Development of a validated method for rapid quantification of up to sixteen cannabinoids using ultra-high-performance liquid chromatography diode-array detector with optional electrospray ionization time-of-flight mass spectrometry detection

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Fig. 1. Chemical structures of seventeen cannabinoids.

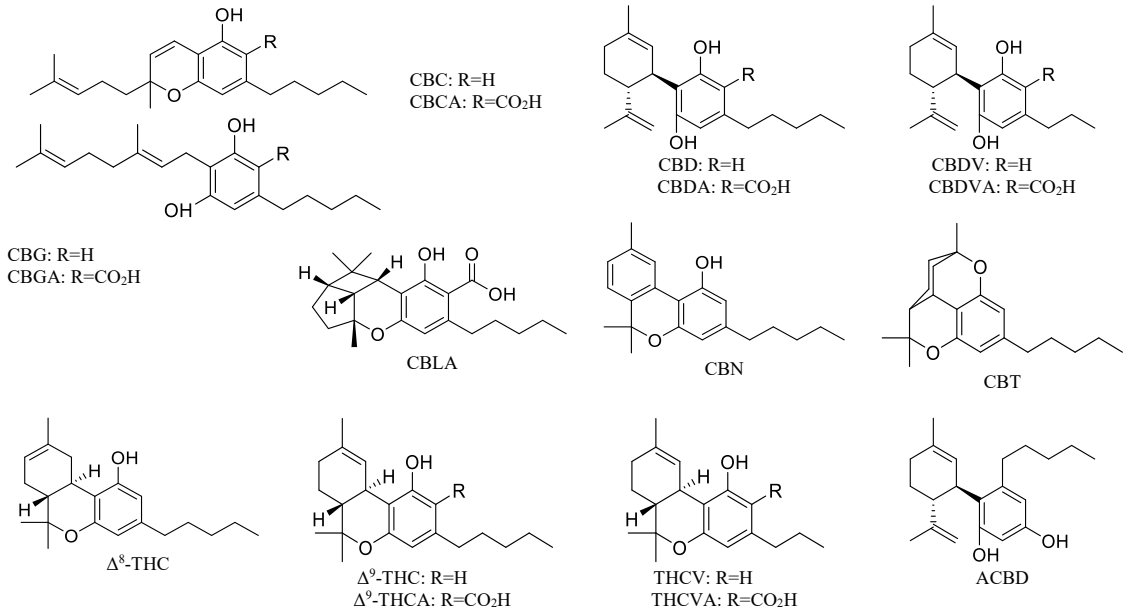
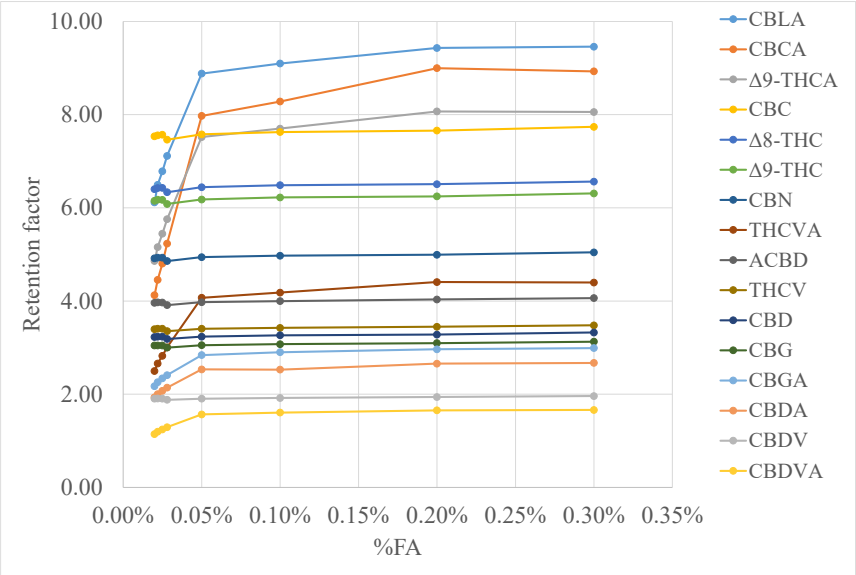
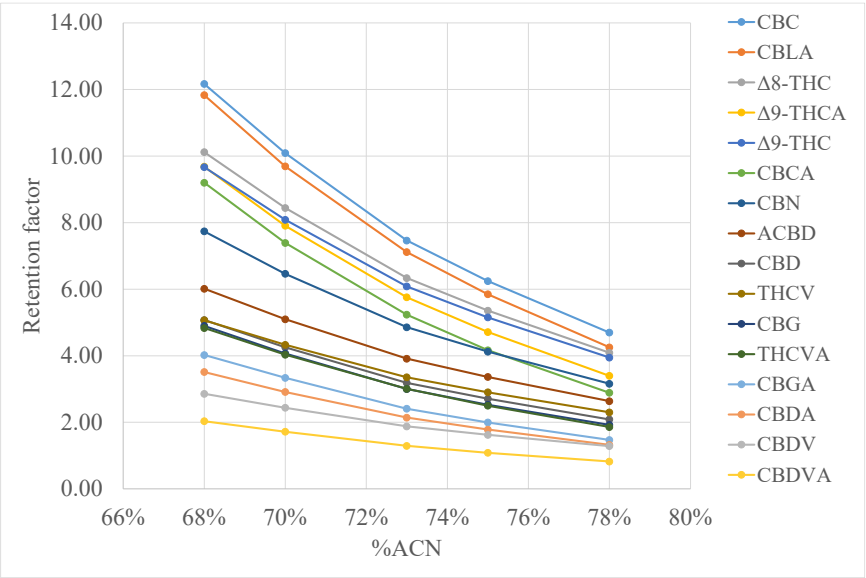


Fig. 2. Effects of (A) %FA in the aqueous solvent of the mobile phase, (B) %ACN in the mobile phase, and (C) column oven temperature on retention factor of cannabinoids.

Default %FA, %ACN and column oven temperature were 0.028%, 73% and 30ºC (all % were in v/v).





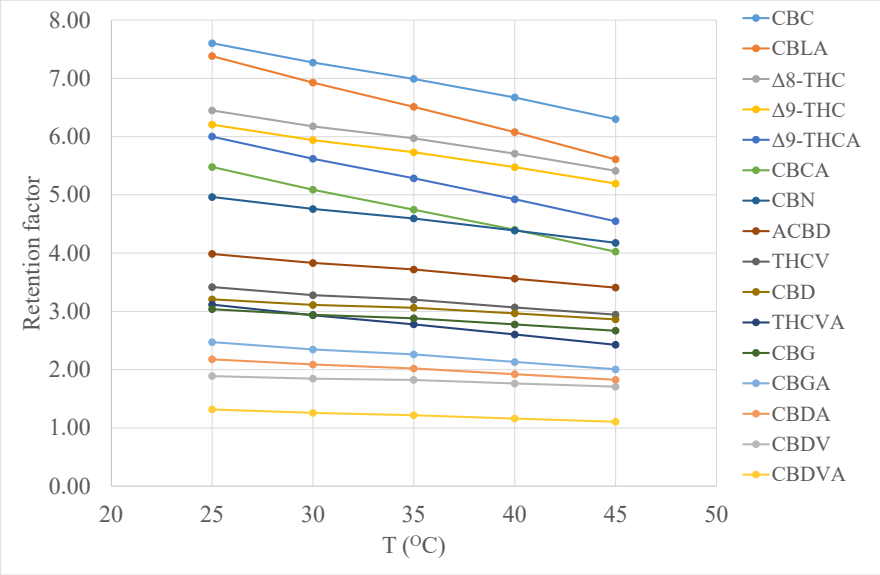
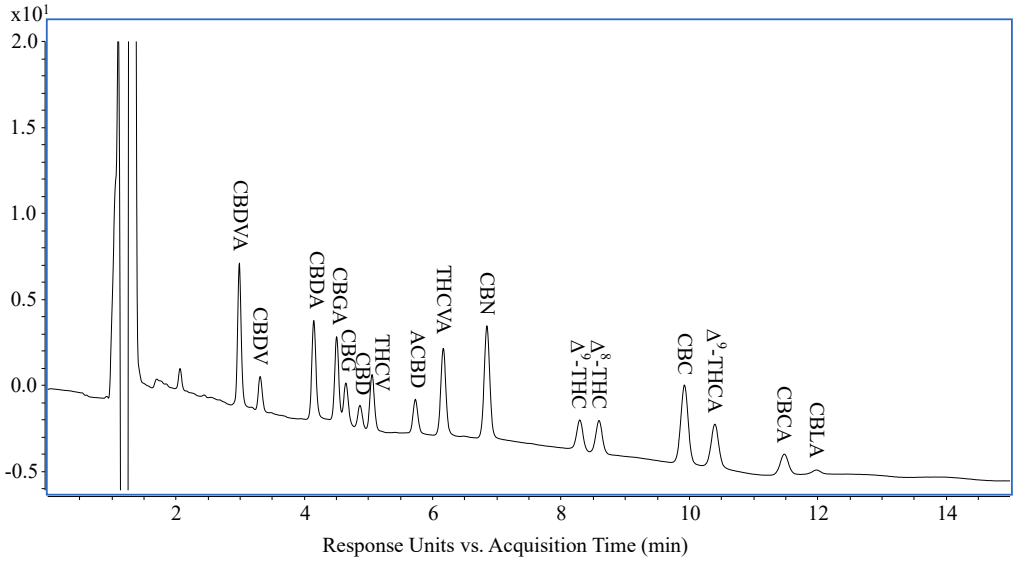
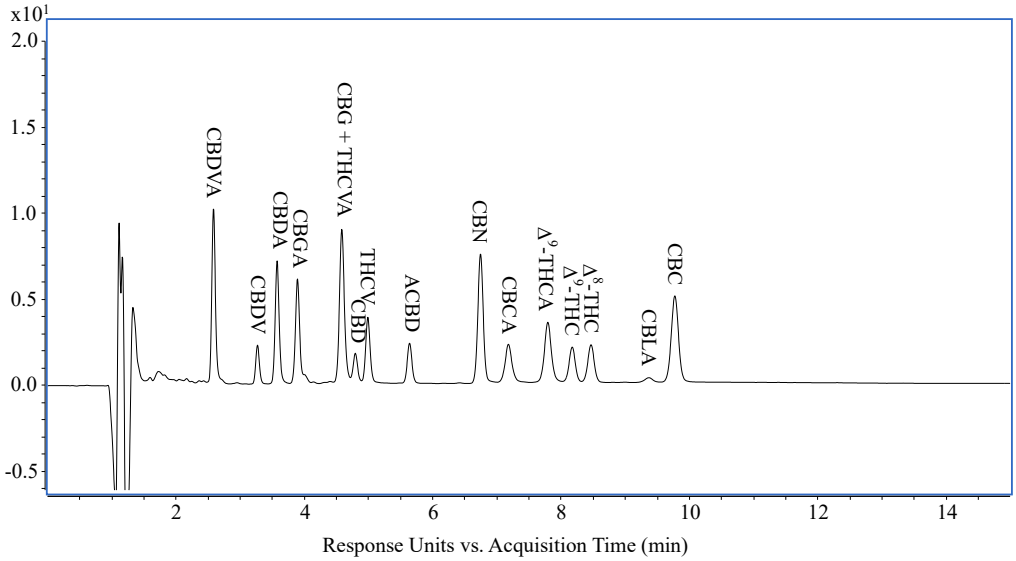
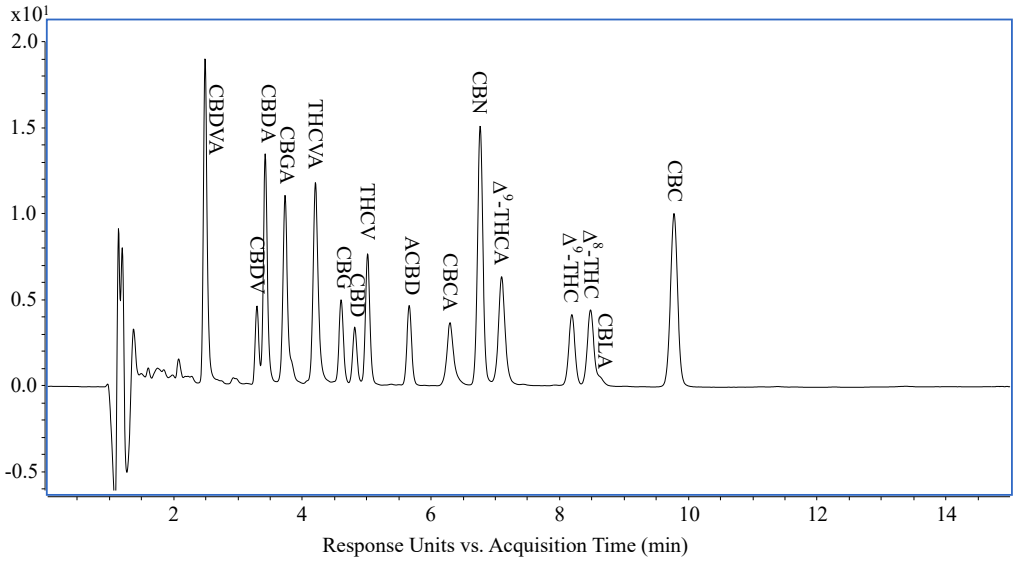

8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Retention factor
20
25
30
35
40
45
50
T (OC)
CBC
CBLA
Δ8-THC
Δ9-THC
Δ9-THCA
CBCA
CBN
ACBD
THCV
CBD
THCVA
CBG
CBGA
CBDA
CBDV
CBDVA

Fig. 3. UHPLC-UV chromatogram at (A) 0.200% FA, 73% ACN and 30ºC; (B) 0.028% FA, 73% ACN and 30ºC; (C) 0.022% FA, 73% ACN and 30ºC; (D) 0.028% FA, 73% ACN and 45ºC (all % were in v/v).






x10¹
2.0
1.5
1.0
0.5
0.0
-0.5
CBDVA
CBDA
CBDV
CBGA
THCVA
CBG
CBD
THCV
ACBD
CBCA
CBN
Δ⁹-THCA
Δ⁹-THC
Δ⁸-THC
CBLA
CBC
2
4
6
8
10
12
14
Response Units vs. Acquisition Time (min)

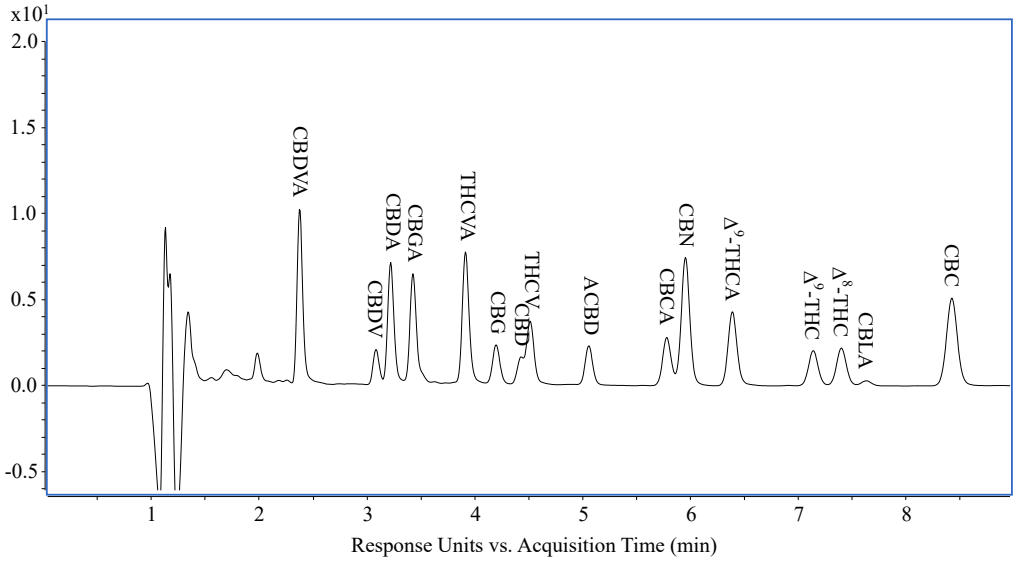

x10¹
2.0
1.5
1.0
0.5
0.0
-0.5
CBDVA
CBDV
CBDA
CBGA
THCVA
CBG
THCV
CBD
ACBD
CBCA
CBN
Δ⁹-THCA
Δ⁹-THC
Δ⁸-THC
CBLA
CBC
1
2
3
4
5
6
7
8
Response Units vs. Acquisition Time (min)

Fig. 4. UHPLC-UV chromatograms of cannabinoids at LLOQ level, i.e., 0.02 µg/mL, except for ACBD at 0.5 µg/mL under optimized LC conditions: 0.028% FA (pH 3.6), 73% ACN and 30ºC (all % were in v/v).

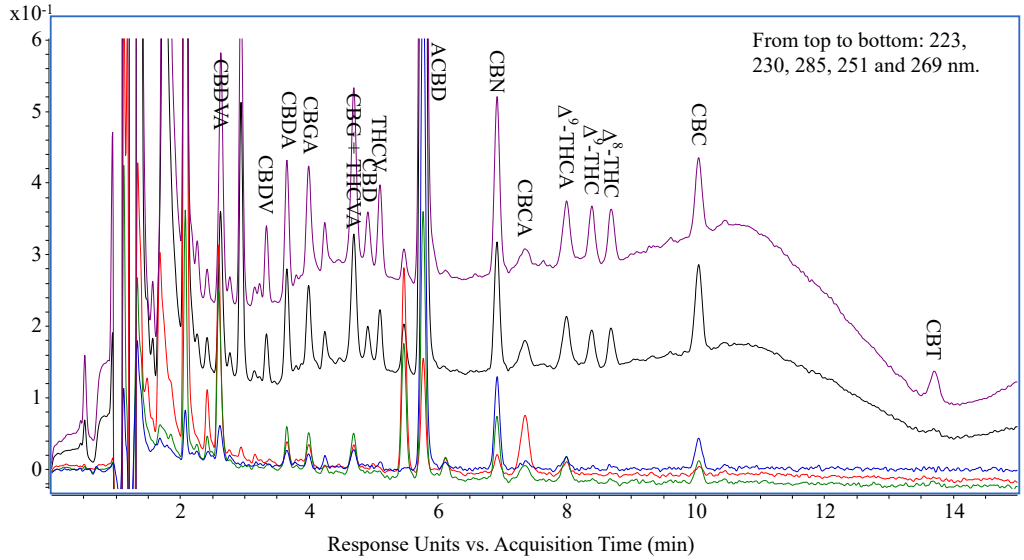
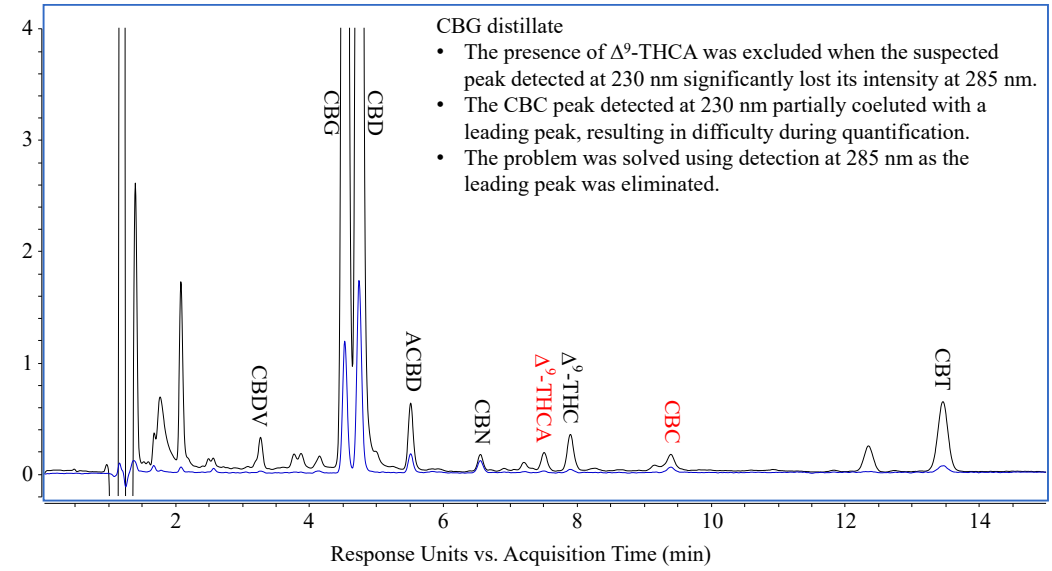
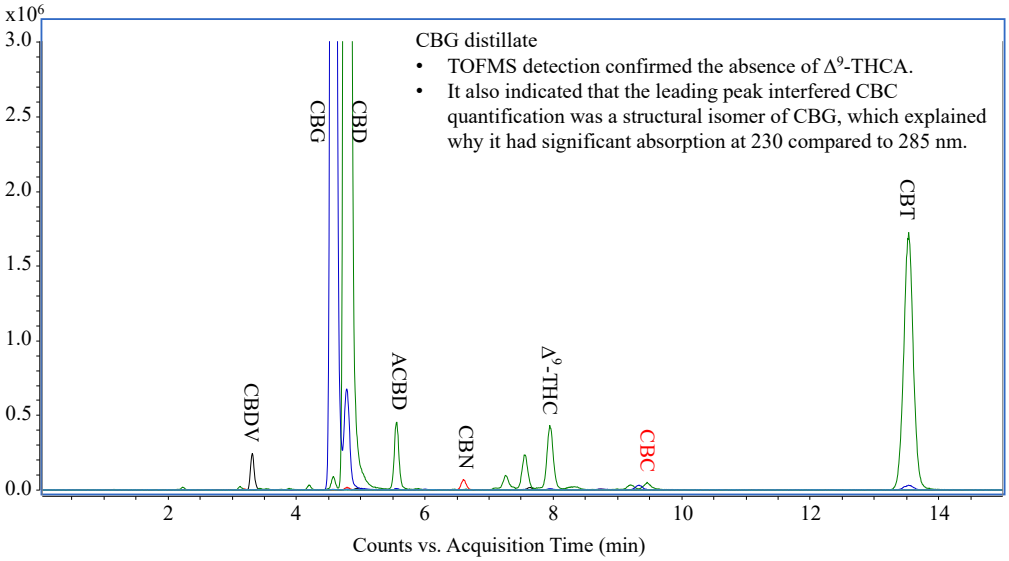
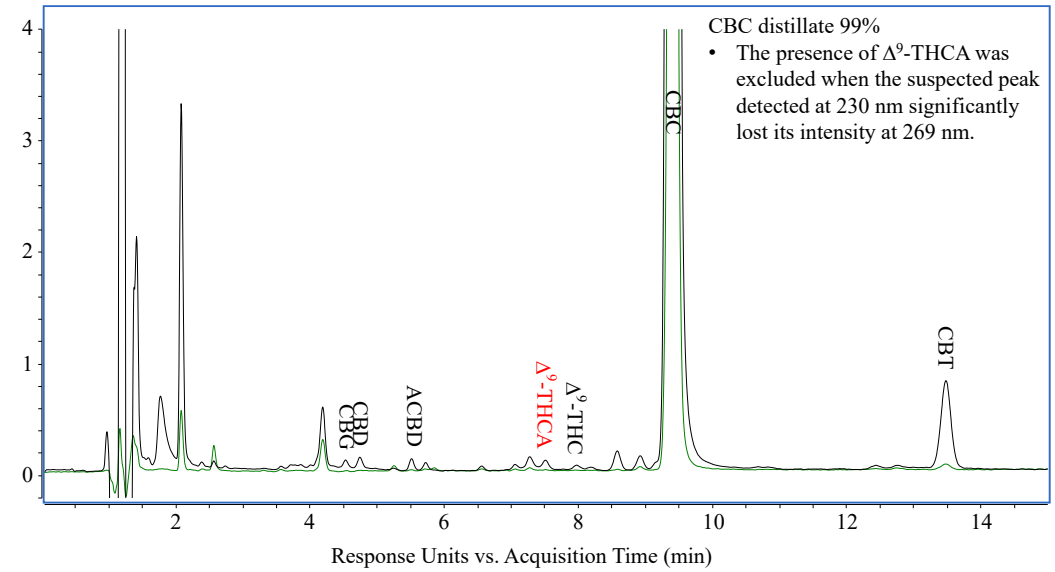
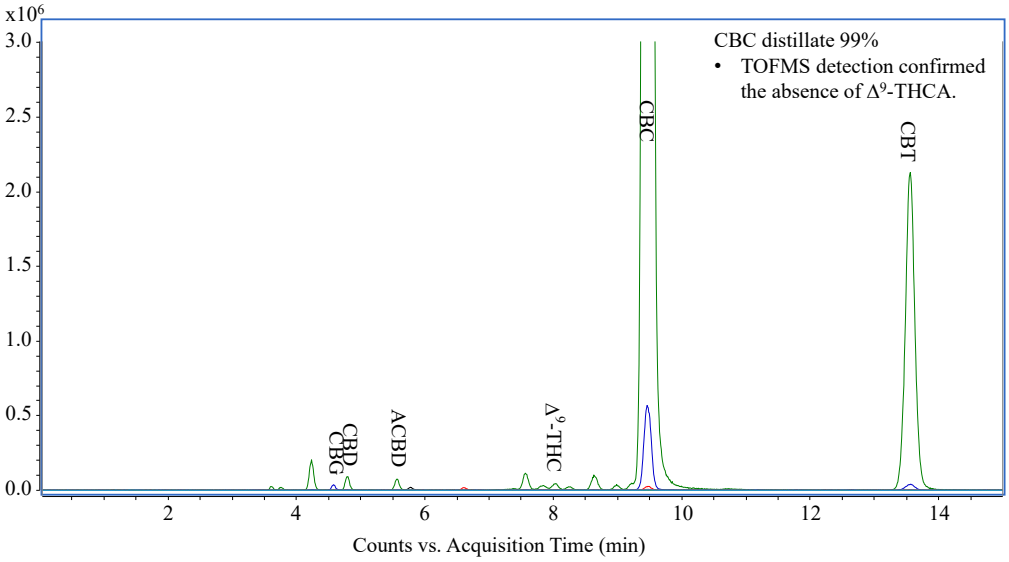


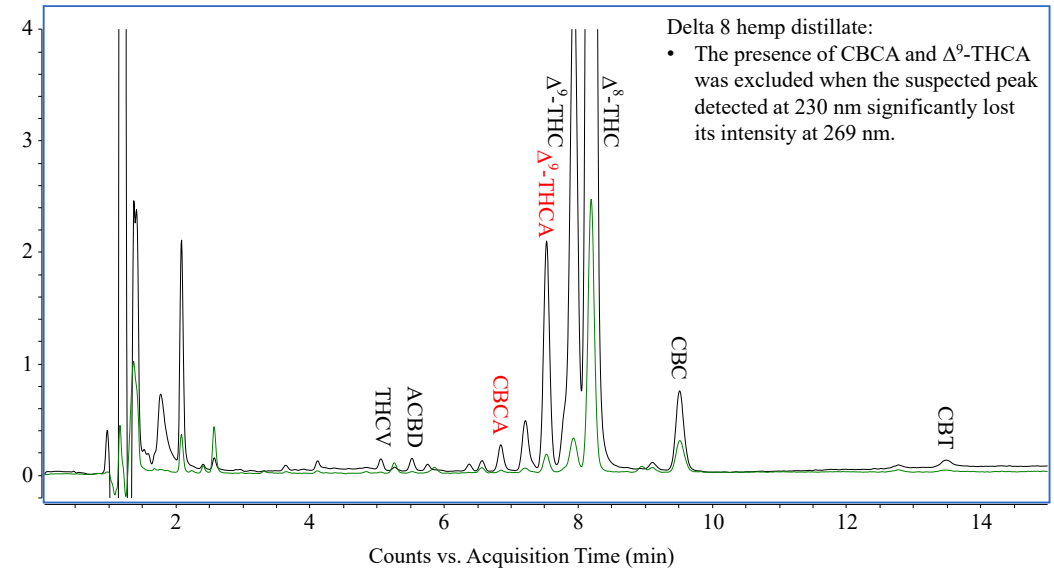
Fig.5. UHPLC-UV chromatogram (black, 230 nm; green, 269 nm; blue, 285 nm) and UHPLC-EICs (cannabinoids are both color coded and labelled) of CBG distillate (A and B), CBC distillate 99% (C and D), Delta 8 hemp distillate (E and F), and Delta 8 hemp shatter (G and H).

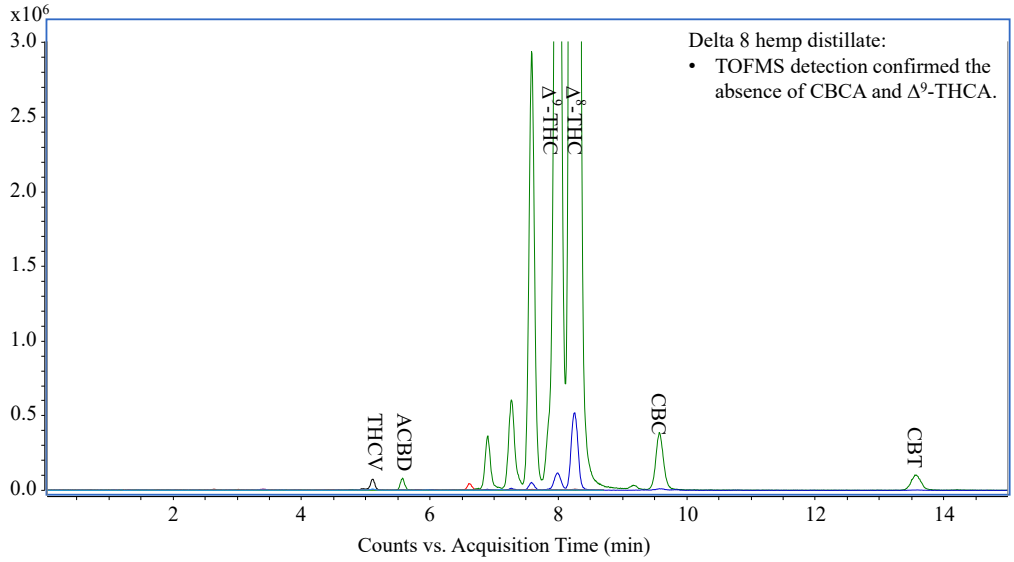


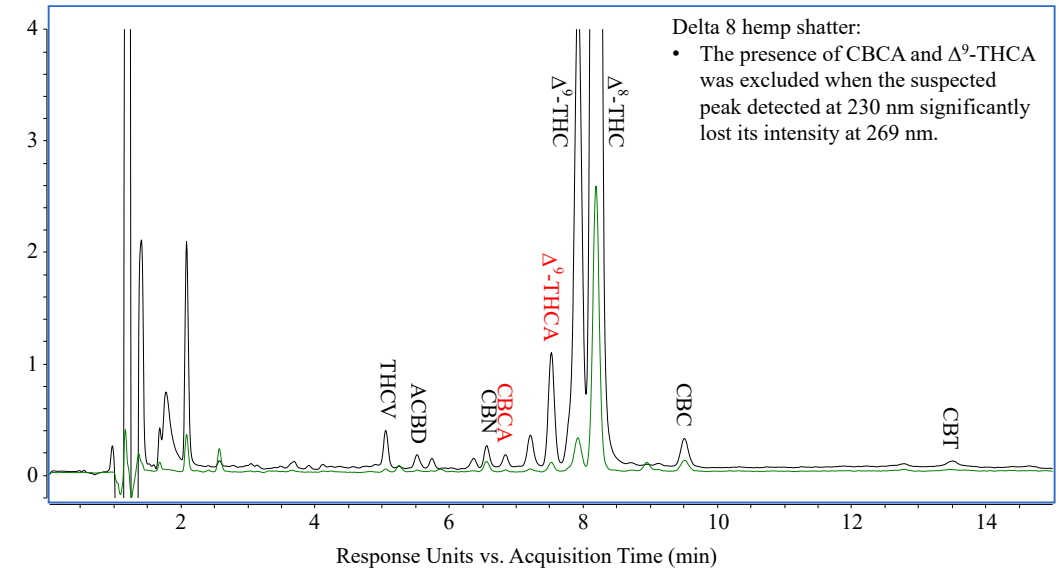



CBC distillate 99%
• The presence of Δ9-THCA was excluded when the suspected peak detected at 230 nm significantly lost its intensity at 269 nm.
CBC
CBT
CBD
CBG
ACBD
Δ9-THCA
Δ9-THC
Response Units vs. Acquisition Time (min)


x10^6
CBC distillate 99%
• TOFMS detection confirmed the absence of Δ9-THCA.
CBC
CBT
CBD
CBG
ACBD
Δ9-THC
Counts vs. Acquisition Time (min)


Delta 8 hemp distillate:
• The presence of CBCA and Δ9-THCA was excluded when the suspected peak detected at 230 nm significantly lost its intensity at 269 nm.
THCV
ACBD
CBCA
Δ9-THCA
Δ9-THC
Δ8-THC
CBC
CBT
Counts vs. Acquisition Time (min)


x10^6
Delta 8 hemp distillate:
• TOFMS detection confirmed the absence of CBCA and Δ9-THCA.
Δ9-THC
Δ8-THC
THCV
ACBD
CBC
CBT
Counts vs. Acquisition Time (min)


Delta 8 hemp shatter:
• The presence of CBCA and Δ9-THCA was excluded when the suspected peak detected at 230 nm significantly lost its intensity at 269 nm.
Δ9-THC
Δ8-THC
Δ9-THCA
THCV
ACBD
CBN
CBCA
CBC
CBT
Response Units vs. Acquisition Time (min)

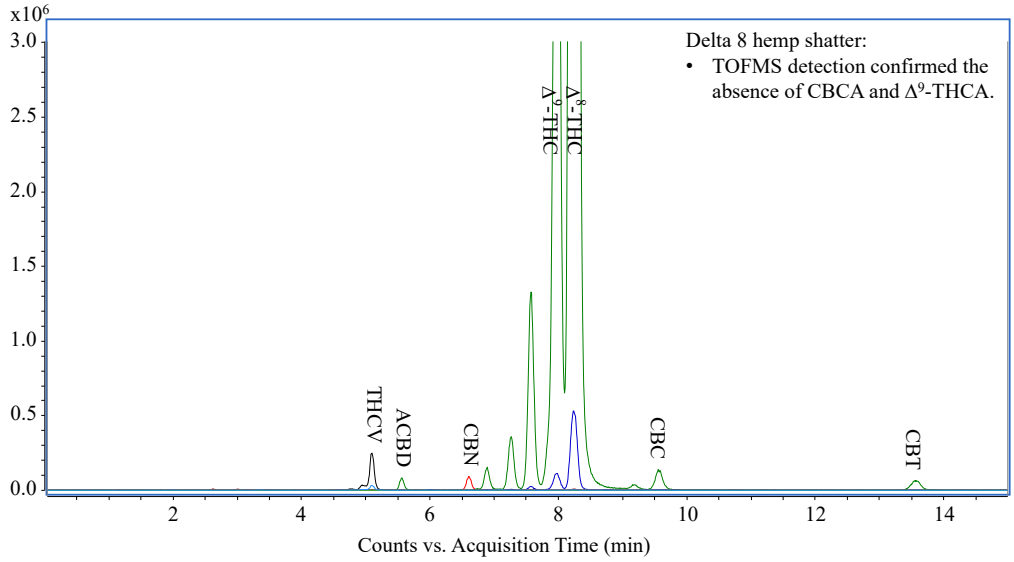

x10^6
Delta 8 hemp shatter:
• TOFMS detection confirmed the absence of CBCA and Δ9-THCA.
Δ9-THC
Δ8-THC
THCV
ACBD
CBN
CBC
CBT
Counts vs. Acquisition Time (min)

Fig.6. Discovery of untargeted cannabinoids**.** The UHPLC-EICs of the samples were extracted using the $[M+H]^+$ ions of Δ^9-THC, i.e., *m/z* 315.2319 ± 20 ppm.

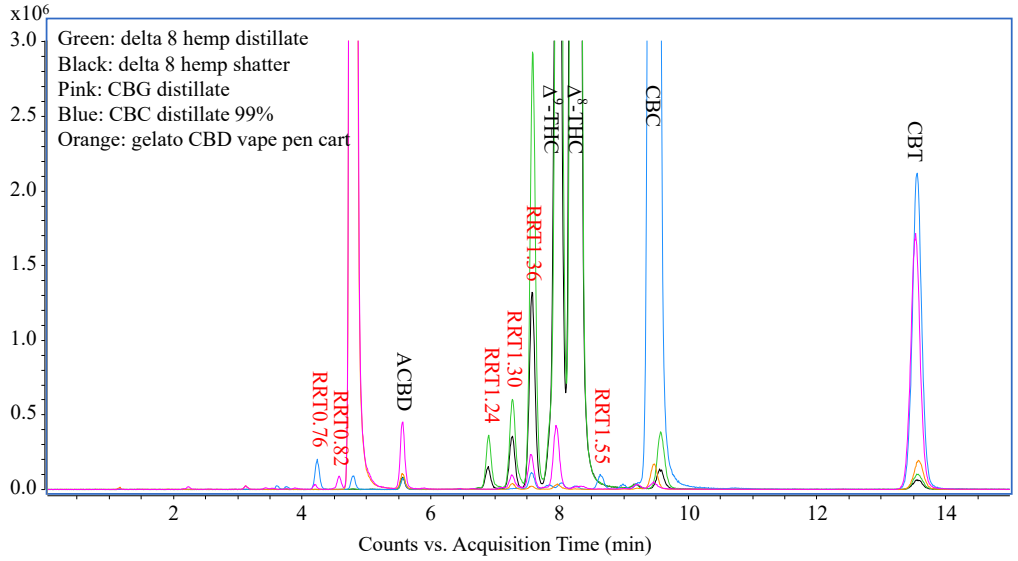


Table 1

Average content (%, w/w) of cannabinoids in hemp concentrates measured in triplicates.

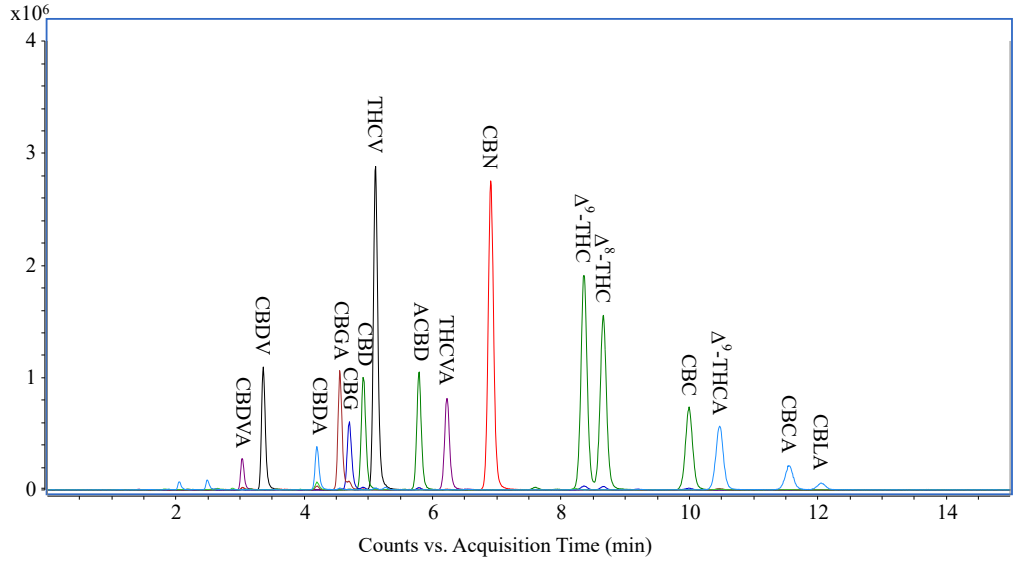
Hemp concentrate	CBDV	CBG	CBD	THCV	CBN	Δ^9-THC	Δ^8-THC	CBC	CBT	Total
CBD distillate (90%+)	0.33	5.81	93.23							99.37
CBG distillate	0.38	39.16	53.26		0.12	0.75		0.15	2.44	96.27
CBC distillate 99%		0.10	0.16			0.11		85.98	3.38	89.72
CBN terpsolate dabs					78.29					78.29
Delta 8 hemp distillate				0.15		9.35	70.96	0.86	0.32	81.63
Delta 8 hemp shatter				0.45	0.15	11.33	72.32	0.31	0.31	84.87
Gelato CBD vape pen cart	0.21	0.48	53.48		0.48	0.11		0.59	0.40	55.74
Clementine CBD vape pen cart	0.15	0.77	46.17		0.60			0.68	0.40	48.77
Full spectrum CBD oil 1000 mg		0.27	6.74						0.22	7.23

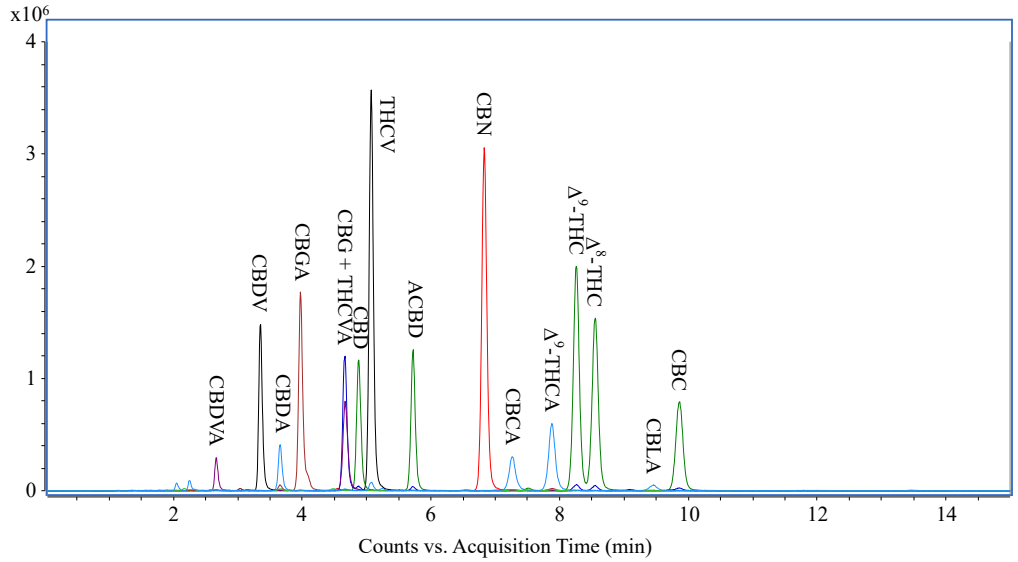
Table 2

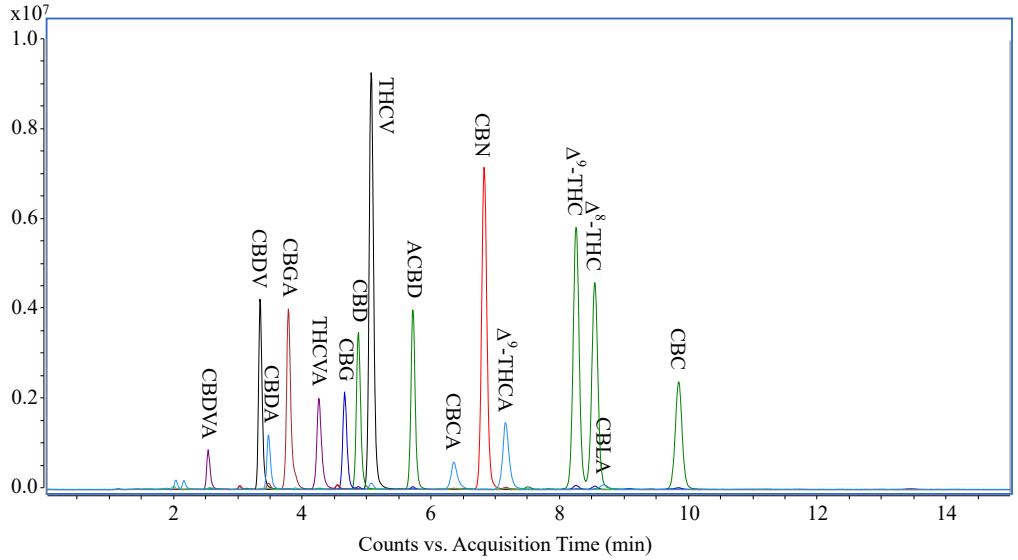
RSD values (%) of cannabinoids in hemp concentrates measured in triplicates.

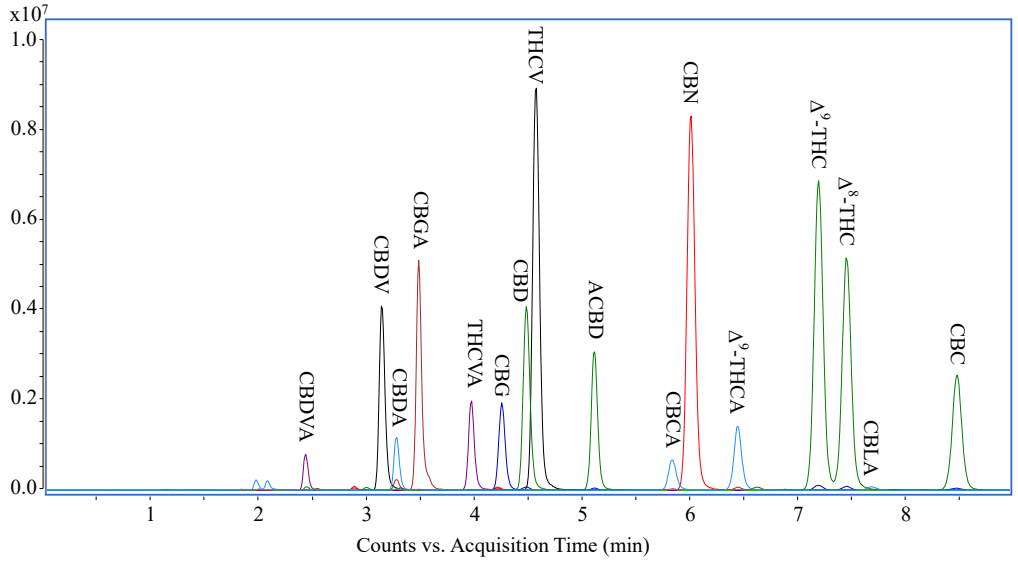
Hemp concentrate	CBDV	CBG	CBD	THCV	CBN	Δ^9-THC	Δ^8-THC	CBC	CBT	Total
CBD distillate (90%+)	3.6	3.2	4.0							4.0
CBG distillate	4.0	3.1	4.0		4.0	3.9		1.3	5.2	3.3
CBC distillate 99%		6.7	11.2			3.1		8.5	7.5	8.5
CBN terpsolate dabs					1.0					1.0
Delta 8 hemp distillate				4.6		1.4	1.4	0.8	6.3	1.4
Delta 8 hemp shatter				3.0	3.2	4.3	2.2	7.9	8.8	2.5
Gelato CBD vape pen cart	2.4	1.4	0.6		1.2	5.2		2.2	4.0	0.6
Clementine CBD vape pen cart	0.8	2.7	2.0		1.7			2.4	2.6	2.0
Full spectrum CBD oil 1000 mg		0.3	3.6						6.7	3.5

Supplementary Fig. S1. From top to bottom, UHPLC-EICs at (A) 0.200% FA, 73% ACN and 30ºC; (B) 0.028% FA, 73% ACN and 30ºC; (C) 0.022% FA, 73% ACN and 30ºC; (D) 0.028% FA, 73% ACN and 45ºC. The peaks were extracted using their $[M+H]^+$ ions except for CBGA using its $[M+H-H_2O]^+$ ion with ±20 ppm.

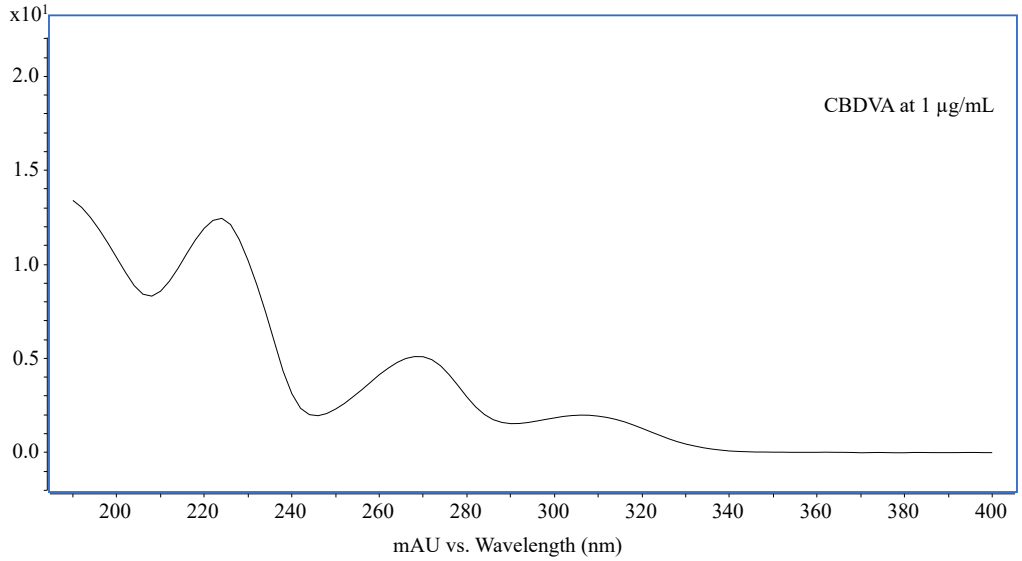


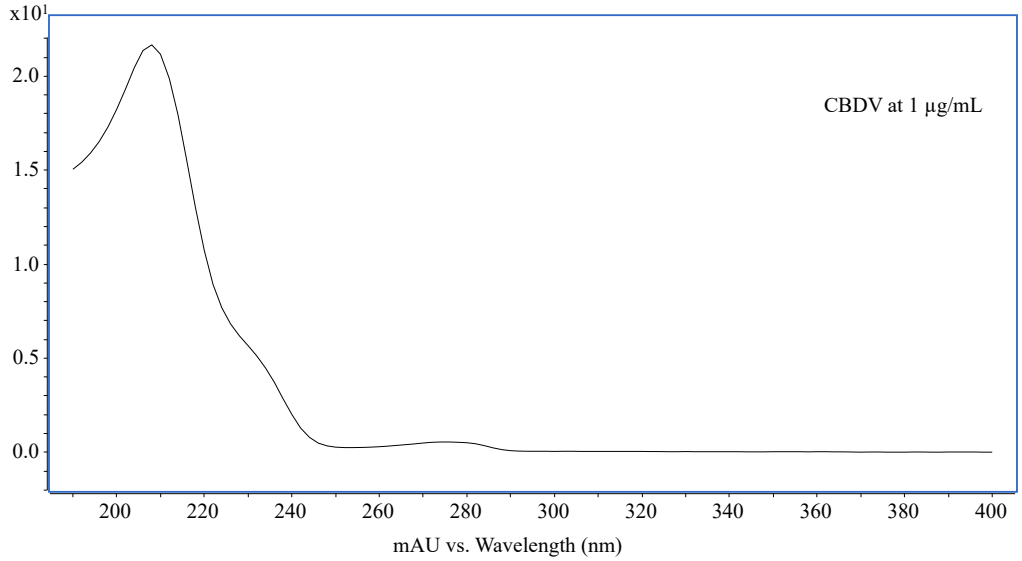


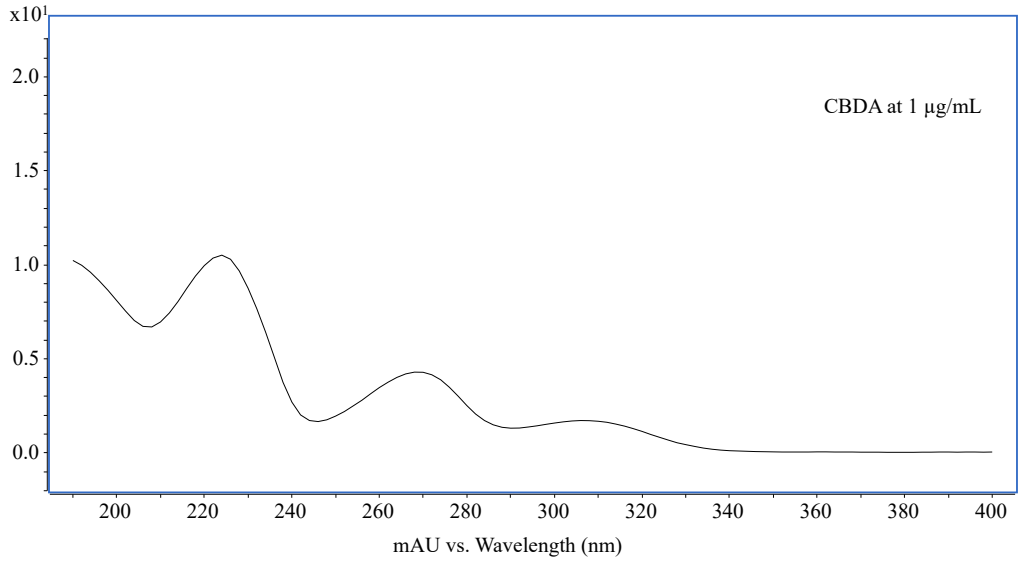

x10^7
1.0
0.8
0.6
0.4
0.2
0.0
CBDVA
CBDV
CBDA
CBGA
THCVA
CBG
CBD
THCV
ACBD
CBCA
CBN
Δ9-THCA
Δ9-THC
Δ8-THC
CBLA
CBC
2
4
6
8
10
12
14
Counts vs. Acquisition Time (min)

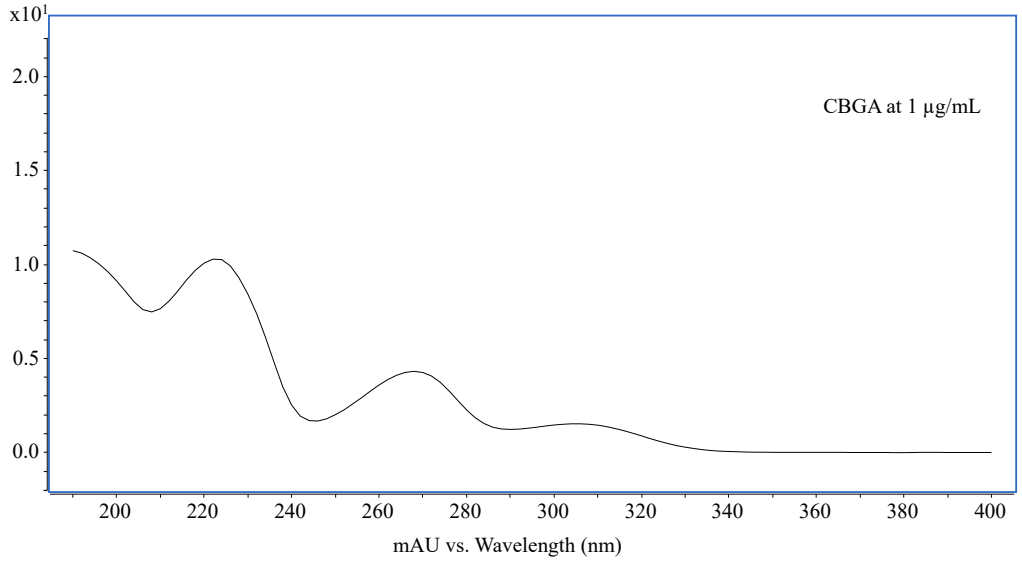

x10^7
1.0
0.8
0.6
0.4
0.2
0.0
CBDVA
CBDV
CBDA
CBGA
THCVA
CBG
CBD
THCV
ACBD
CBCA
CBN
Δ9-THCA
Δ9-THC
Δ8-THC
CBLA
CBC
1
2
3
4
5
6
7
8
Counts vs. Acquisition Time (min)

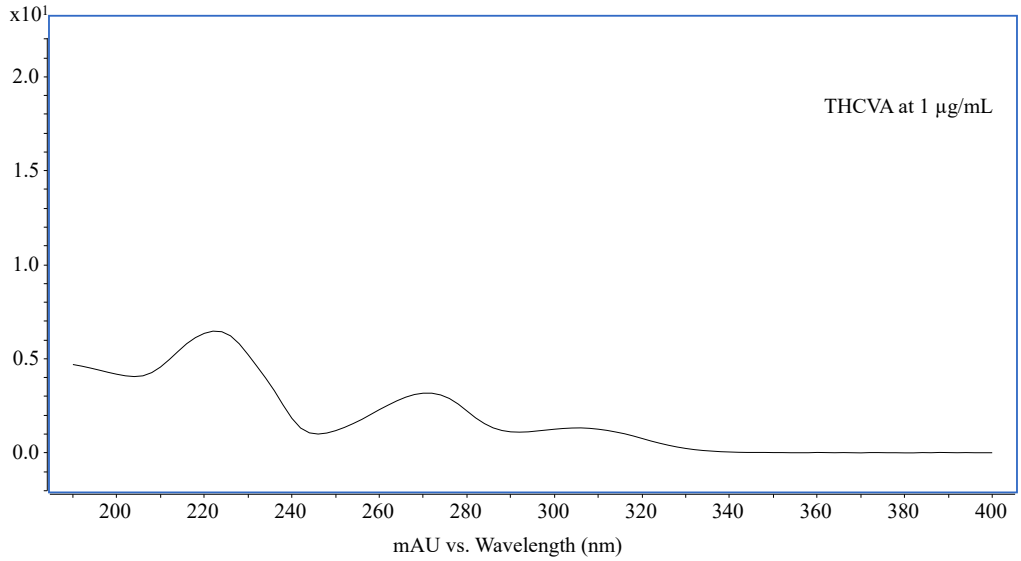
Supplementary Fig. S2. UV absorption spectra of the seventeen cannabinoids.

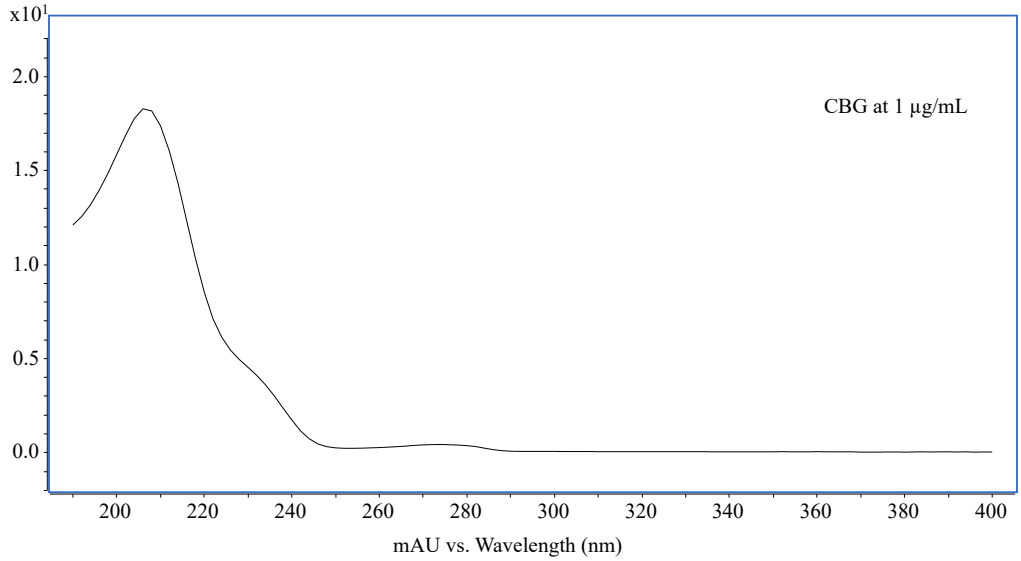


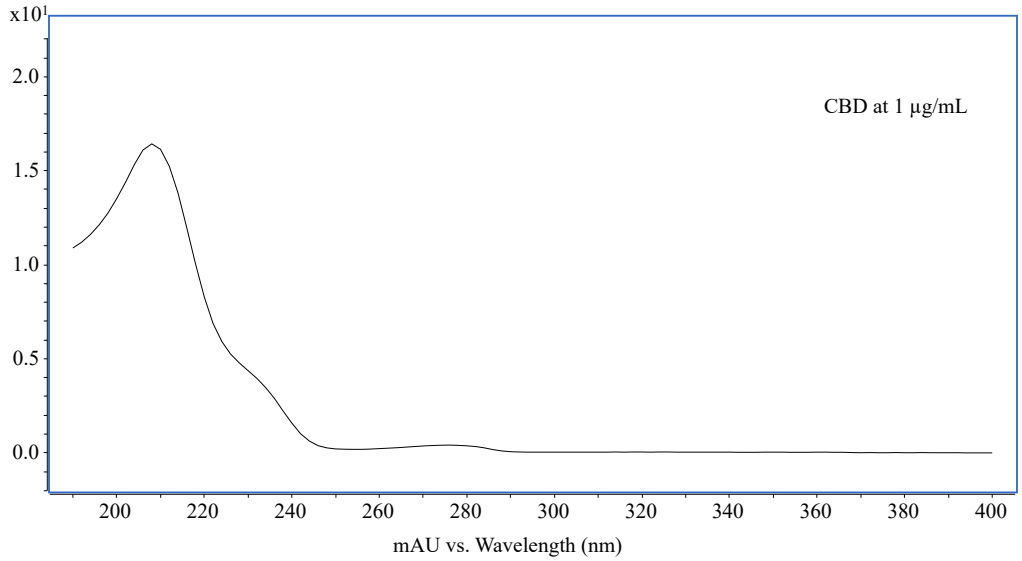


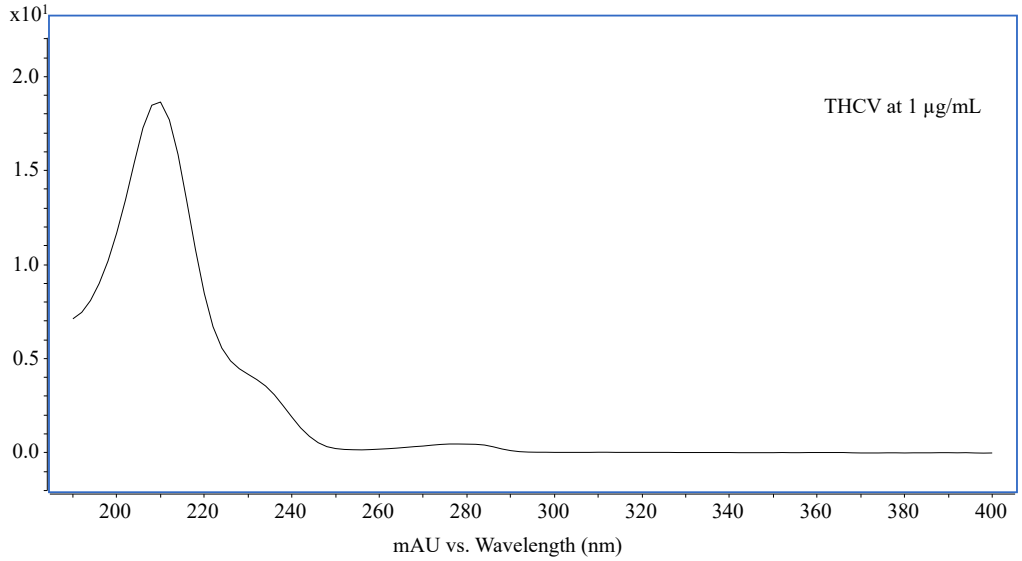

x10¹
2.0
1.5
1.0
0.5
0.0
CBDA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

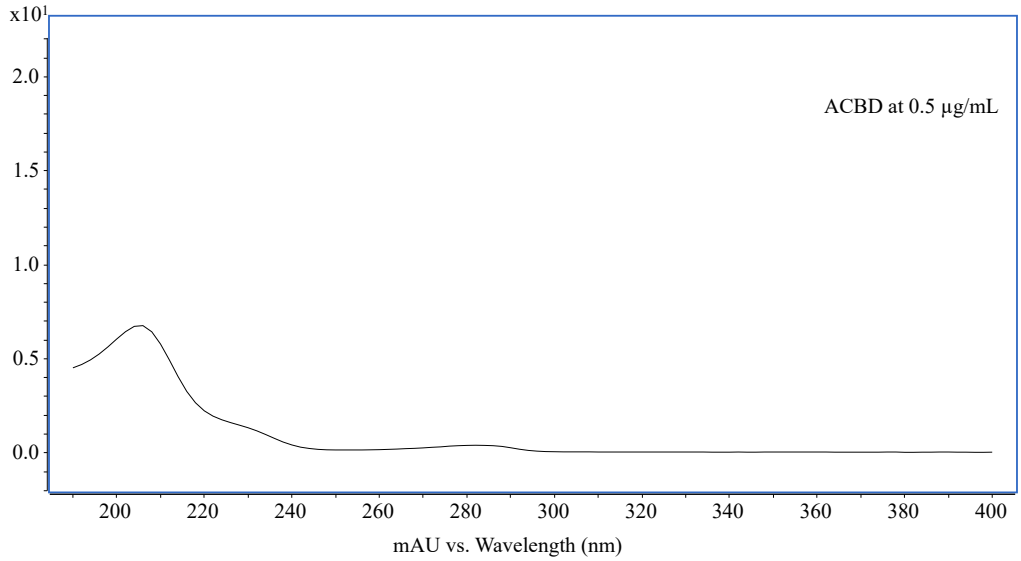

x10¹
2.0
1.5
1.0
0.5
0.0
CBGA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

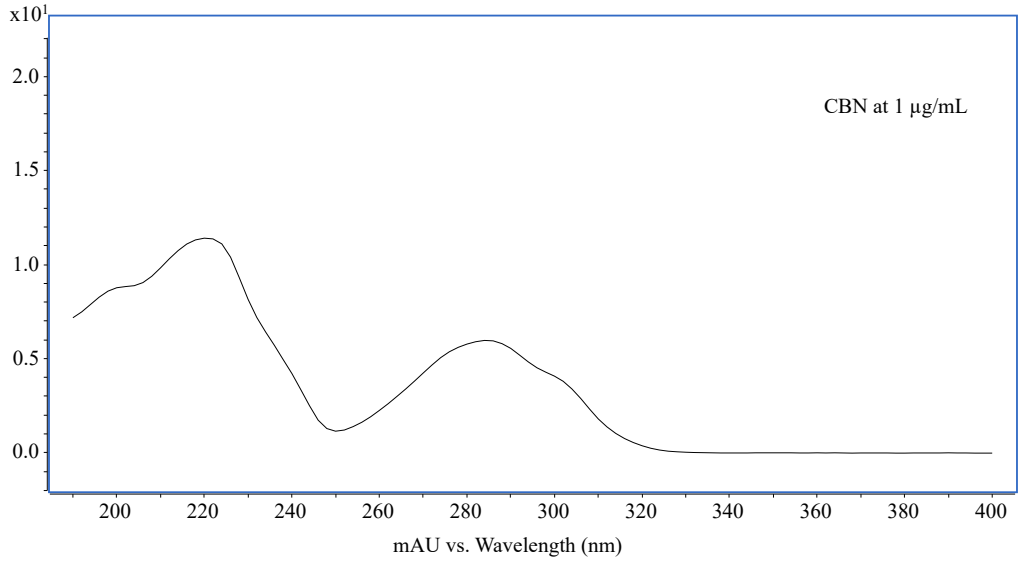

x10¹
2.0
1.5
1.0
0.5
0.0
THCVA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

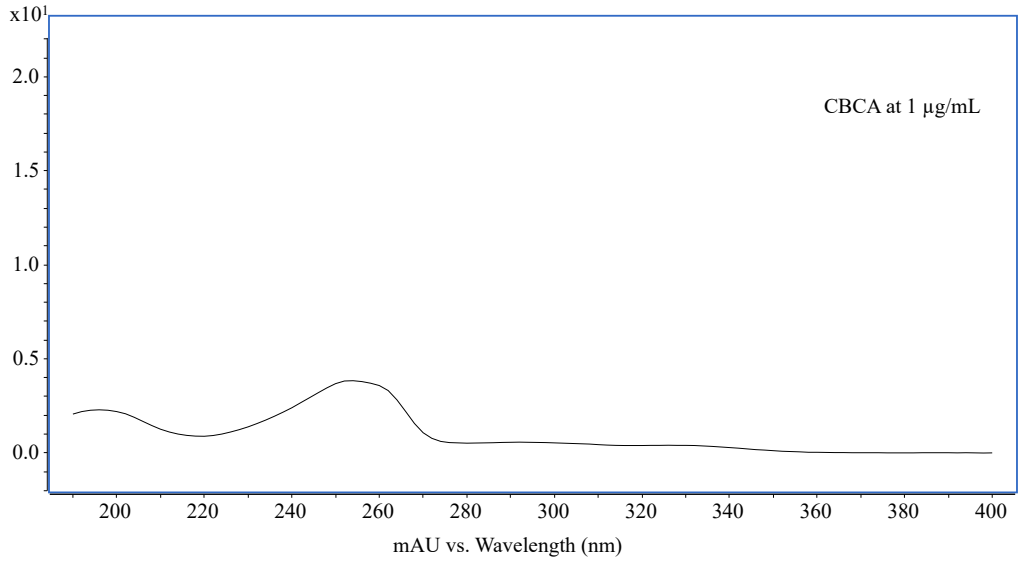

x10¹
2.0
1.5
1.0
0.5
0.0
CBG at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

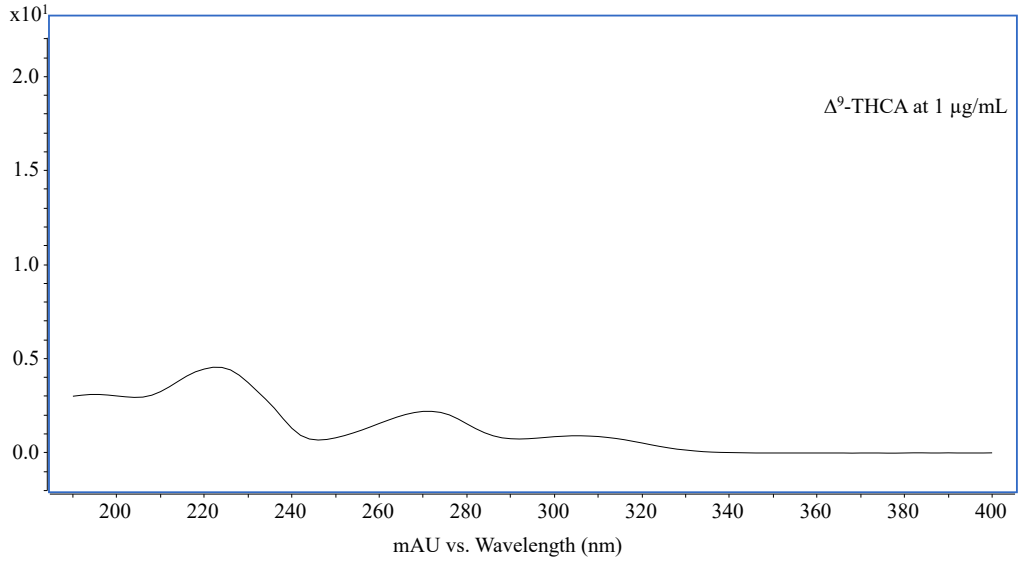

x10¹
2.0
1.5
1.0
0.5
0.0
CBD at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

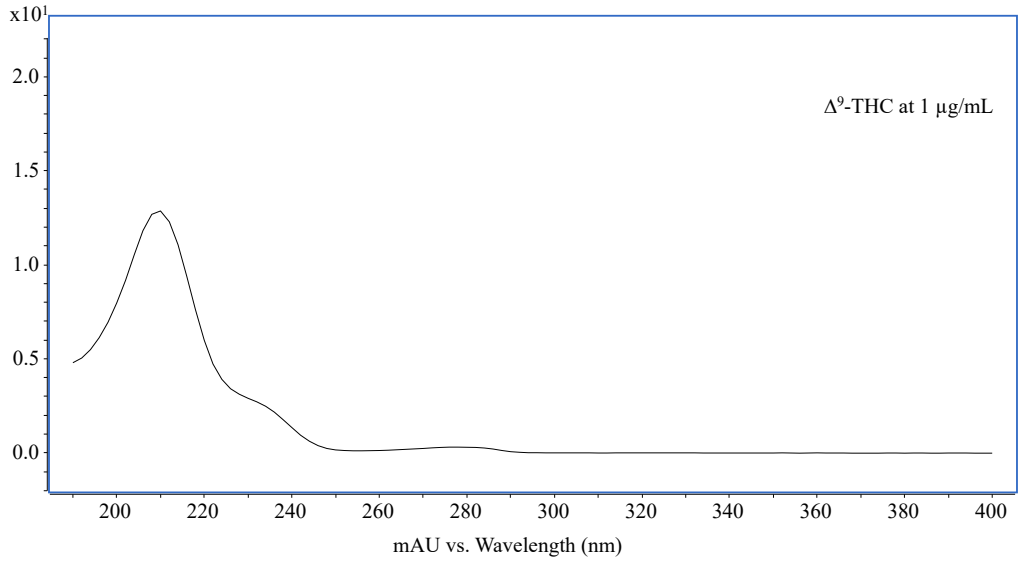

x10¹
2.0
1.5
1.0
0.5
0.0
THCV at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

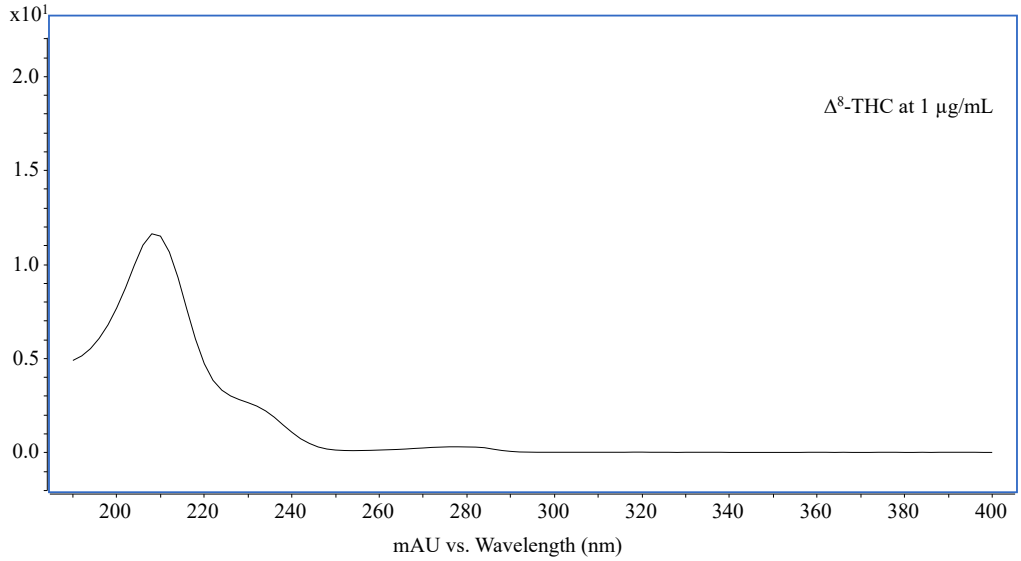

x10¹
2.0
1.5
1.0
0.5
0.0
ACBD at 0.5 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

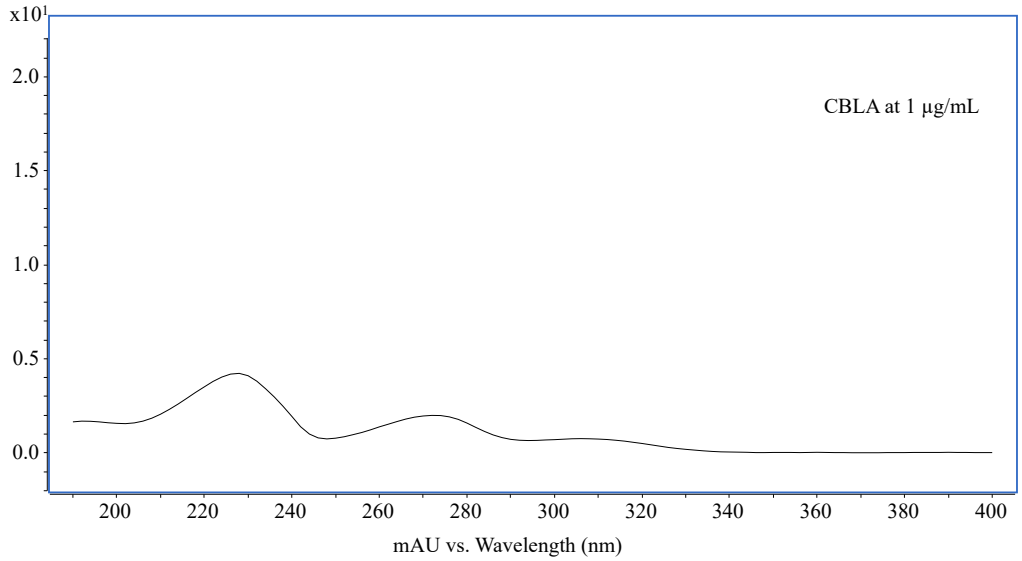

x10¹
2.0
1.5
1.0
0.5
0.0
CBN at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

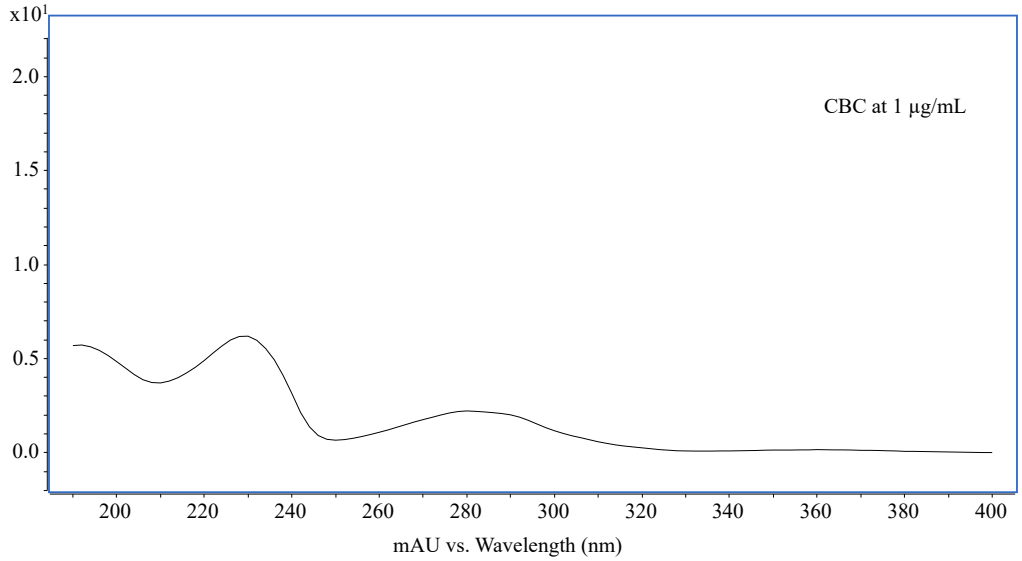

x10¹
2.0
1.5
1.0
0.5
0.0
CBCA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

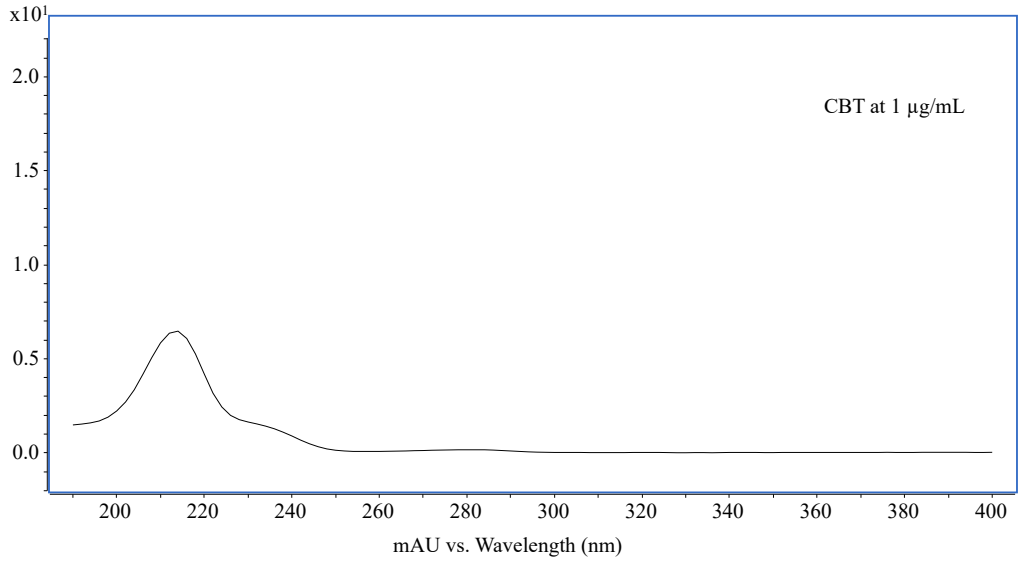

x10¹
2.0
1.5
1.0
0.5
0.0
Δ⁹-THCA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)


x10¹
2.0
1.5
1.0
0.5
0.0
Δ⁹-THC at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

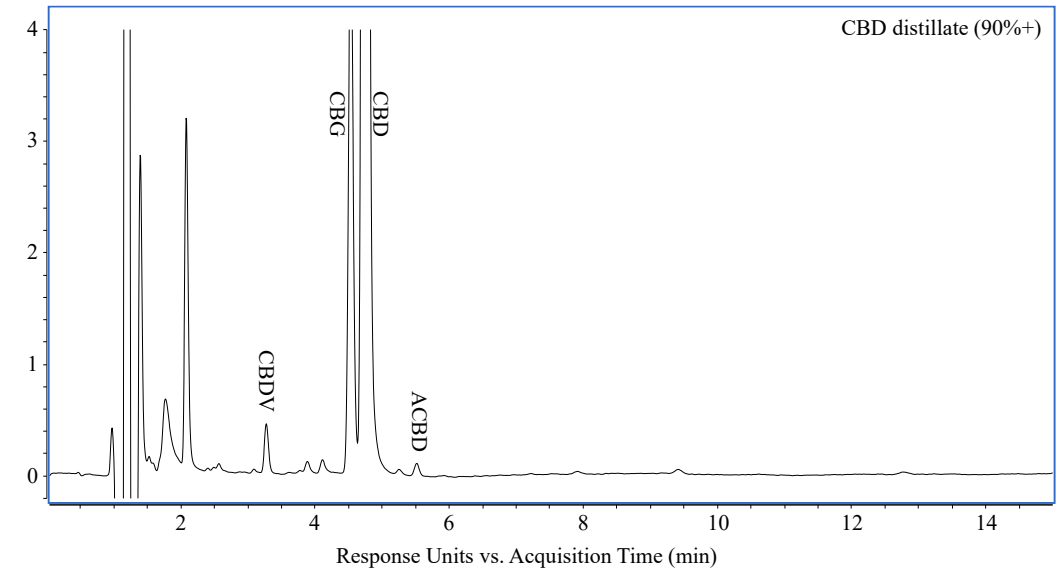

x10¹
2.0
1.5
1.0
0.5
0.0
Δ⁸-THC at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

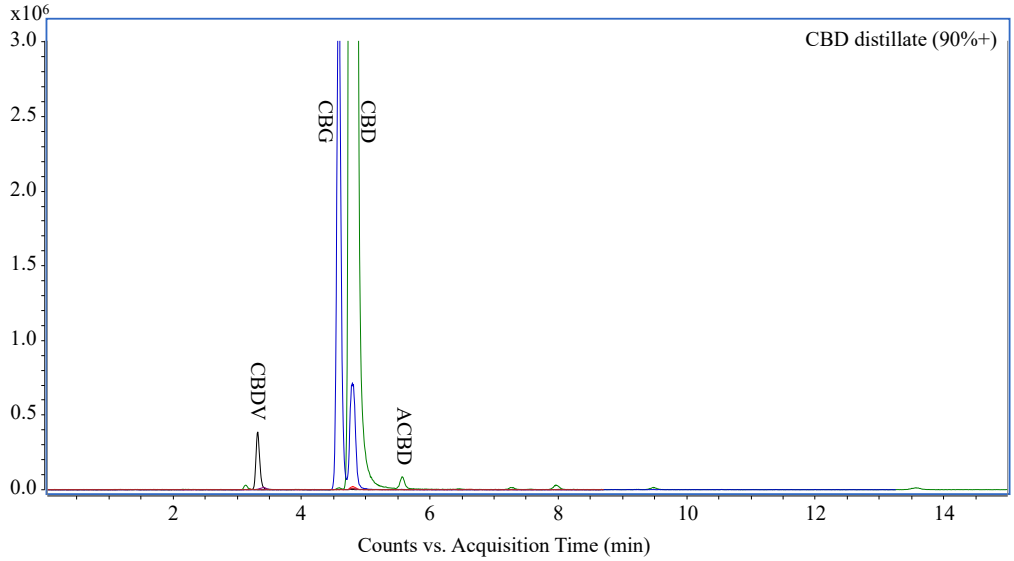

x10¹
2.0
1.5
1.0
0.5
0.0
CBLA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

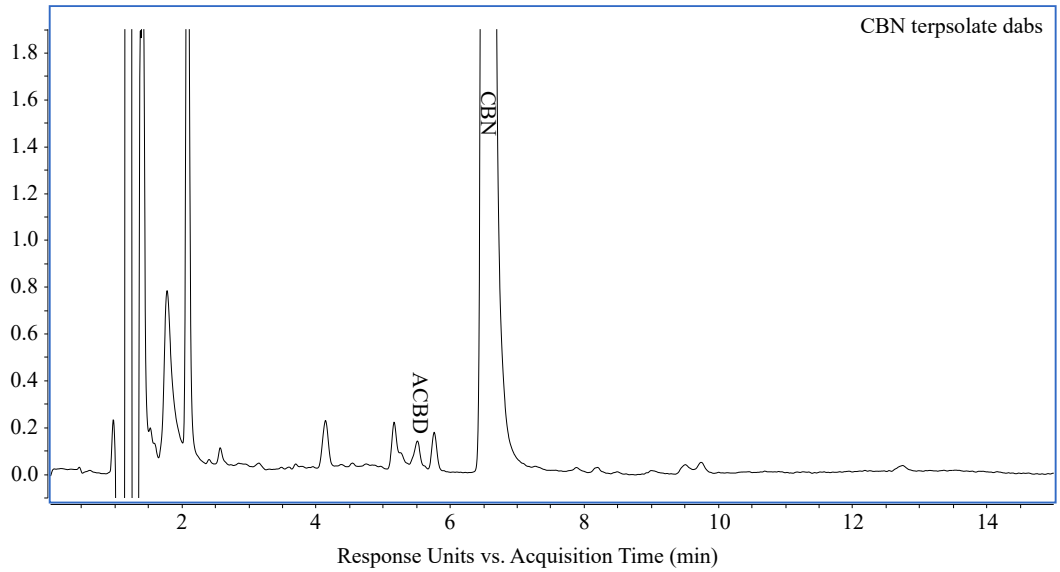

x10¹
2.0
1.5
1.0
0.5
0.0
CBC at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

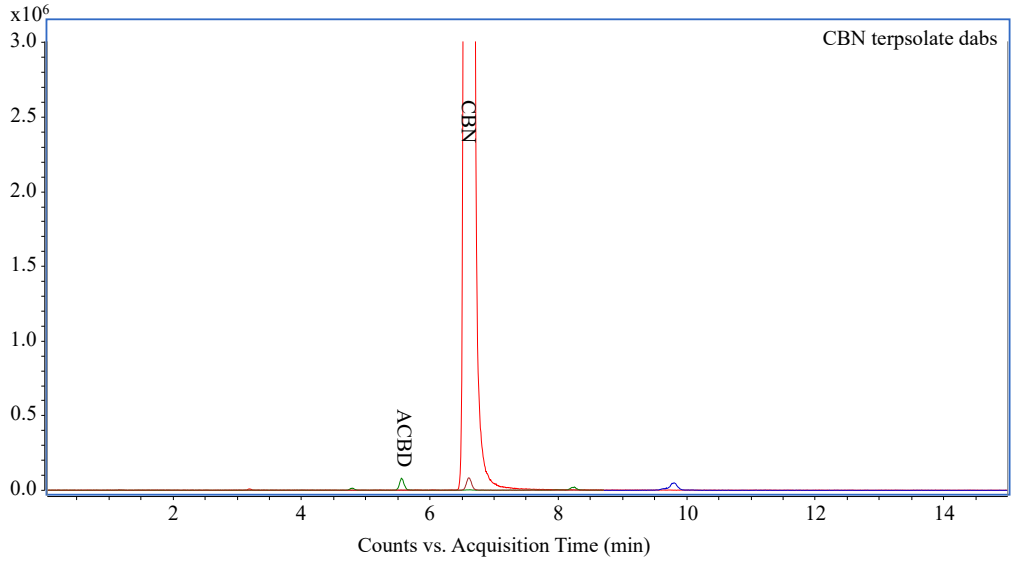

x10¹
2.0
1.5
1.0
0.5
0.0
CBT at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

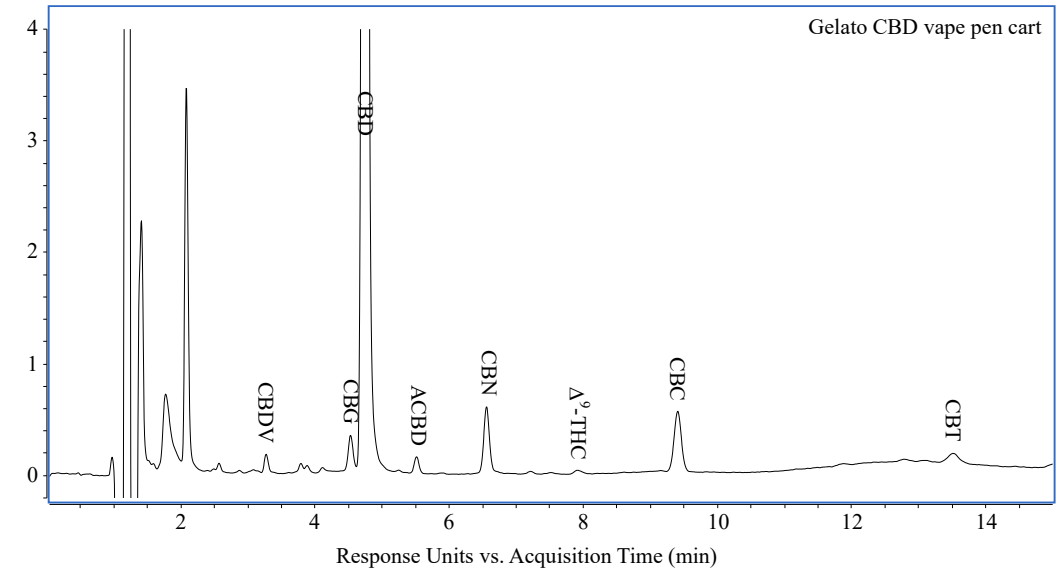
Supplementary Fig. S3. From top to bottom, UHPLC-UV chromatogram (black, 230 nm; green, 269 nm; blue, 285 nm) and UHPLC-EICs (cannabinoids are both color coded and labelled) of CBD distillate (90%+) (A and B), CBN terpsolate dabs (C and D), Gelato CBD vape pen cart (E and F), Clementine CBD vape pen cart (G and H), and full spectrum CBD oil 1000 mg (I and J).

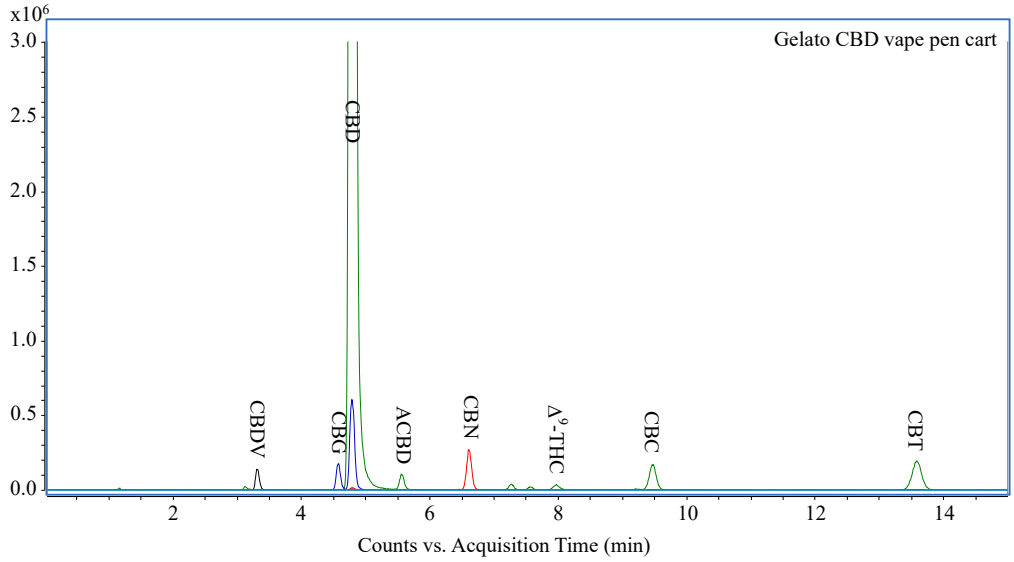


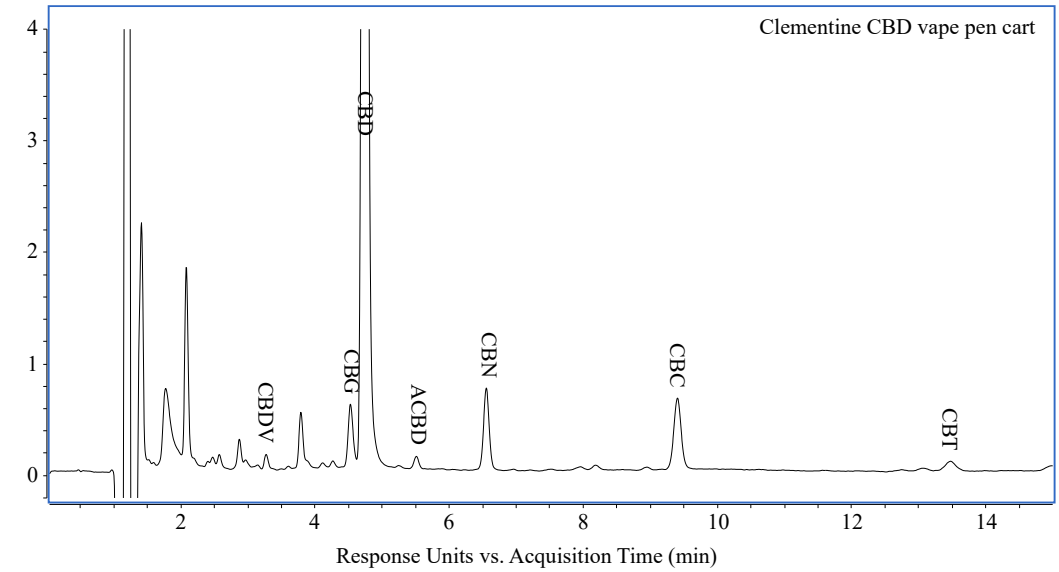


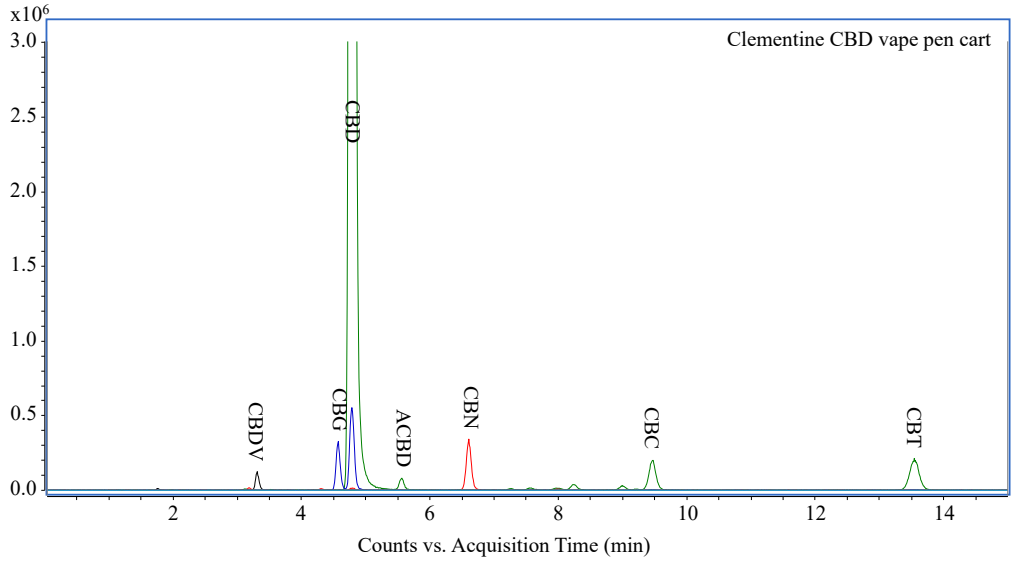

CBN terpsolate dabs
CBN
ACBD
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2
4
6
8
10
12
14
Response Units vs. Acquisition Time (min)

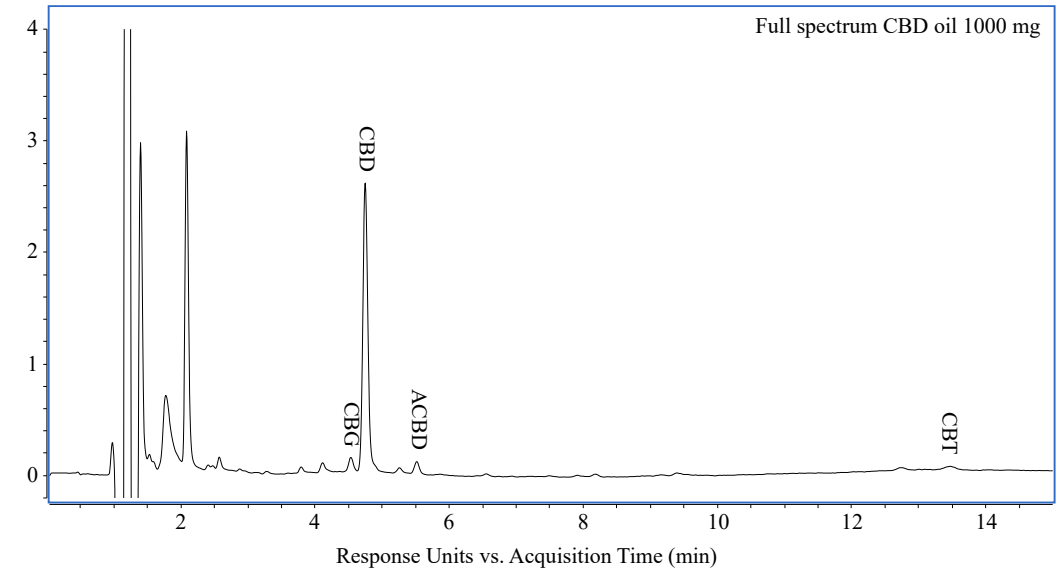

x10^6
CBN terpsolate dabs
CBN
ACBD
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2
4
6
8
10
12
14
Counts vs. Acquisition Time (min)

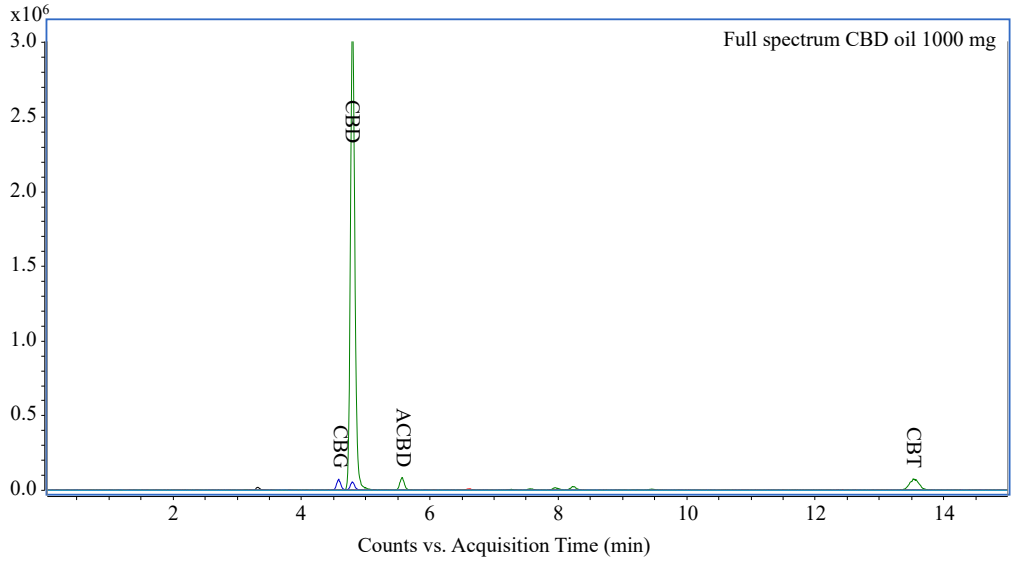

Gelato CBD vape pen cart
CBDV
CBG
CBD
ACBD
CBN
Δ9-THC
CBC
CBT
Response Units vs. Acquisition Time (min)


x10^6
Gelato CBD vape pen cart
CBDV
CBG
CBD
ACBD
CBN
Δ9-THC
CBC
CBT
Counts vs. Acquisition Time (min)


Clementine CBD vape pen cart
CBDV
CBG
CBD
ACBD
CBN
CBC
CBT
Response Units vs. Acquisition Time (min)


x10^6
Clementine CBD vape pen cart
CBDV
CBG
CBD
ACBD
CBN
CBC
CBT
Counts vs. Acquisition Time (min)


Full spectrum CBD oil 1000 mg
CBD
CBG
ACBD
CBT
Response Units vs. Acquisition Time (min)


x10^6
Full spectrum CBD oil 1000 mg
CBD
CBG
ACBD
CBT
Counts vs. Acquisition Time (min)

Supplementary Table S1

Precision of the QC samples: RSD values were computed using triplicate measurements for both intraday and interday.

Concentration	RSD (%)	CBDVA	CBDV	CBDA	CBGA	THCVA	CBG	CBD	THCV	CBN	CBCA	Δ^9-THCA	Δ^9-THC	Δ^8-THC	CBC
0.04 μg/mL	Day 1	1.1	4.2	0.3	0.7	0.3	1.8	0.5	2.9	0.2	7.1	9.6	4.1	8.4	1.6
	Day 2	4.8	1.2	1.0	5.3	7.9	5.3	0.7	3.8	1.7	1.3	2.5	1.0	2.0	1.9
	Day 3	2.3	3.1	2.1	10.3	4.3	5.4	3.0	2.3	1.2	7.8	3.1	3.5	3.9	4.2
	Interday	4.7	8.8	1.4	0.2	2.6	1.6	1.9	14.0	1.8	3.7	5.6	2.6	4.3	3.6
1 μg/mL	Day 1	1.5	0.7	0.4	1.1	0.8	2.1	0.7	2.0	0.2	3.4	2.5	2.1	0.4	0.5
	Day 2	1.8	1.0	0.8	0.8	1.8	1.3	2.3	7.1	0.5	3.1	1.8	1.4	8.9	0.6
	Day 3	0.6	0.4	2.4	0.8	0.8	0.7	2.1	9.8	1.3	0.9	2.1	0.7	8.7	0.2
	Interday	0.8	1.0	1.8	1.1	0.7	0.9	1.0	1.3	0.9	2.8	0.9	1.1	5.8	1.0
25 μg/mL	Day 1	1.3	1.3	0.5	0.5	0.2	0.1	4.4	0.4	0.4	1.1	0.2	0.6	0.5	0.6
	Day 2	0.4	0.4	0.2	0.8	2.3	0.2	0.5	0.3	0.5	1.3	1.1	0.8	3.0	0.5
	Day 3	0.5	1.0	0.5	0.7	1.1	3.0	1.2	2.7	0.6	0.6	1.9	1.7	4.1	0.9
	Interday	1.4	1.0	0.7	1.7	2.9	2.1	3.8	4.2	1.1	1.7	0.9	0.7	3.4	0.8

Supplementary Table S2

Accuracy of the QC samples: average recovery values were computed using triplicate measurements for both intraday and interday.

Concentration	Accuracy (%)	CBDVA	CBDV	CBDA	CBGA	THCVA	CBG	CBD	THCV	CBN	CBCA	Δ^9-THCA	Δ^9-THC	Δ^8-THC	CBC
0.04 μg/mL	Day 1	93.9	84.5	93.9	96.9	99.4	101.1	98.6	114.5	93.9	99.9	102.6	104.6	90.5	94.7
	Day 2	86.8	99.9	91.6	97.0	97.4	103.2	96.1	90.8	92.2	95.0	93.1	99.5	94.5	100.8
	Day 3	94.5	97.1	91.6	97.2	94.5	100.1	99.6	90.3	95.6	93.1	93.6	100.7	98.5	101.1
	Interday	91.7	93.8	92.4	97.0	97.1	101.5	98.1	98.5	93.9	96.0	96.4	101.6	94.5	98.9
1 μg/mL	Day 1	98.0	100.4	97.7	96.2	96.4	99.6	98.8	98.6	98.7	97.5	94.4	97.0	98.3	98.9
	Day 2	96.8	98.9	97.8	97.1	97.7	98.0	98.5	100.7	98.2	94.8	96.0	98.7	89.6	97.1
	Day 3	96.6	98.6	94.7	95.0	97.2	99.4	100.3	98.4	97.0	92.2	94.9	98.9	88.5	98.7
	Interday	97.1	99.3	96.7	96.1	97.1	99.0	99.2	99.2	98.0	94.8	95.1	98.2	92.1	98.2
25 μg/mL	Day 1	110.4	107.6	107.6	106.3	102.6	101.5	101.5	100.4	105.4	114.7	109.8	104.7	108.6	107.5
	Day 2	107.6	106.5	109.1	109.8	108.5	105.1	109.2	108.8	107.5	111.9	107.8	106.0	103.2	106.5
	Day 3	108.4	105.5	108.1	108.5	104.8	105.6	107.6	107.0	105.6	114.8	109.0	104.8	101.8	108.2
	Interday	108.8	106.5	108.2	108.2	105.3	104.0	106.1	105.4	106.2	114.1	108.9	105.2	104.5	107.4