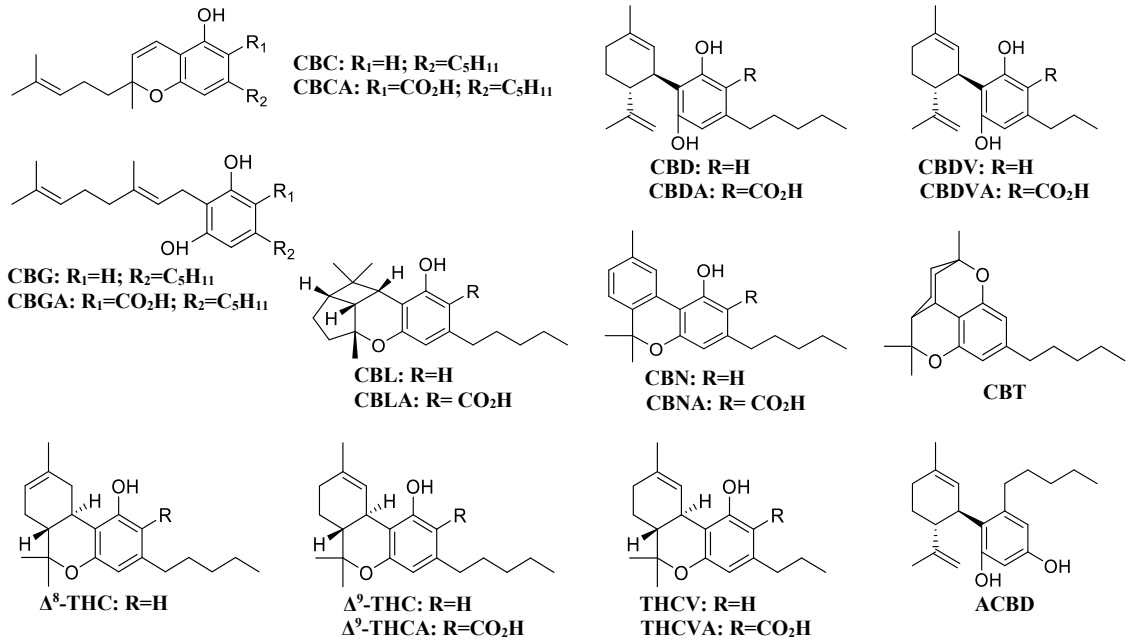
Sample	Recovery	Matrix Effect
D1	95.9±2.2%	97.7±2.4%
D2	94.1±0.8%	96.3±1.4%
O1	102.3±5.2%	98.6±8.3%
O2	98.2±1.6%	94.2±2.6%
T1	100.1±4.5%	107.4±18.9%
T2	100.5±2.8%	91.3±3.2%
T3	106.3±0.7%	95.2±2.2%
T4	100.8±2.4%	100.3±9.8%
G1	96.2±2.1%	119.0±3.5%
G2	97.6±8.0%	100.1±11.0%
HC1	92.9±2.5%	120.2±3.1%
C1	94.4±1.8%	107.5±3.9%
S1	97.7±4.0%	97.8±5.5%
S2	101.6±6.5%	96.6±7.0%
S3	100.4±2.3%	99.6±5.4%
S4	103.9±2.5%	101.2±3.2%
PT1	100.8±9.0%	102.0±11.6%
PT2	92.9±2.0%	94.7±3.0%

Table 3

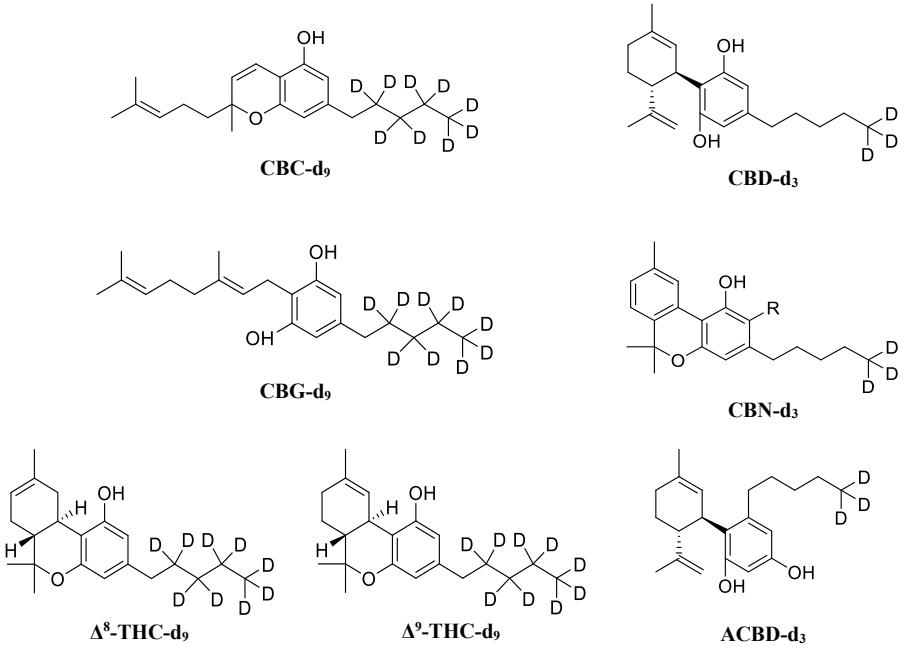
Average content (%, w/w) of cannabinoids in hemp-derived products measured in triplicates.

Sample	CBDV	CBG	CBD	CBN	Δ^9-THC	CBC	CBT	Total
D1			0.00491		0.00011	0.00013		0.00516
D2			0.00332		0.00010	0.00010		0.00352
O1		1.514	0.491					2.006
O2			0.010	0.372				0.382
T1	0.024	0.025	3.184		0.066	0.120	0.015	3.434
T2			0.051					0.051
T3			0.119					0.119
T4		0.009	0.764		0.030	0.046		0.850
G1			0.411		0.010		0.018	0.438
G2		0.048	0.044					0.092
HC1			0.036					0.036
C1			0.027					0.027
S1			0.020					0.020
S2			0.199			0.012		0.211
S3			0.118					0.118
S4			0.026					0.026
PT1			0.021					0.021
PT2			0.087					0.087

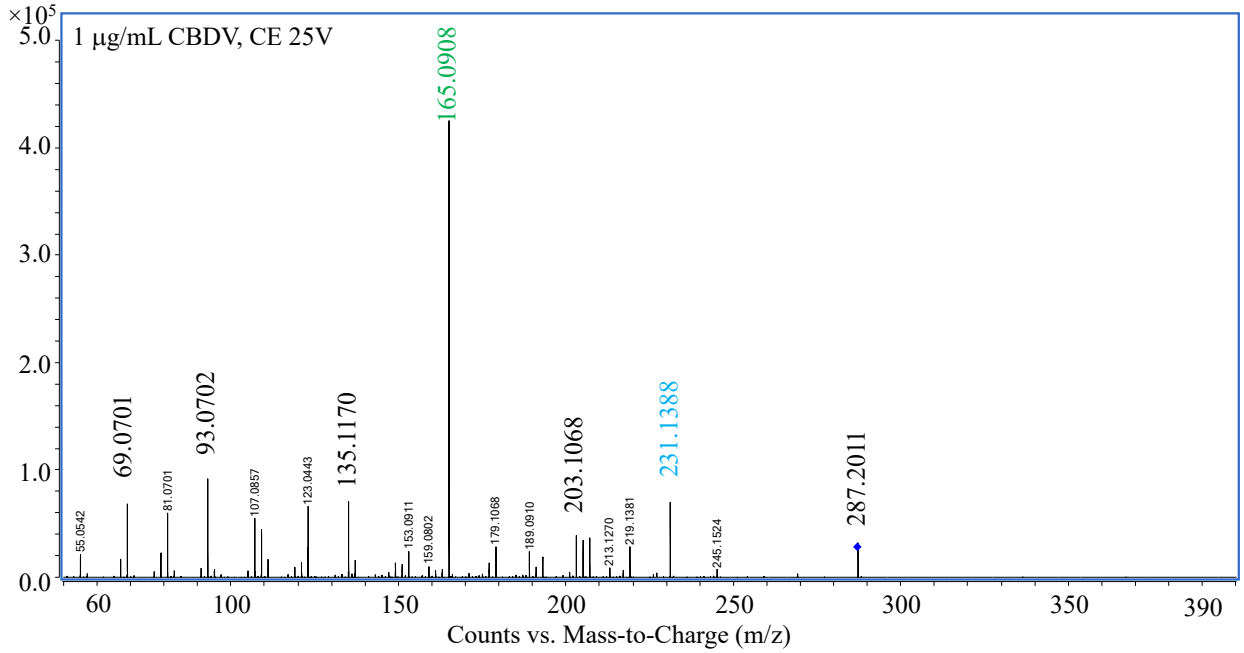
Supplementary Figure S1. Chemical structure of the nineteen cannabinoids used in this study.

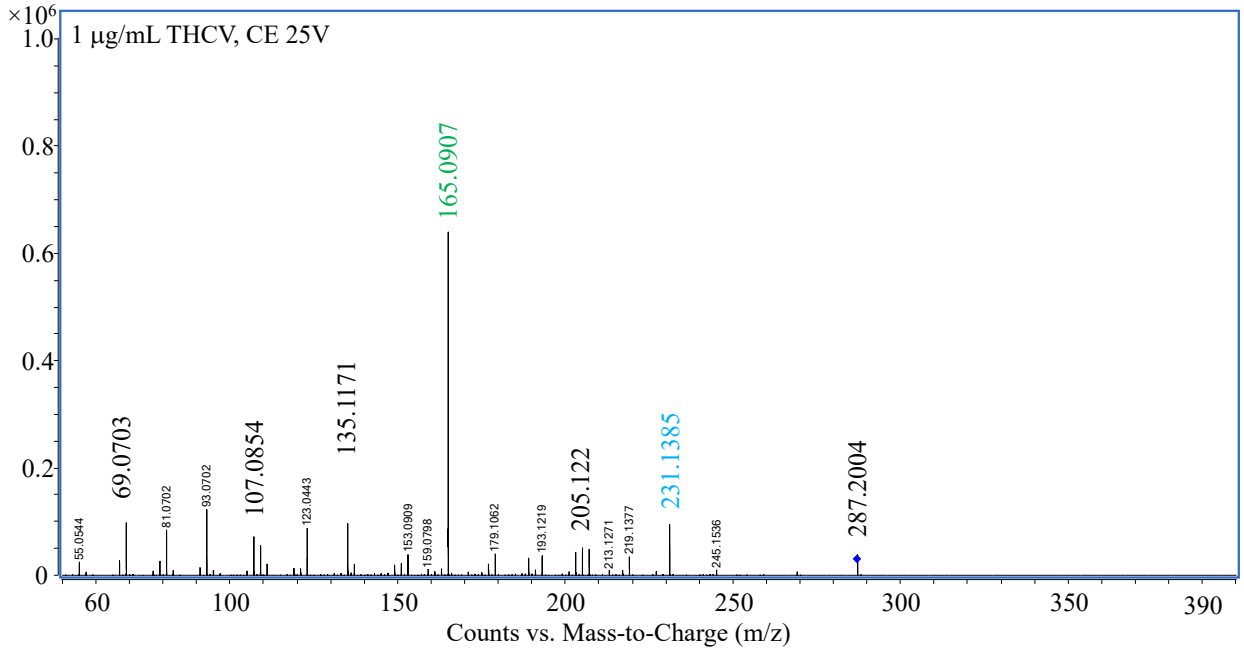


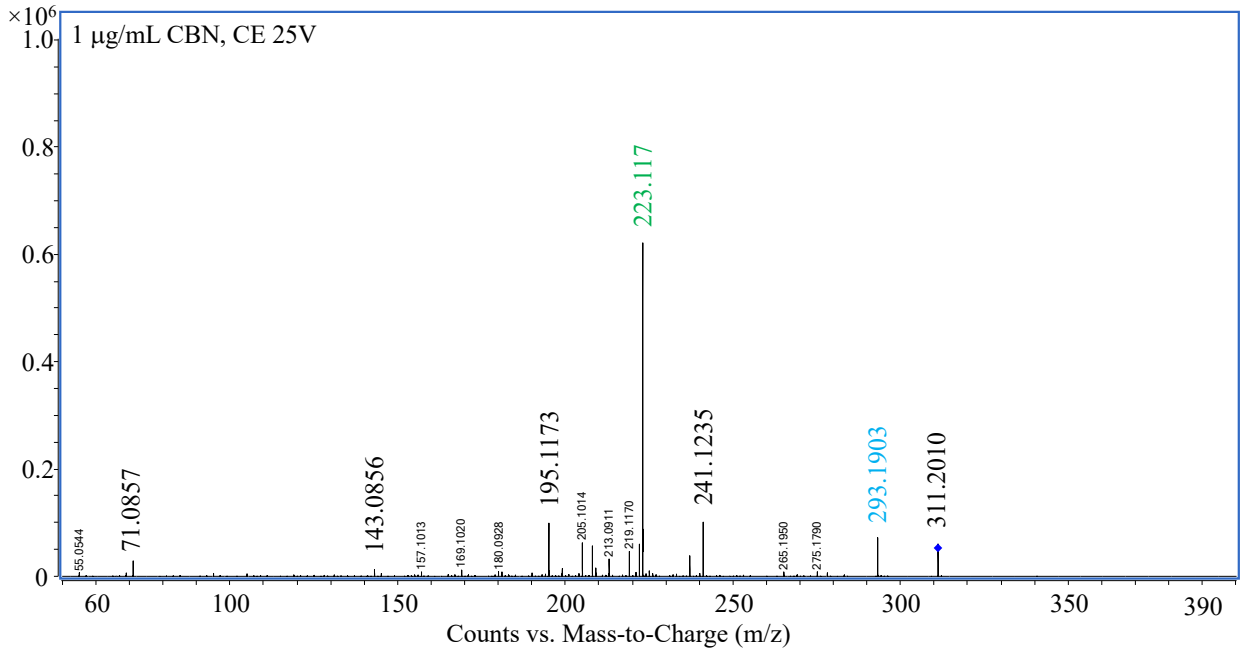
Supplementary Figure S2. Chemical structure of the seven isotopically labelled cannabinoids used in this study.

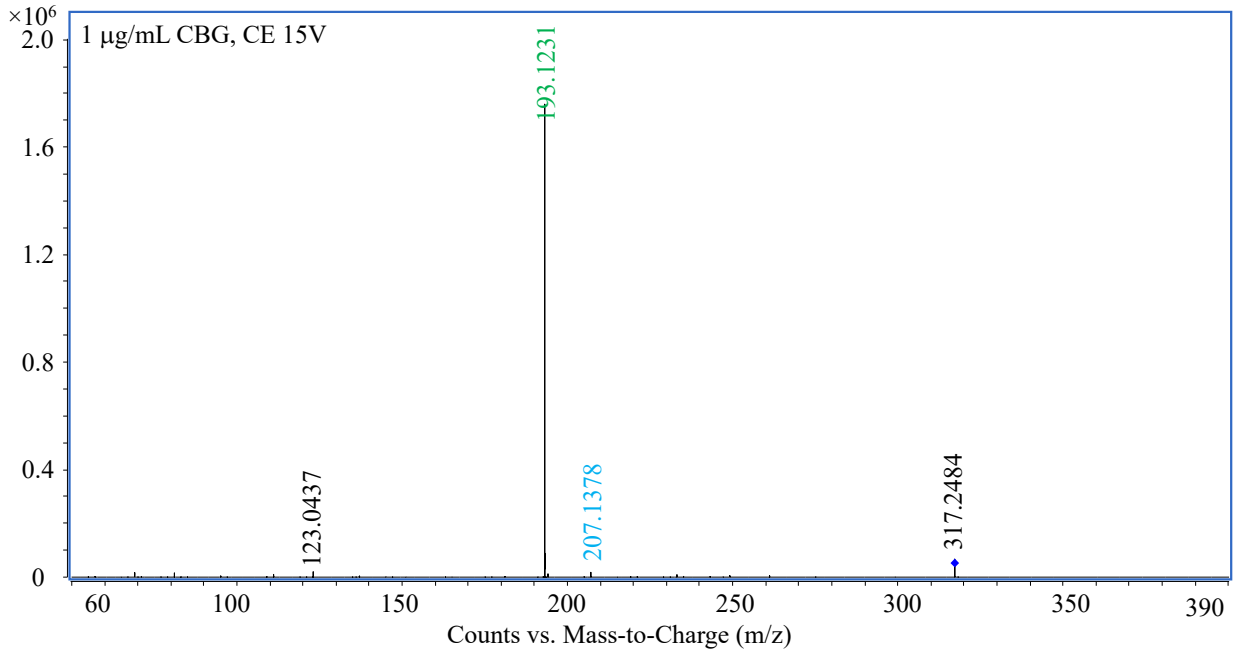


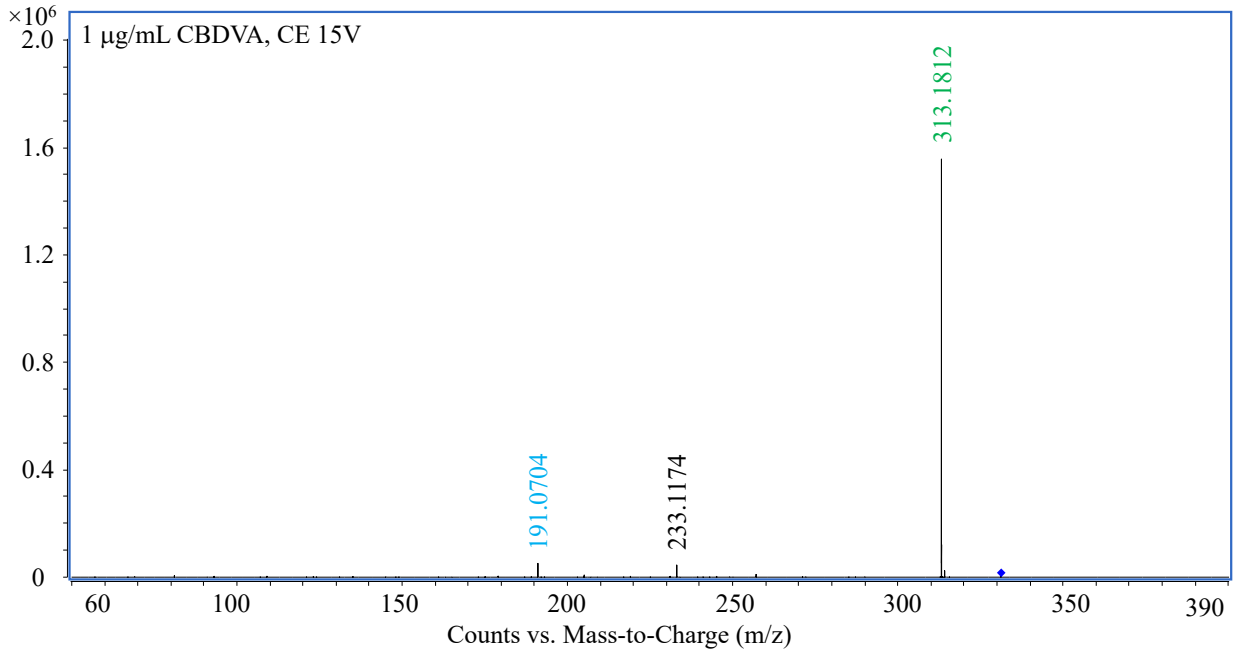
Supplementary Figure S3. MS/MS spectra of twelve cannabinoids at 1 µg/mL. Precursor ions are marked with a blue diamond symbol, while quantifier and qualifier ions are in green and cyan, respectively.






×10^6
1.0
0.8
0.6
0.4
0.2
0
1 µg/mL CBN, CE 25V
55.0544
71.0857
143.0856
157.1013
169.1020
180.0928
195.1173
205.1014
213.0911
219.1170
223.117
241.1235
265.1950
275.1790
293.1903
311.2010
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)


×10^6
1 µg/mL CBG, CE 15V
2.0
1.6
1.2
0.8
0.4
0
193.1231
123.0437
207.1378
317.2484
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)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1.2
0.8
0.4
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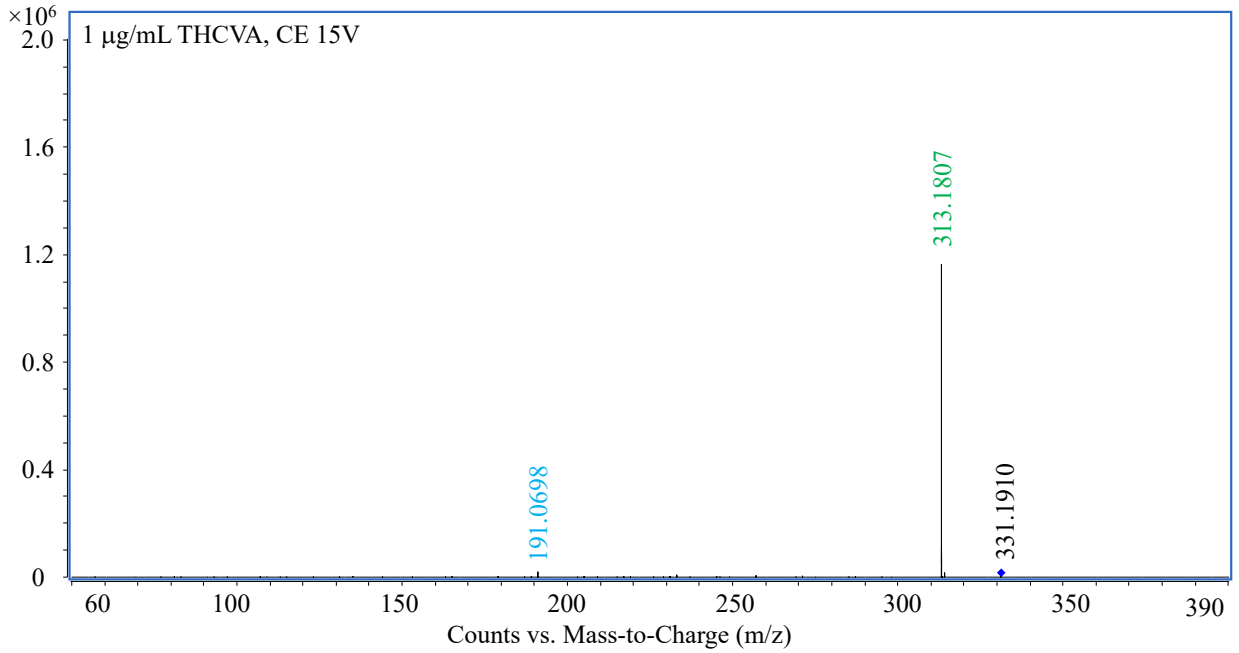
300
350
390
Counts vs. Mass-to-Charge (m/z)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1.2
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0.4
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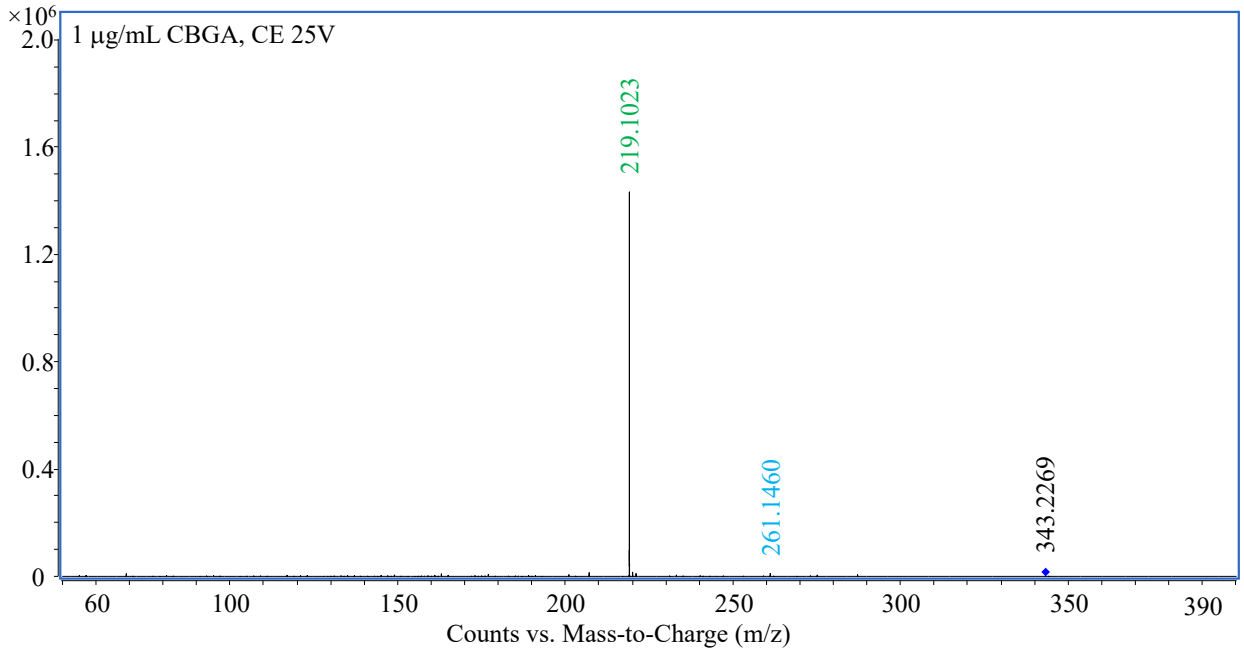
Counts vs. Mass-to-Charge (m/z)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1.2
0.8
0.4
0
1 µg/mL CBGA, CE 25V
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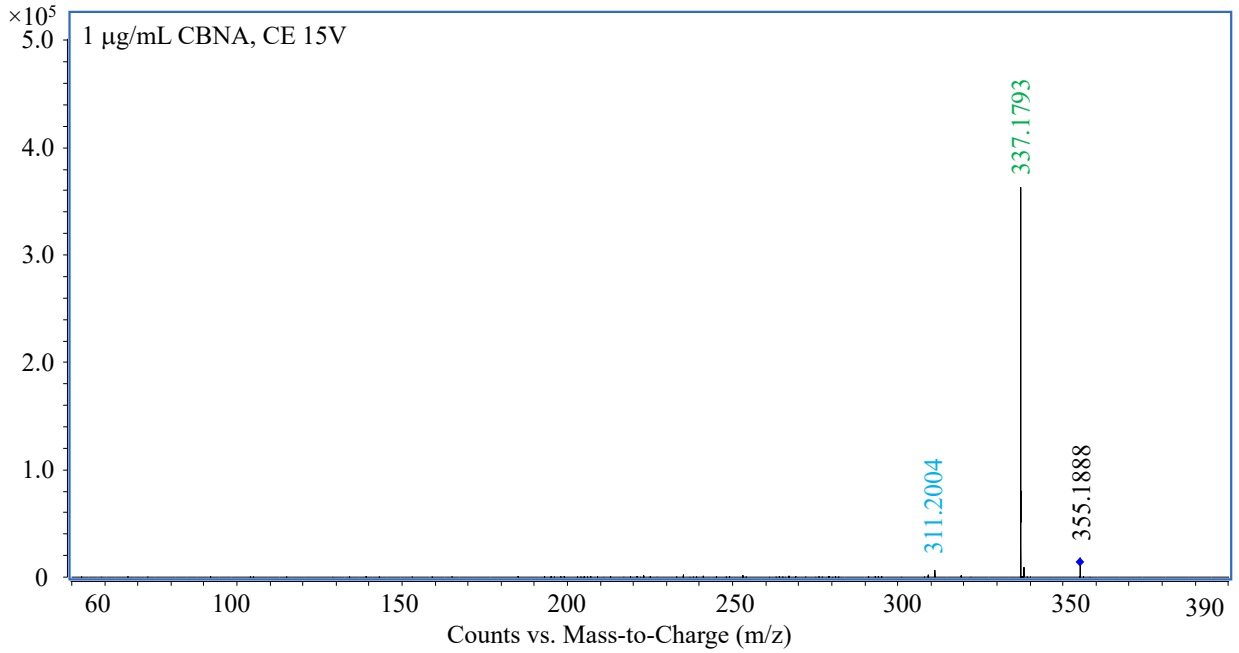
300
350
390
Counts vs. Mass-to-Charge (m/z)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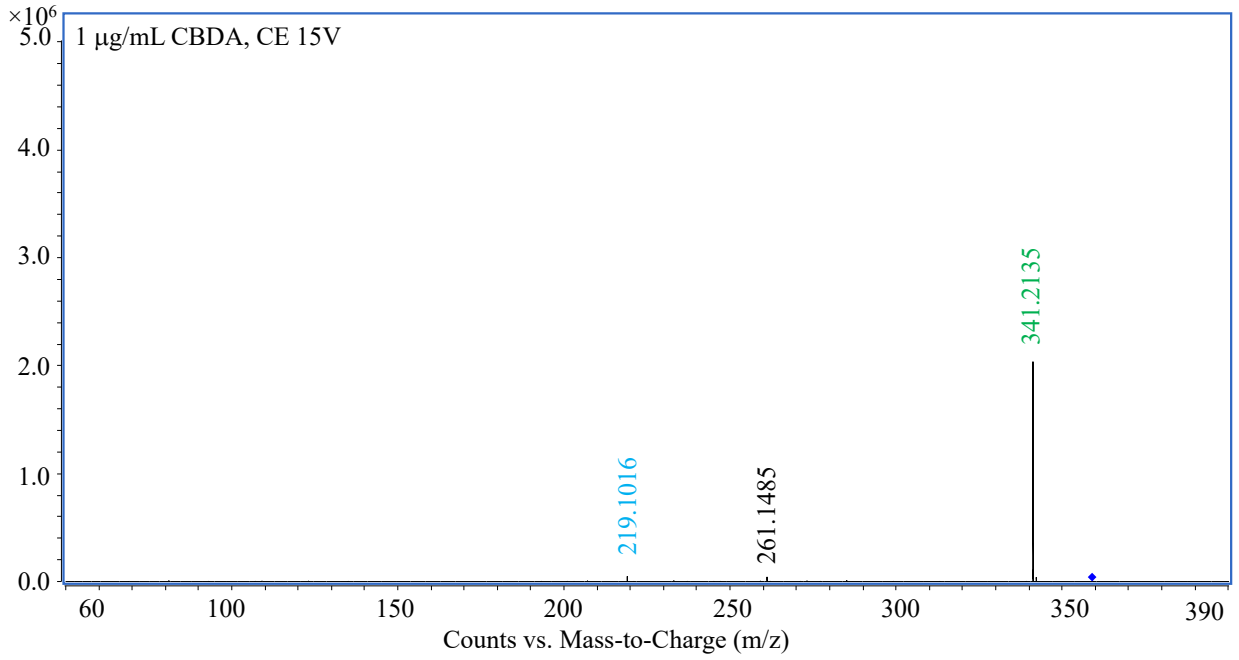
Counts vs. Mass-to-Charge (m/z)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2.0
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350
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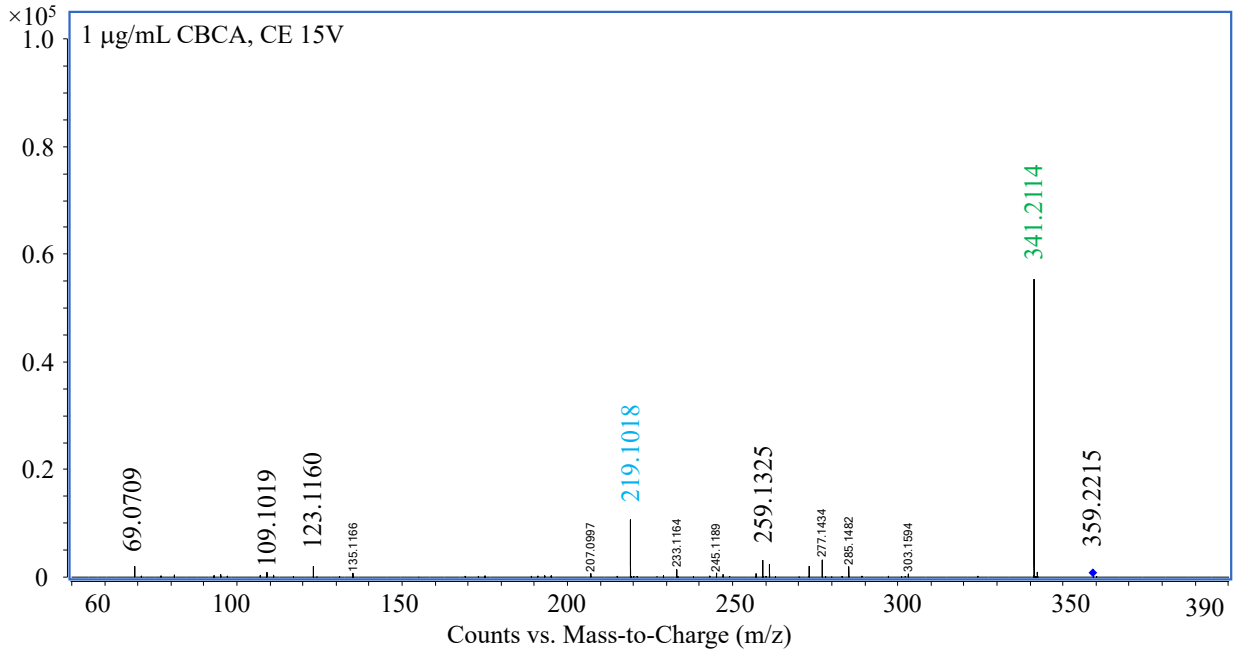
Counts vs. Mass-to-Charge (m/z)


×10^5
1 µg/mL CBCA, CE 15V
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0.8
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0.4
0.2
0
69.0709
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123.1160
135.1166
207.0997
219.1018
233.1164
245.1189
259.1325
277.1434
285.1482
303.1594
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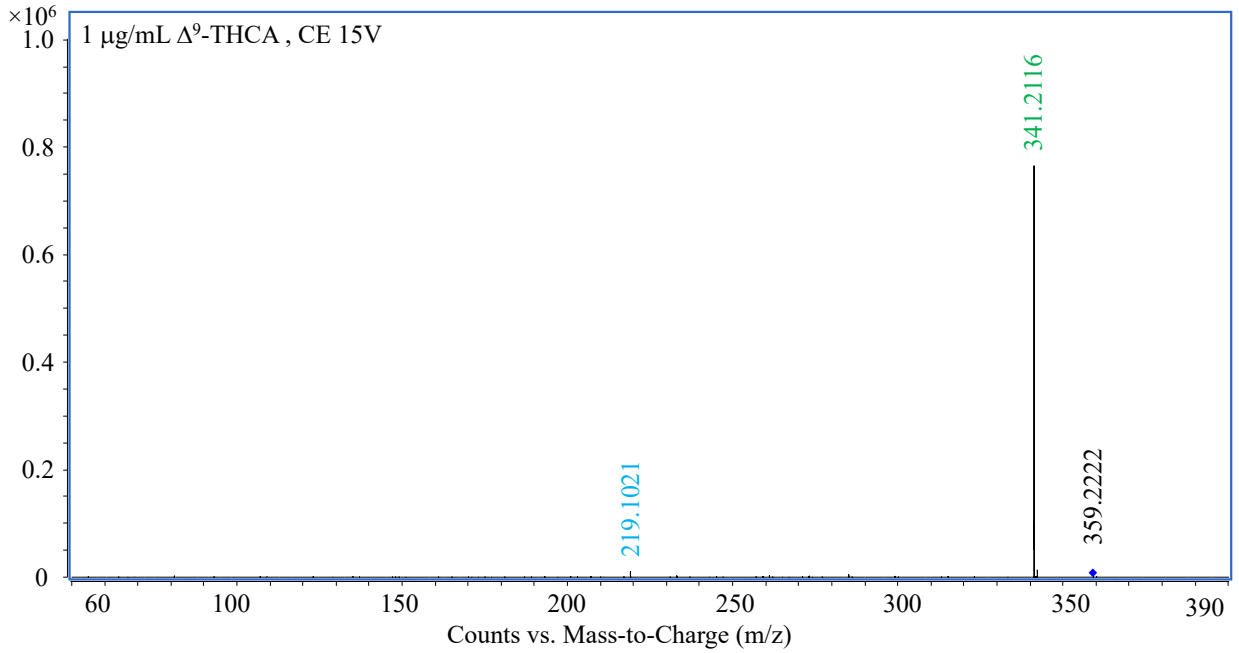
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)


×10^6
1 µg/mL Δ9-THCA , CE 15V
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0.8
0.6
0.4
0.2
0
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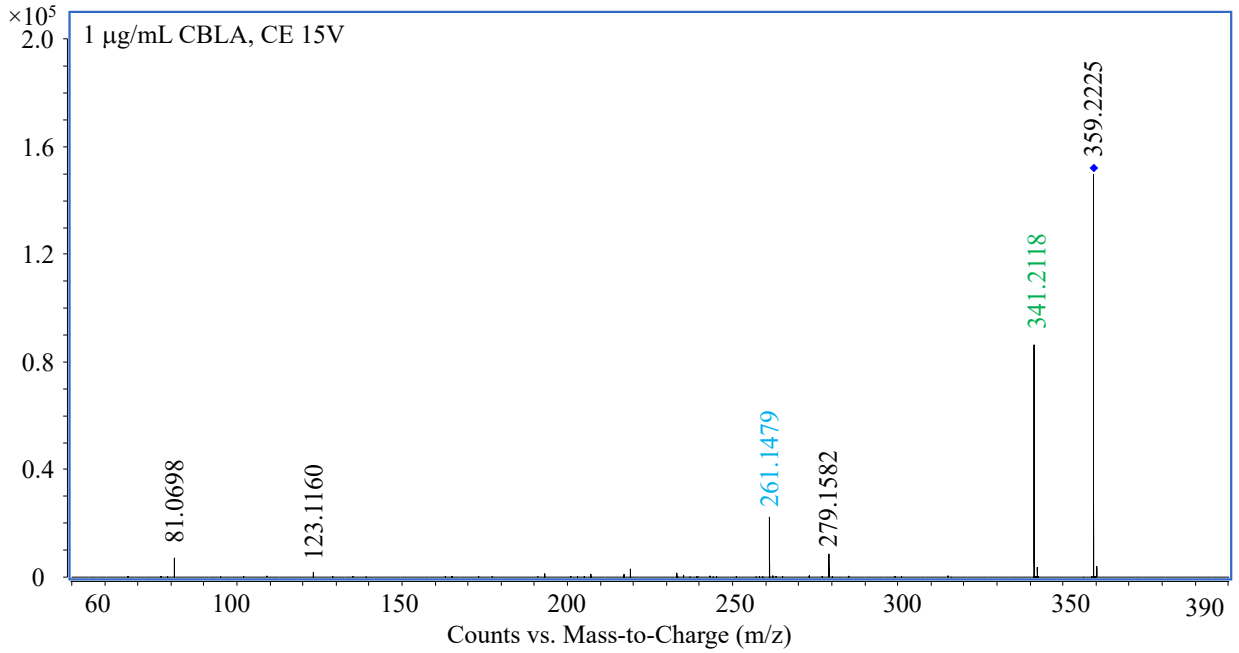
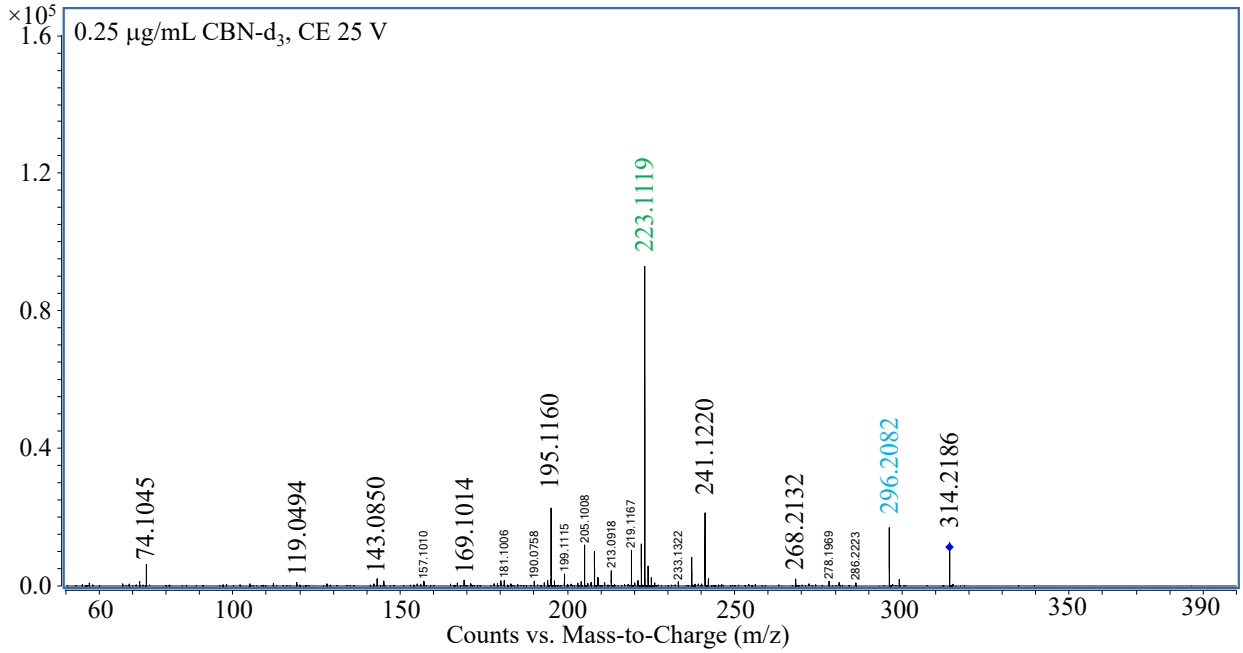
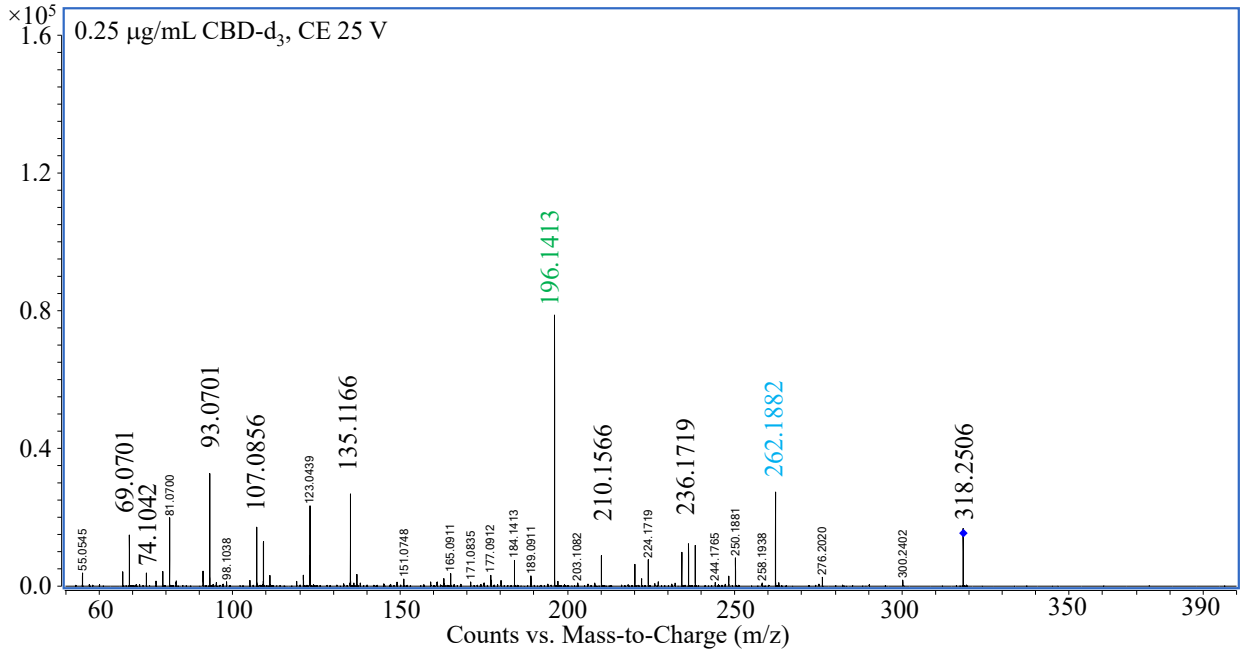
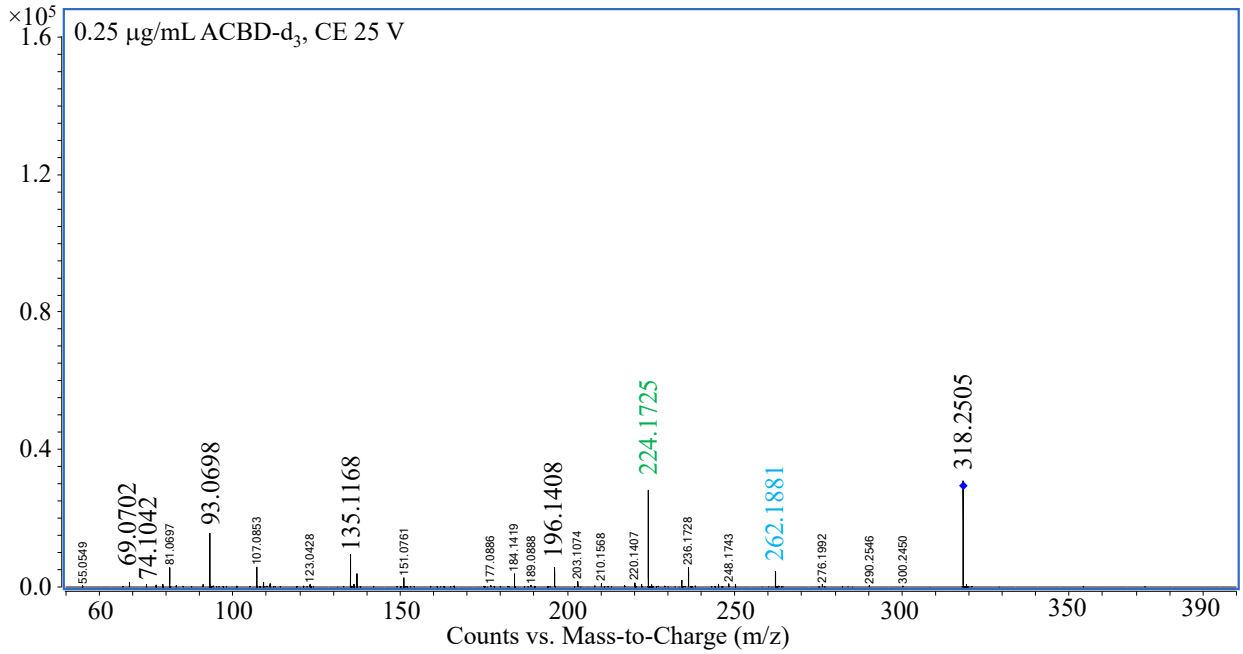
300
350
390
Counts vs. Mass-to-Charge (m/z)


×10^5
2.0
1.6
1.2
0.8
0.4
0
1 µg/mL CBLA, CE 15V
81.0698
123.1160
261.1479
279.1582
341.2118
359.2225
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

Supplementary Figure S4. MS/MS spectra of seven isotopically labeled cannabinoids at 0.5 µg/mL. Precursor ions are marked with a blue diamond symbol, while quantifier and qualifier ions are in green and cyan, respectively.






0.25 µg/mL ACBD-d3, CE 25 V
×10^5
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1.2
0.8
0.4
0.0
55.0549
69.0702
74.1042
81.0697
93.0698
107.0853
123.0428
135.1168
151.0761
177.0886
184.1419
189.0888
196.1408
203.1074
210.1568
220.1407
224.1725
236.1728
248.1743
262.1881
276.1992
290.2546
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318.2505
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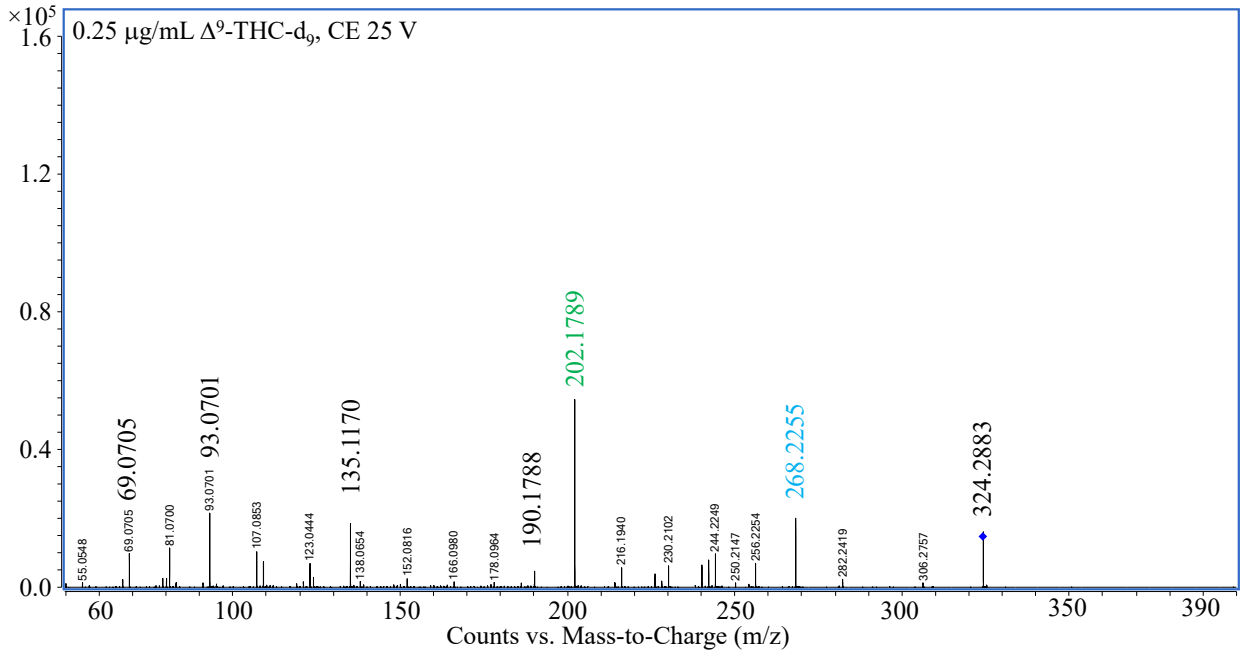
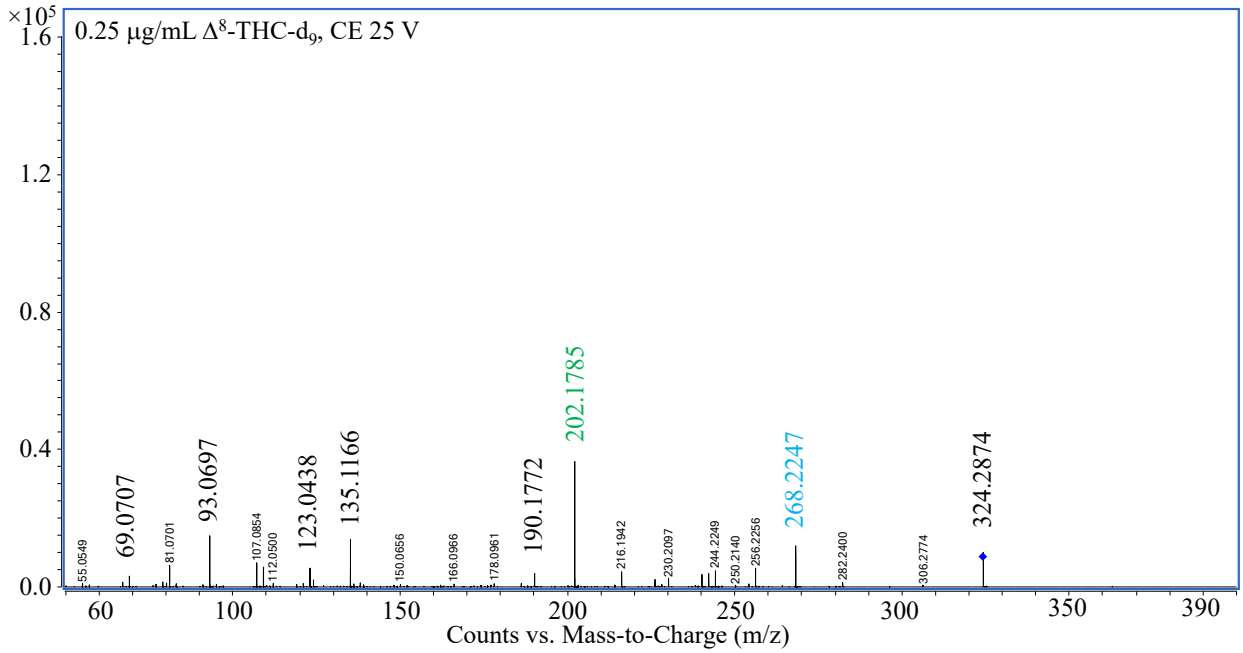
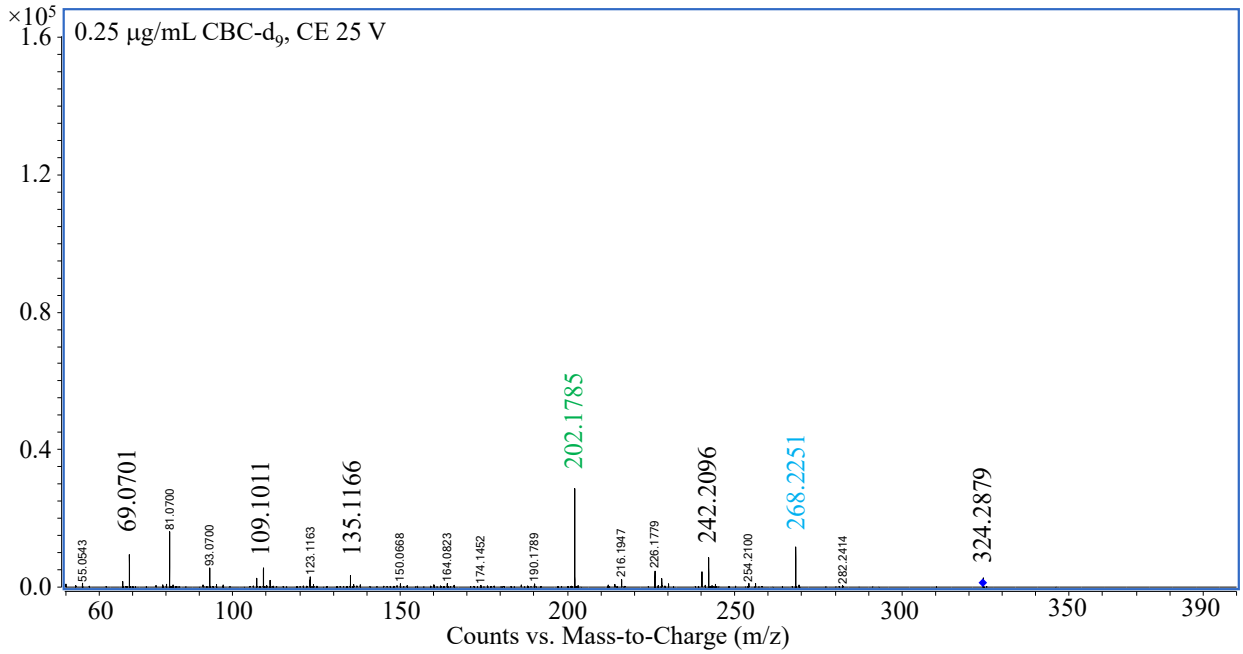
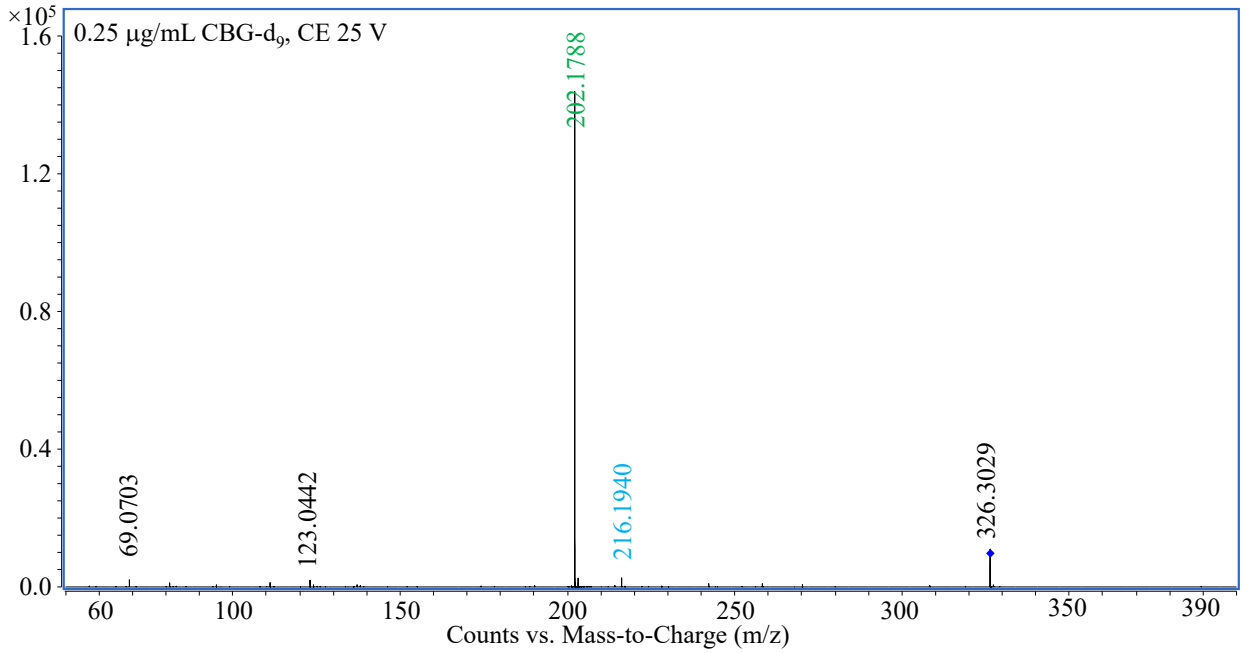
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

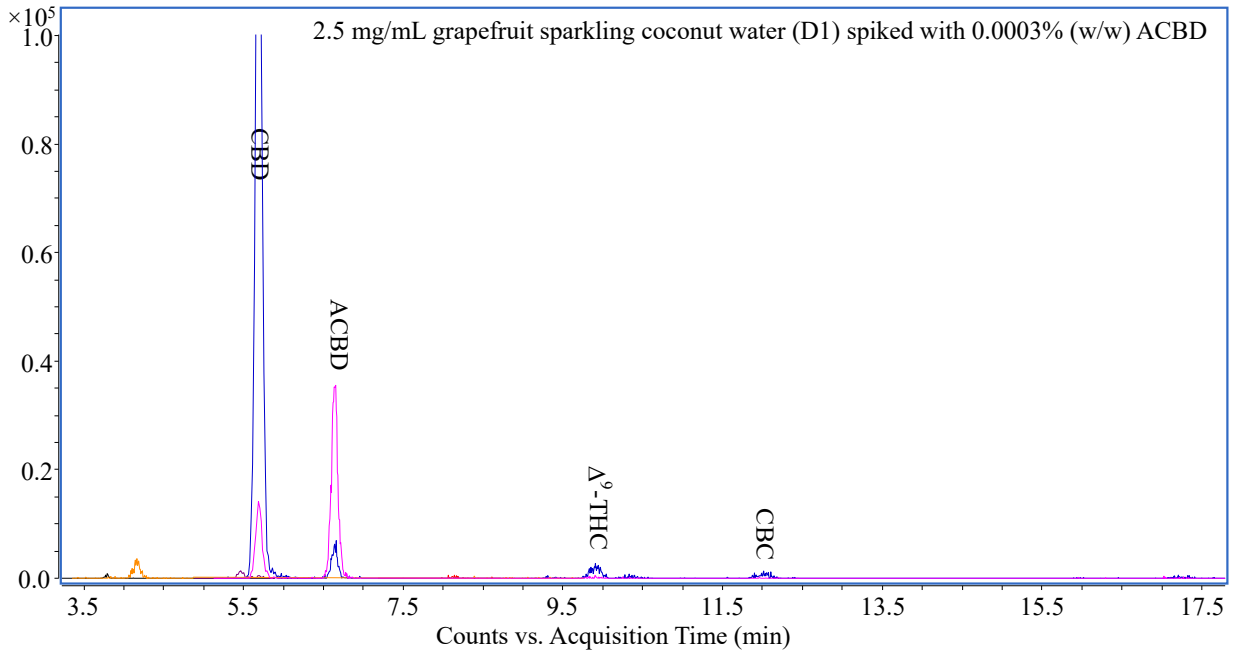

0.25 µg/mL Δ9-THC-d9, CE 25 V
×10^5
1.6
1.2
0.8
0.4
0.0
55.0548
69.0705
69.0705
81.0700
93.0701
93.0701
107.0853
123.0444
135.1170
138.0654
152.0816
166.0980
178.0964
190.1788
202.1789
216.1940
230.2102
244.2249
250.2147
256.2254
268.2255
282.2419
306.2757
324.2883
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

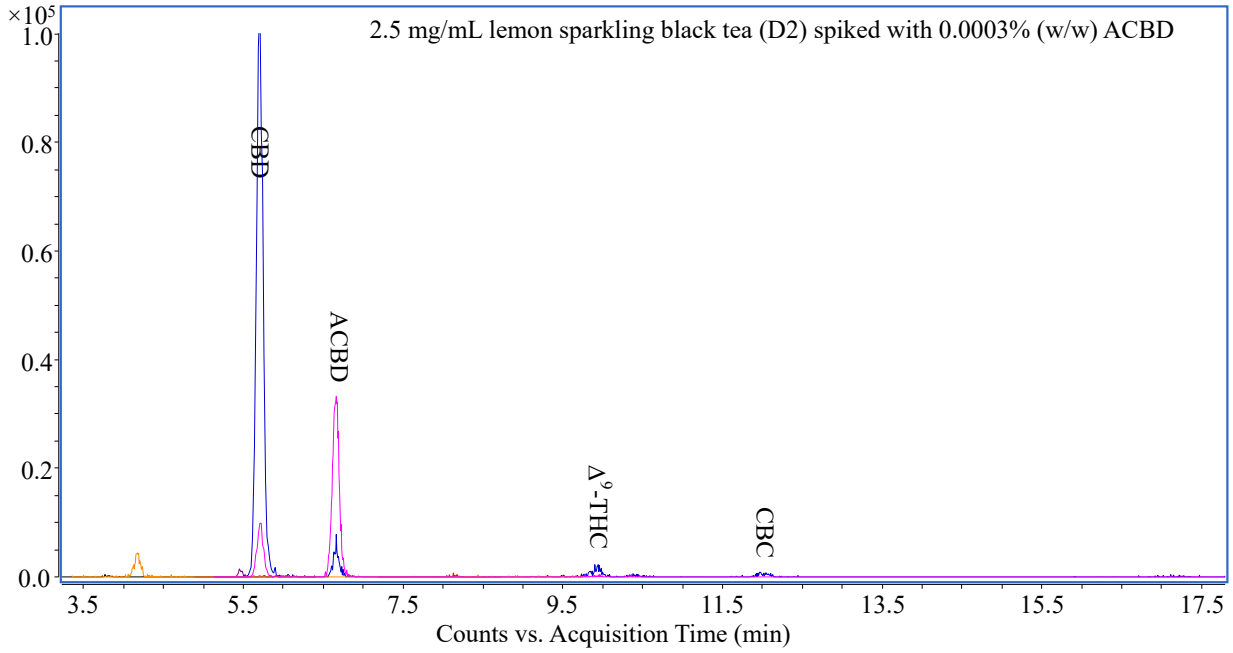

0.25 µg/mL Δ8-THC-d9, CE 25 V
×10^5
1.6
1.2
0.8
0.4
0.0
55.0549
69.0707
81.0701
93.0697
107.0854
112.0500
123.0438
135.1166
150.0656
166.0966
178.0961
190.1772
202.1785
216.1942
230.2097
244.2249
250.2140
256.2256
268.2247
282.2400
306.2774
324.2874
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

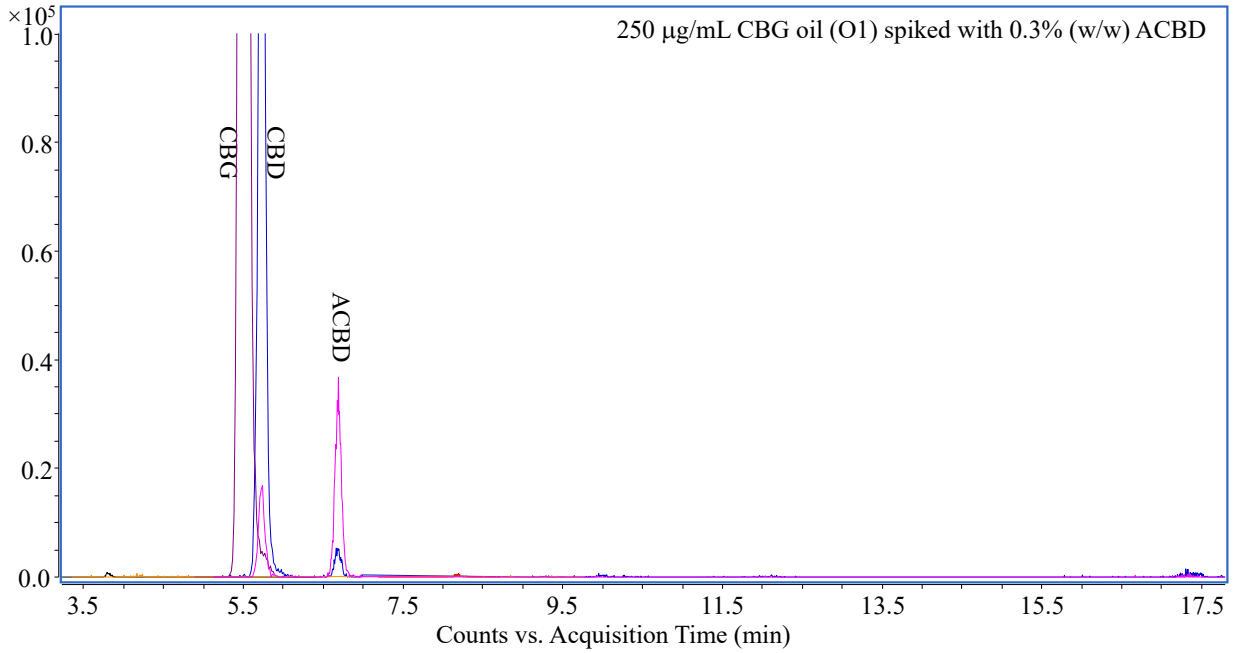

0.25 µg/mL CBC-d9, CE 25 V
×10^5
1.6
1.2
0.8
0.4
0.0
55.0543
69.0701
81.0700
93.0700
109.1011
123.1163
135.1166
150.0668
164.0823
174.1452
190.1789
202.1785
216.1947
226.1779
242.2096
254.2100
268.2251
282.2414
324.2879
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

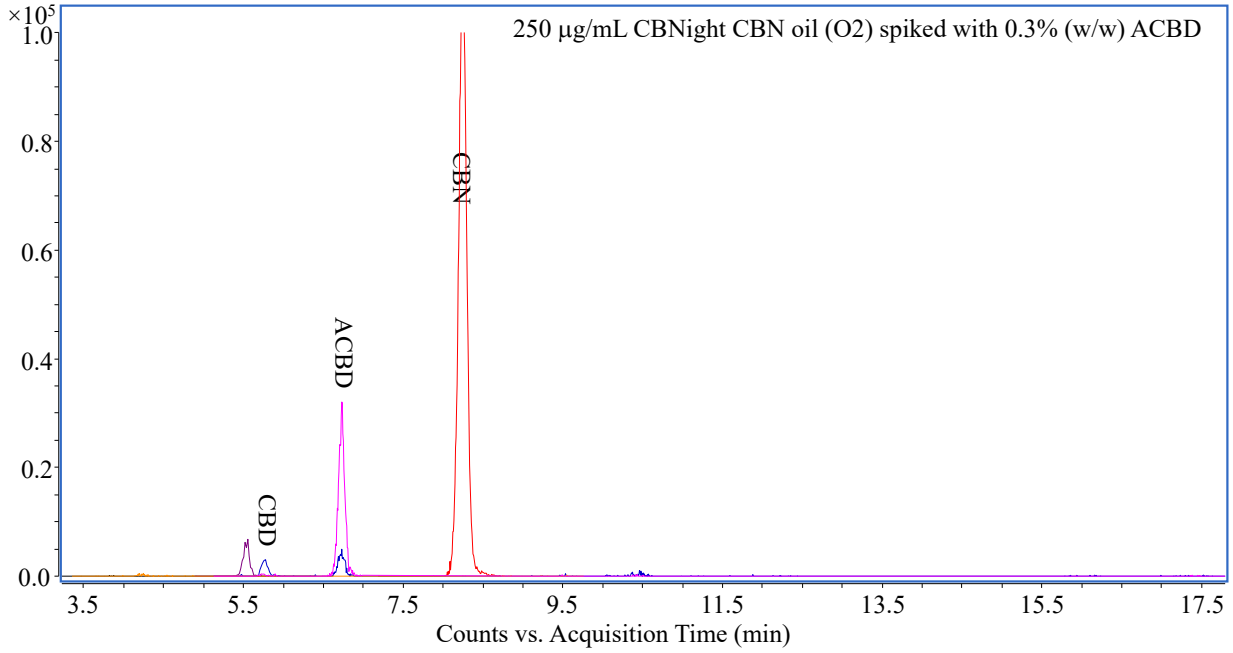

×10^5
0.25 µg/mL CBG-d9, CE 25 V
1.6
1.2
0.8
0.4
0.0
202.1788
69.0703
123.0442
216.1940
326.3029
60
100
150
200
250
300
350
390
Counts vs. Mass-to-Charge (m/z)

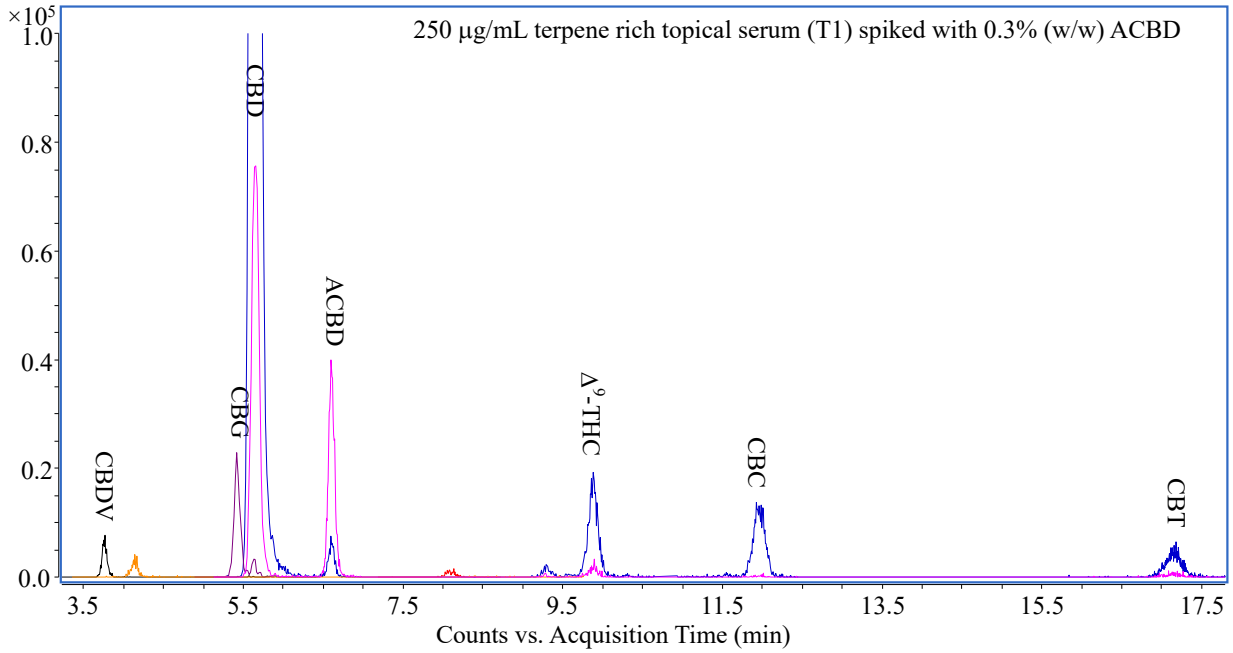
Supplementary Figure S5. LC-ESI/MS/MS extracted ion chromatograms of eighteen hemp infused products spiked with ACBD. Precursor to quantifier ion transition of each cannabinoid is listed in **Table 1** with ±20 ppm mass error (peaks were both color coded and labelled for easy distinguish).

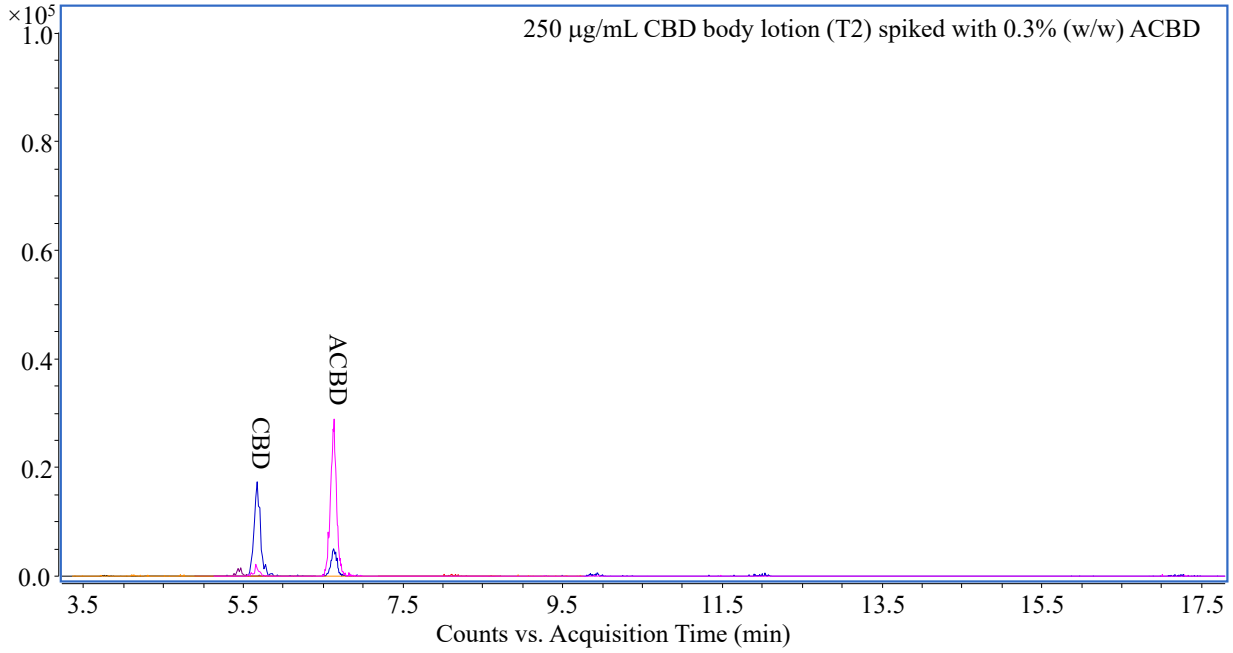


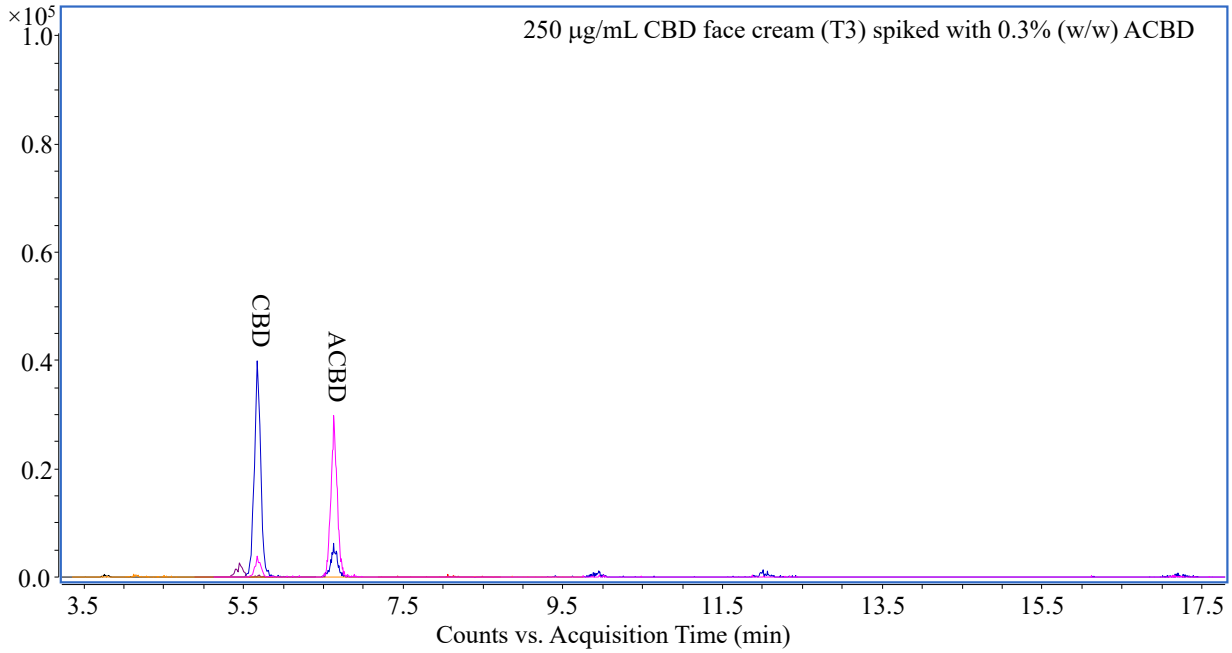


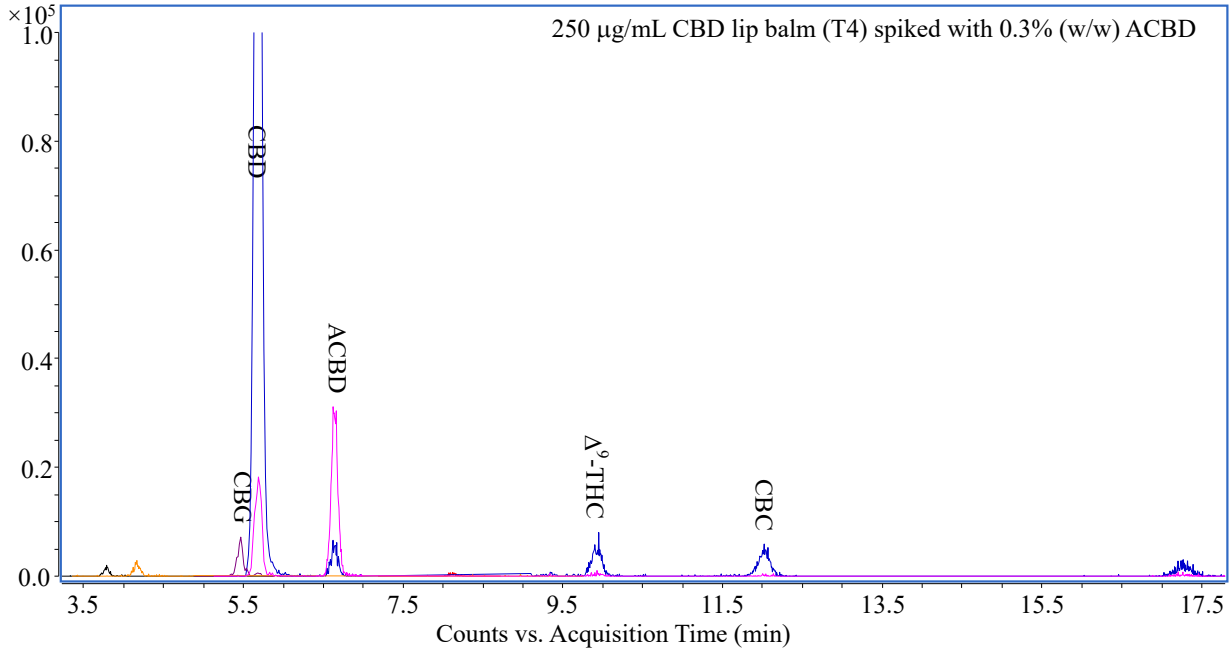

250 µg/mL CBG oil (O1) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBG
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

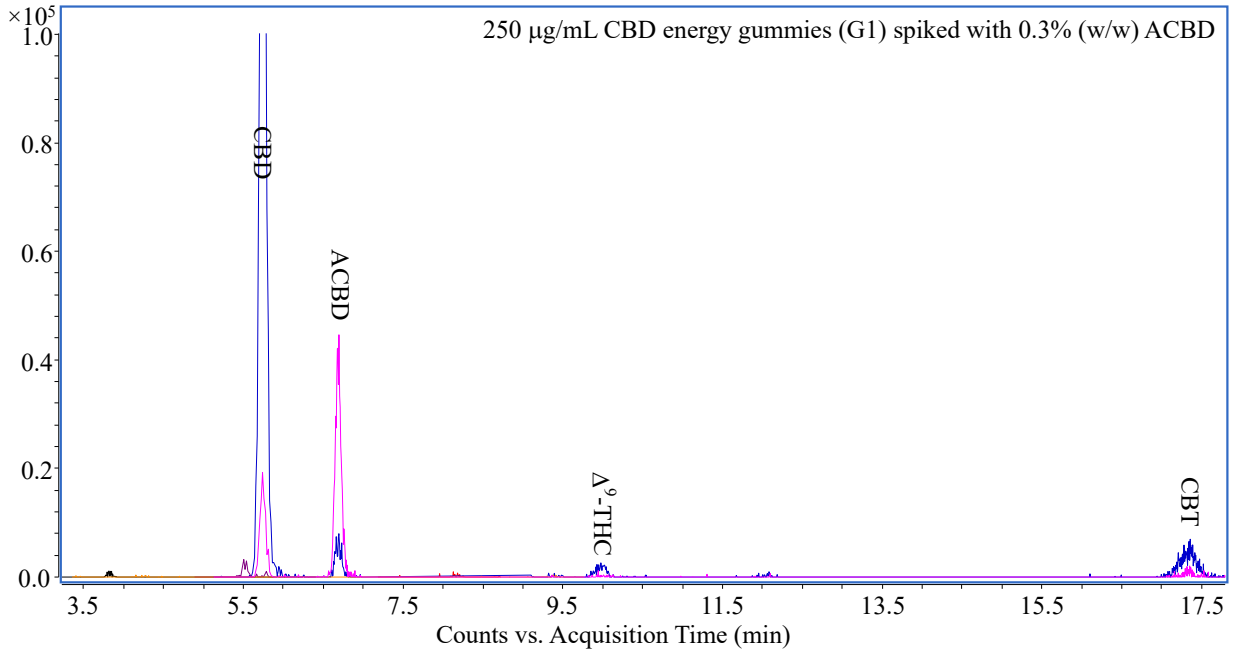

250 µg/mL CBNight CBN oil (O2) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBN
ACBD
CBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

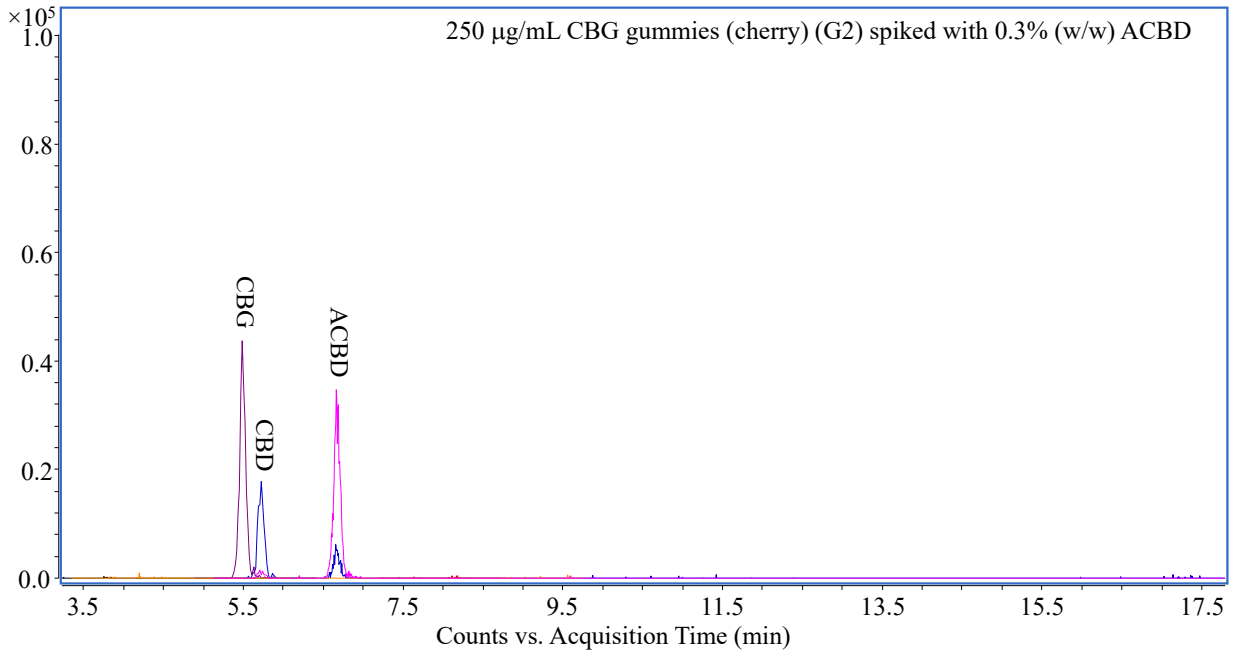

250 µg/mL terpene rich topical serum (T1) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBDV
CBG
CBD
ACBD
Δ9-THC
CBC
CBT
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

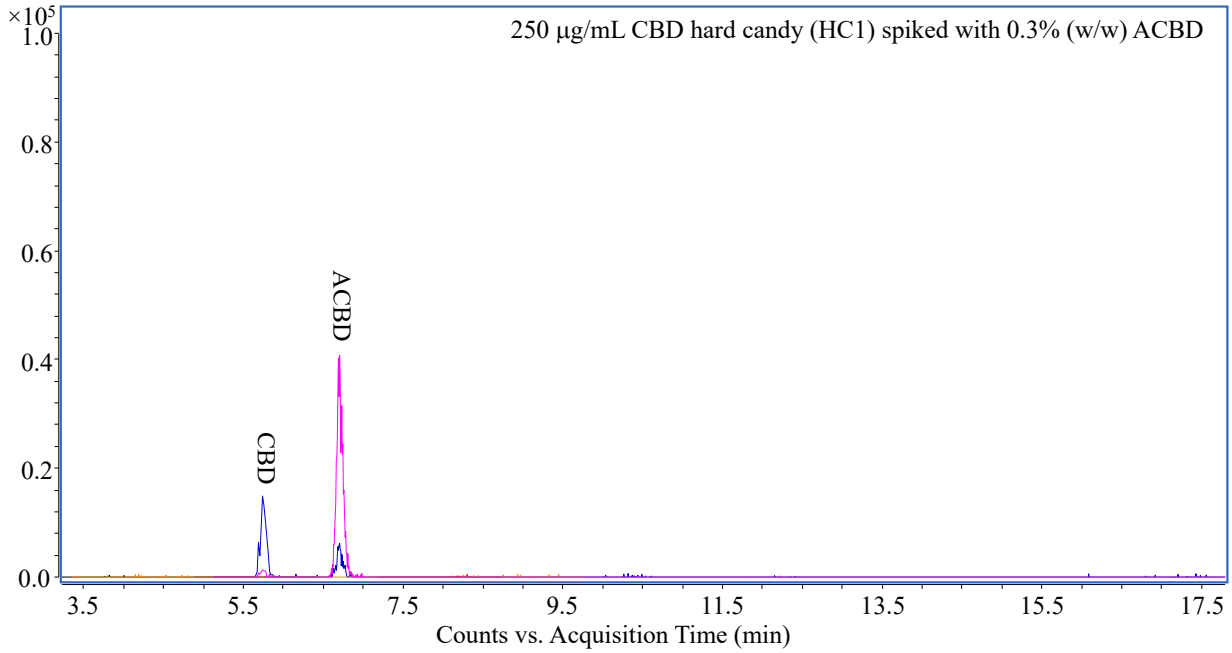

250 µg/mL CBD body lotion (T2) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

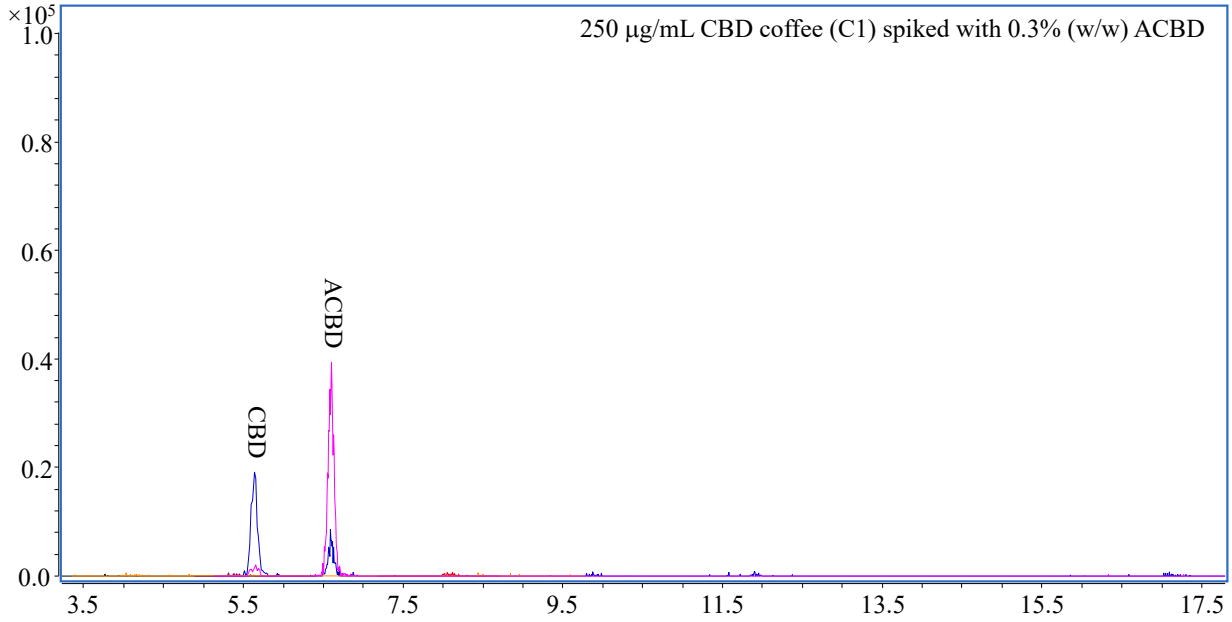

250 µg/mL CBD face cream (T3) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

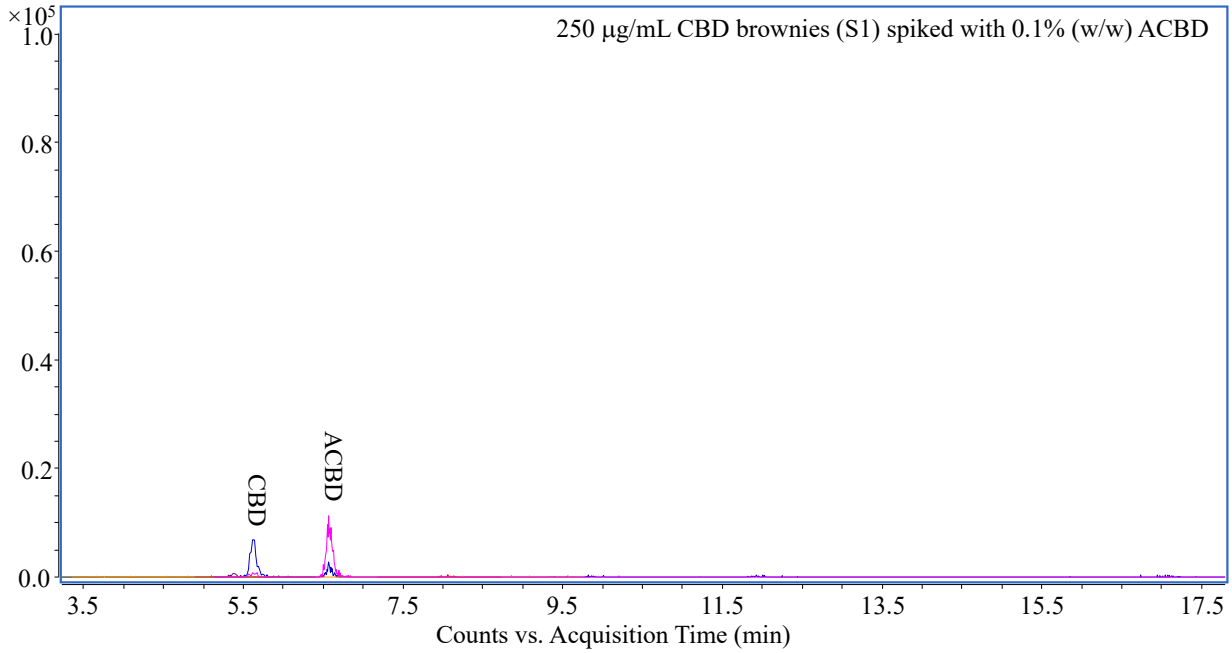

250 µg/mL CBD lip balm (T4) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
CBG
Δ9-THC
CBC
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

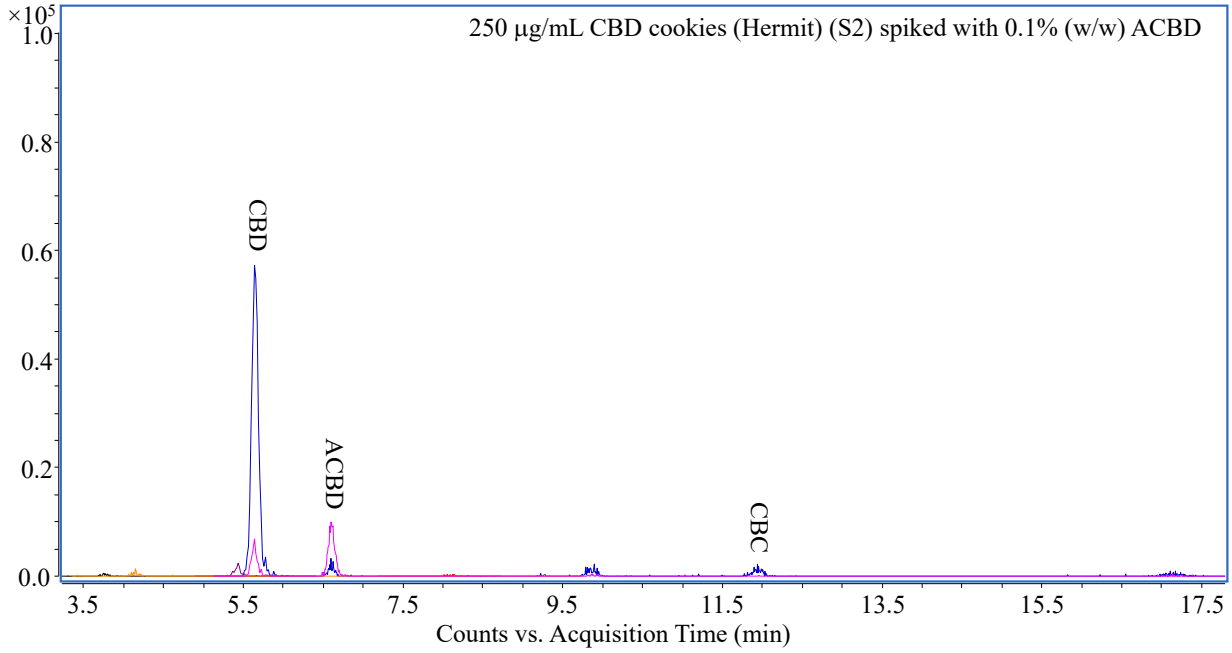

250 µg/mL CBD energy gummies (G1) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
Δ9-THC
CBT
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

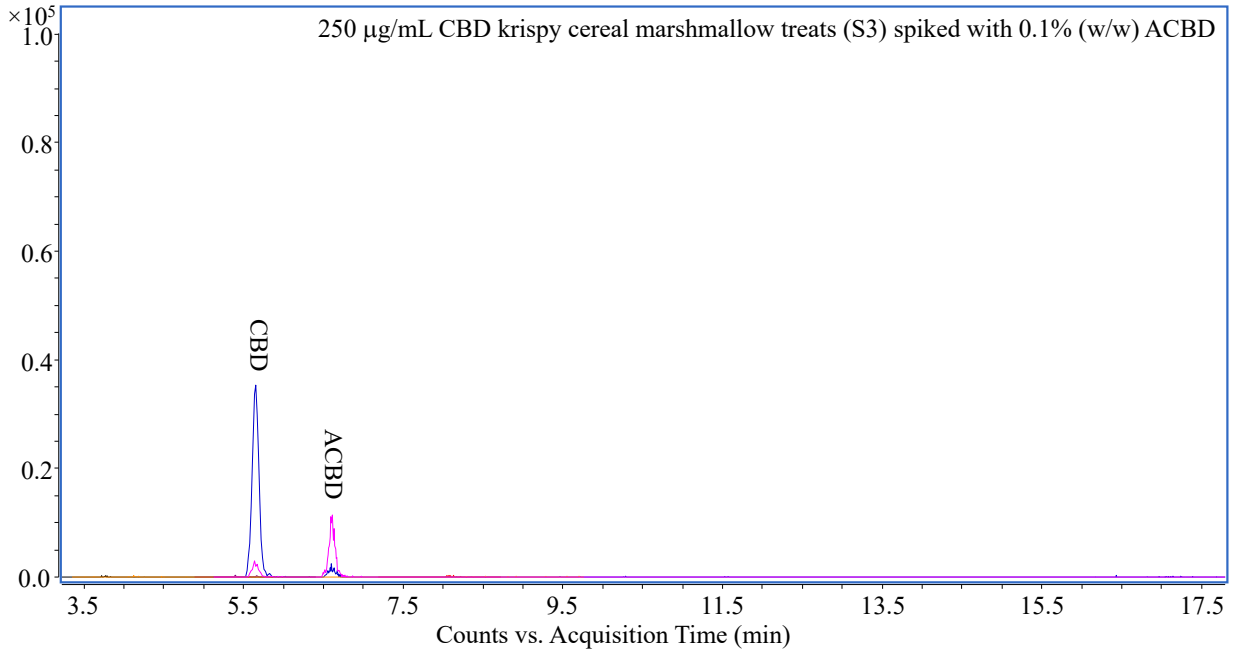

250 µg/mL CBG gummies (cherry) (G2) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBG
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

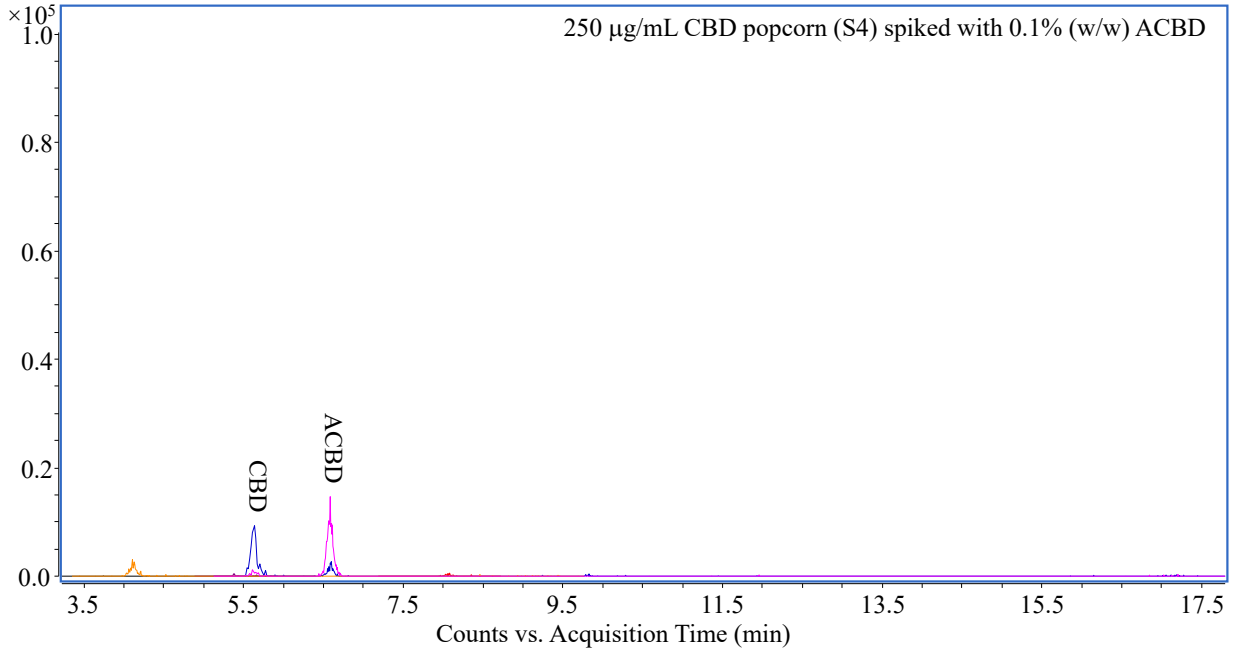

250 µg/mL CBD hard candy (HC1) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

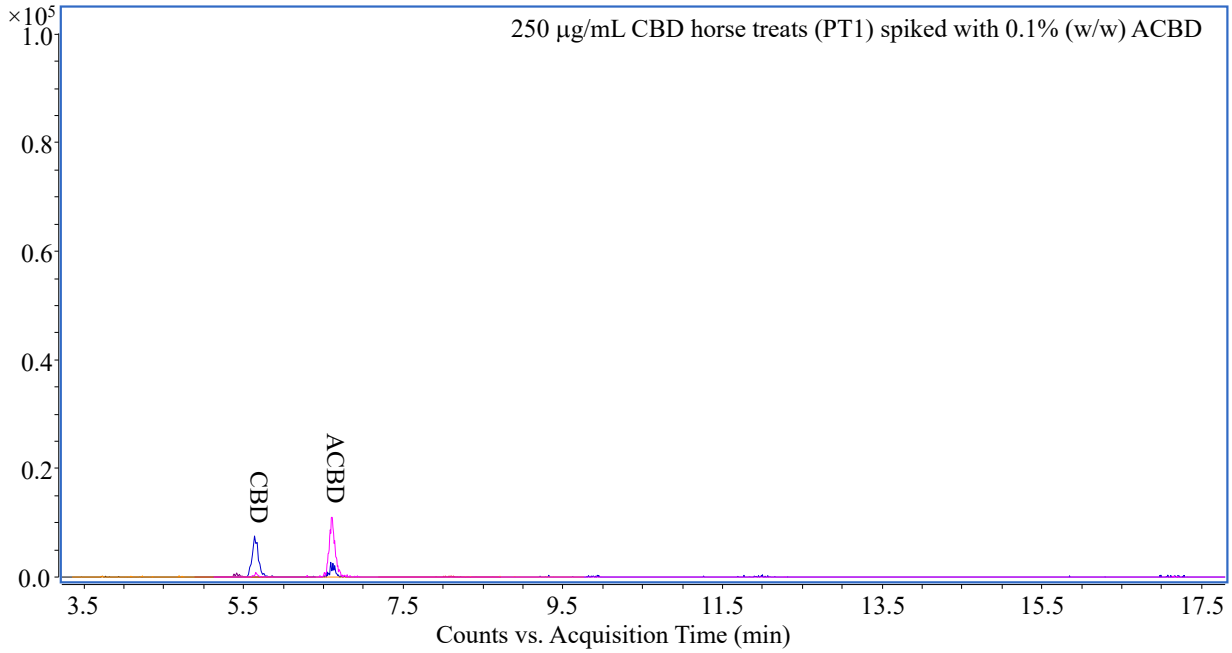

250 µg/mL CBD coffee (C1) spiked with 0.3% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5

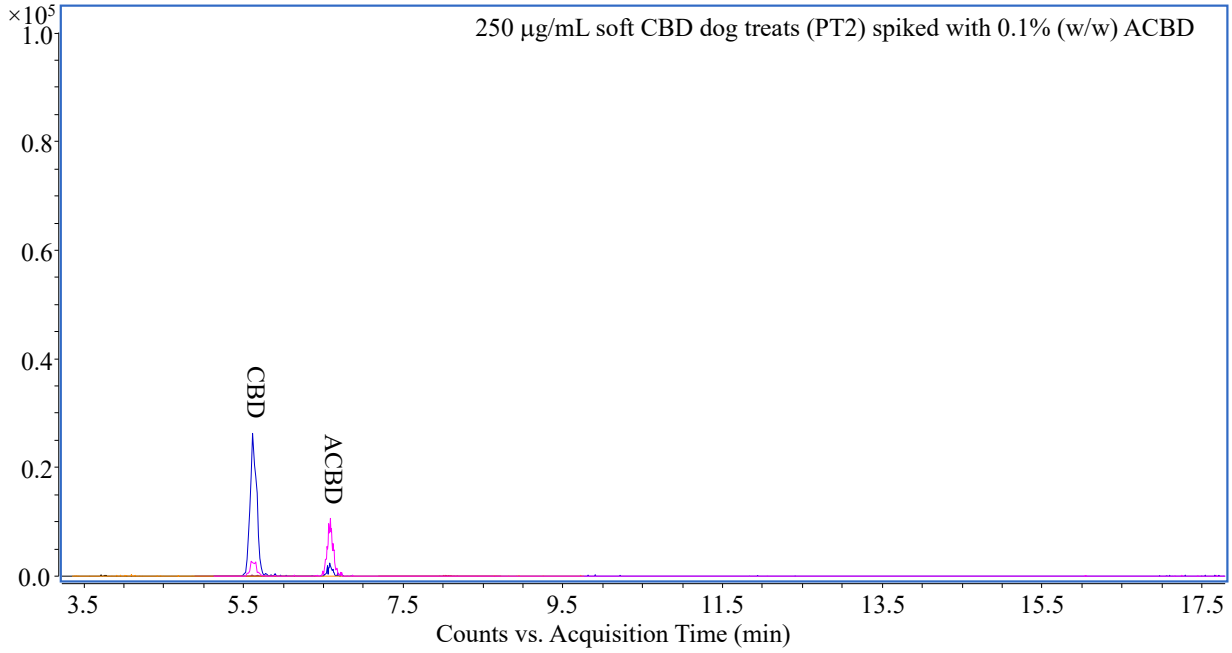

250 µg/mL CBD brownies (S1) spiked with 0.1% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)


250 µg/mL CBD cookies (Hermit) (S2) spiked with 0.1% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
CBC
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)


250 µg/mL CBD krispy cereal marshmallow treats (S3) spiked with 0.1% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)


250 µg/mL CBD popcorn (S4) spiked with 0.1% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)


250 µg/mL CBD horse treats (PT1) spiked with 0.1% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)


250 µg/mL soft CBD dog treats (PT2) spiked with 0.1% (w/w) ACBD
×10^5
1.0
0.8
0.6
0.4
0.2
0.0
CBD
ACBD
3.5
5.5
7.5
9.5
11.5
13.5
15.5
17.5
Counts vs. Acquisition Time (min)

Supplementary Table S1. Detailed parameters of targeted MS/MS acquisition and quantification used in method validation and sample analysis.

Parameter	Acquisition				Quantification			
	Precursor	RT	Δ_{RT}	CE	RT	Quantifier	Qualifier	ISTD
CBDV	287.2006	3.735	1.0	25	3.735	165.089	231.133	CBD-d_3
CBDA	359.2217	4.124	1.0	15	4.124	341.211	219.102	CBD-d_3
CBG-d_9	326.3034	5.352	1.0	15	5.352	202.177	216.194	
CBG	317.2475	5.416	1.0	15	5.416	193.120	207.136	CBG-d_9
CBD-d_3	318.2507	5.616	0.9	25	5.616	196.142	262.184	
CBD	315.2319	5.654	0.9	25	5.654	193.120	259.163	CBD-d_3
THCV	287.2006	5.851	1.0	25	5.851	165.089	231.133	CBD-d_3
ACBD-d_3	318.2507	6.557	0.9	15	6.557	224.172	262.184	
ACBD	315.2319	6.588	0.9	15	6.588	221.152	259.164	ACBD-d_3
CBN-d_3	314.2194	8.006	1.5	25	8.006	223.111	296.207	
CBN	311.2006	8.071	1.5	25	8.071	223.111	293.187	CBN-d_3
Δ^9-THCA	359.2217	9.138	1.5	15	9.138	341.211	219.102	Δ^9-THC-d_9
Δ^9-THC-d_9	324.2883	9.821	1.6	25	9.628	202.177	268.220	
Δ^9-THC	315.2319	10.021	1.6	25	9.826	193.120	259.163	Δ^9-THC-d_9
Δ^8-THC-d_9	324.2883			25	10.013	202.177	268.220	
Δ^8-THC	315.2319			25	10.215	193.120	259.163	Δ^8-THC-d_9
CBC-d_9	324.2883	11.635	1.5	25	11.635	202.177	268.220	
CBC	315.2319	11.891	1.5	25	11.891	193.120	259.163	CBC-d_9
CBT	315.2319	17.089	2.0	25	17.089	193.120	259.163	CBC-d_9

Supplementary Table S2. Accuracy of the quality control samples: average recovery values were computed using triplicate measurements for both intraday and interday.

Concentration	Accuracy (%)	CBDV	CBDA	CBG	CBD	THCV	CBN	Δ^9-THCA	Δ^9-THC	Δ^8-THC	CBC	CBT
0.01	Day 1	91.8	110.9	107.9	105.8	106.5	110.3	112.9	112.6	103.7	111.2	108.3
	Day 2	102.5	103.0	103.1	103.8	98.1	98.0	107.4	101.8	117.7	102.3	102.5
	Day 3	102.6	102.2	106.8	96.2	100.8	98.2	100.7	97.6	118.9	100.5	101.2
	Interday	99.0	105.4	105.9	101.9	101.8	102.2	107.0	104.0	113.4	104.7	104.0
0.25	Day 1	104.2	90.1	99.2	98.4	105.2	98.6	87.8	104.9	104.0	87.0	97.4
	Day 2	102.6	92.0	96.9	93.9	98.8	96.3	98.6	108.6	97.9	90.8	101.1
	Day 3	111.1	95.5	90.1	95.1	104.1	97.7	91.1	93.8	90.1	87.8	97.1
	Interday	106.0	92.5	95.4	95.8	102.7	97.5	92.5	102.4	97.3	88.5	98.5
6.25	Day 1	91.3	101.0	97.2	100.4	90.6	98.6	105.8	87.9	91.1	108.6	
	Day 2	93.1	98.9	101.7	99.6	98.0	101.2	107.4	103.9	104.8	109.7	
	Day 3	97.1	104.3	92.8	95.5	102.1	96.9	101.2	93.3	89.9	108.1	
	Interday	93.8	101.4	97.2	98.5	96.9	98.9	104.8	95.0	95.3	108.8	

Supplementary Table S3. Precision of the quality control samples: RSD values were computed using triplicate measurements for both intraday and interday.

Concentration	Precision (%)	CBDV	CBDA	CBG	CBD	THCV	CBN	Δ^9-THCA	Δ^9-THC	Δ^8-THC	CBC	CBT
0.01	Day 1	2.5	8.2	7.7	17.9	9.0	2.1	5.2	4.7	2.4	13.7	2.3
	Day 2	8.5	7.3	6.2	7.5	9.8	5.7	3.8	6.0	4.7	1.3	5.6
	Day 3	6.6	4.5	5.8	17.4	1.2	6.0	1.5	1.8	6.9	15.4	4.1
	Interday	6.3	4.5	2.4	5.0	4.2	6.9	5.7	7.4	7.4	5.5	3.6
0.25	Day 1	2.3	2.0	1.1	2.0	1.3	2.3	3.1	3.1	0.5	1.1	0.8
	Day 2	0.6	1.1	3.9	1.4	0.5	2.0	3.5	1.3	1.5	2.1	1.4
	Day 3	0.4	1.2	0.6	1.5	1.7	0.7	1.3	4.2	1.3	4.0	0.9
	Interday	4.3	3.0	4.9	2.5	3.3	1.2	6.0	7.5	7.2	2.2	2.2
6.25	Day 1	2.5	2.9	0.7	3.3	2.9	1.2	1.5	3.6	2.5	1.6	
	Day 2	2.7	2.8	1.0	1.2	1.9	0.4	2.3	1.6	3.3	1.9	
	Day 3	7.3	6.0	1.0	6.2	6.3	0.7	0.5	4.1	1.0	0.3	
	Interday	3.2	2.7	4.6	2.7	6.0	2.2	3.1	8.6	8.7	0.7	

Supplementary Table 4. RSD values (%) of cannabinoids in hemp-derived products measured in triplicates.

Sample	CBDV	CBG	CBD	CBN	Δ^9-THC	CBC	CBT	Total
D1			2.1		1.8	16.7		2.3
D2			1.6		7.5	16.2		2.0
O1		9.2	7.3					8.6
O2			13.4	4.1				3.8
T1	7.5	13.8	9.7		4.6	3.3	15.5	9.3
T2			3.1					3.1
T3			5.7					5.5
T4		5.4	0.4		9.2	1.6		0.2
G1			2.9		7.0		5.2	2.5
G2		7.0	0.9					4.1
HC1			11.9					11.9
C1			3.2					3.2
S1			12.8					12.8
S2			2.9			2.6		2.4
S3			5.6					5.6
S4			0.6					0.6
PT1			4.3					4.3
PT2			3.8					3.8