Potency testing of up to twenty cannabinoids by liquid chromatography diode-array detector with optional electrospray ionization time-of-flight mass spectrometry

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Fig. 1. Chemical structures of 21 cannabinoids.

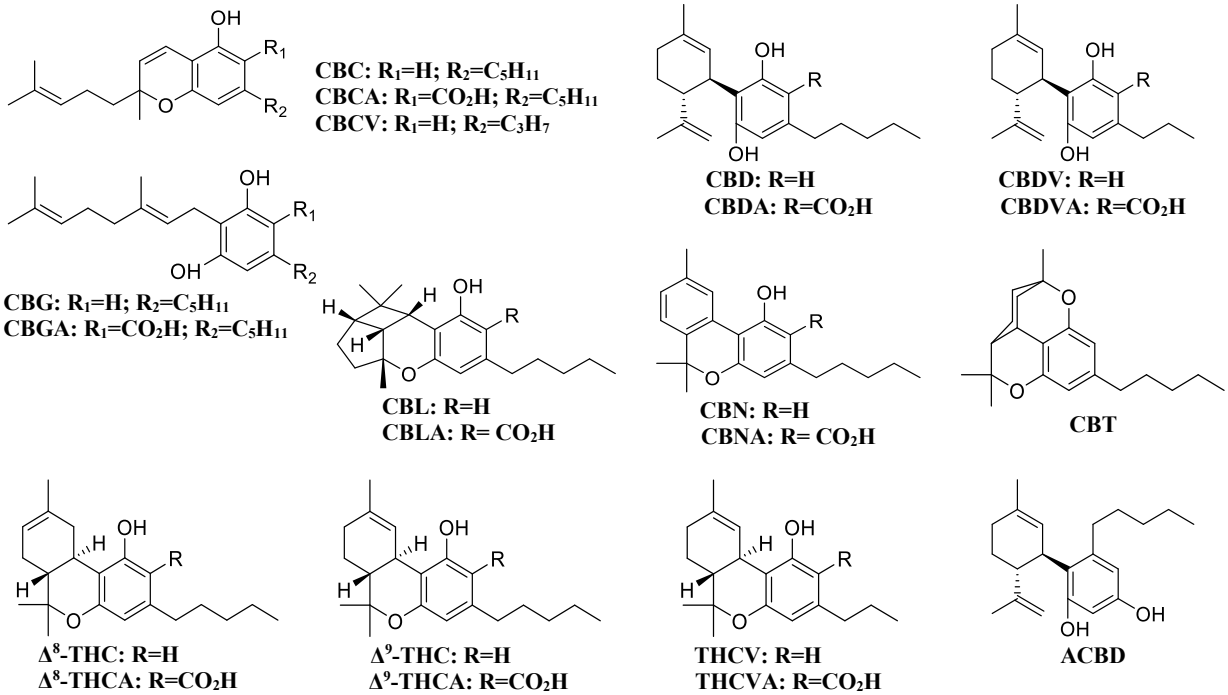
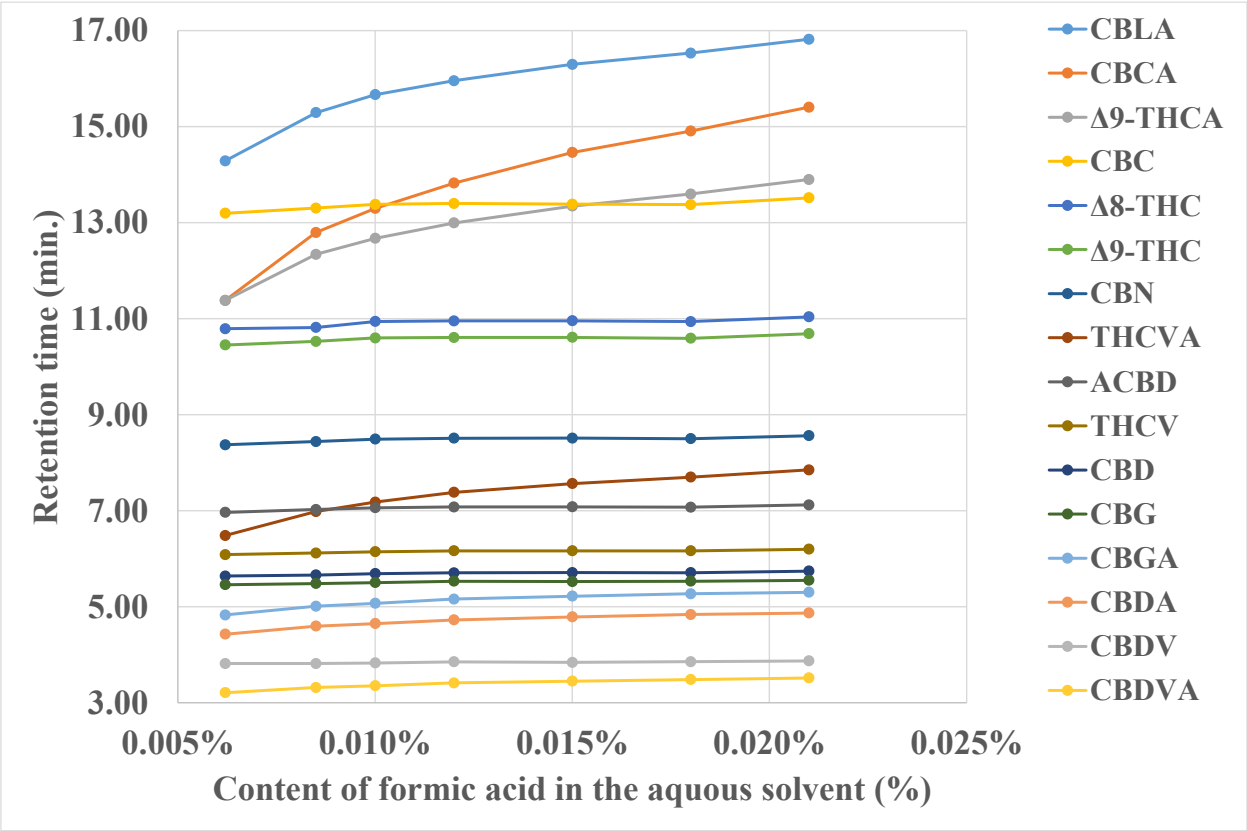


Fig. 2. Effects of (A) content of formic acid in the aqueous solvent containing 1 mM ammonium formate (73% (v/v) acetonitrile in the mobile phase) and (B) content of acetonitrile in the mobile phase (the aqueous solvent containing 1 mM ammonium acetate and 0.021% (v/v) formic acid) on retention time of cannabinoids.



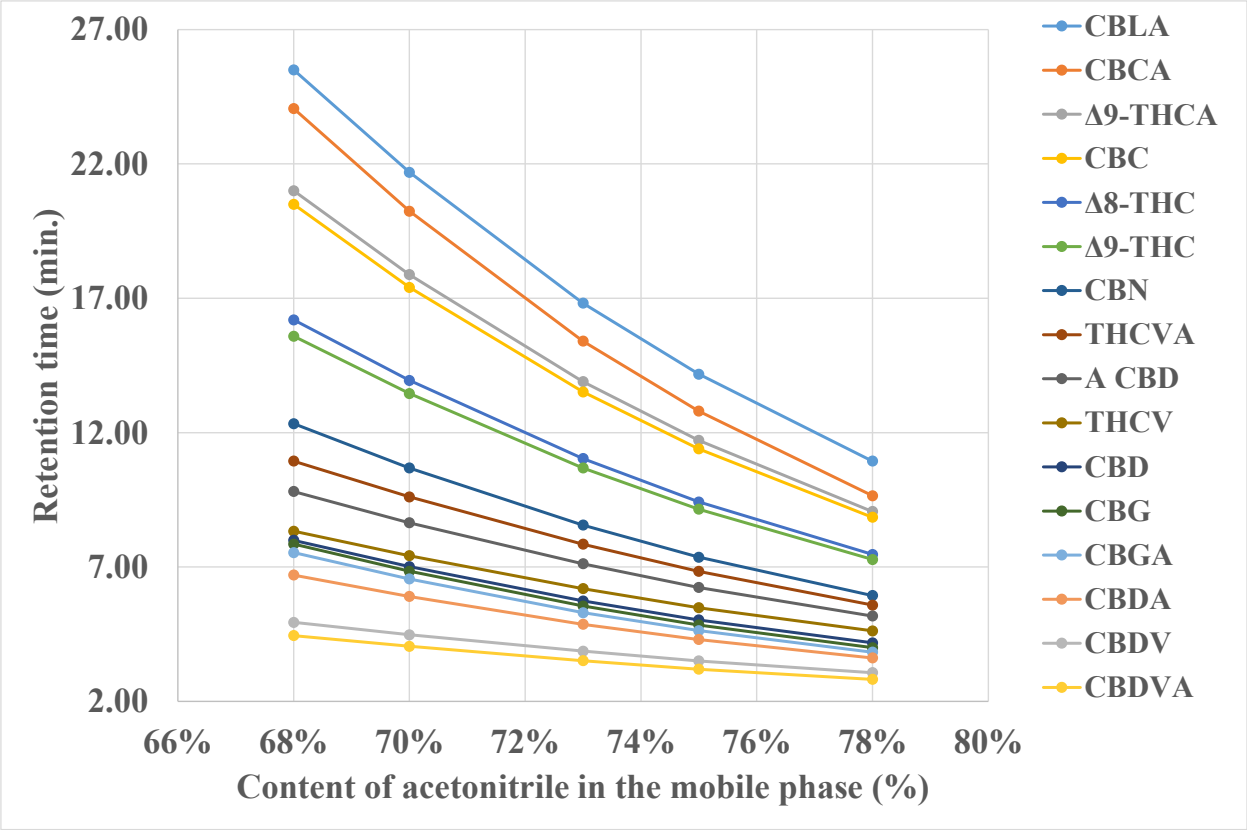

27.00
22.00
17.00
12.00
7.00
2.00
Retention time (min.)
66%
68%
70%
72%
74%
76%
78%
80%
Content of acetonitrile in the mobile phase (%)
CBLA
CBCA
Δ9-THCA
CBC
Δ8-THC
Δ9-THC
CBN
THCVA
A CBD
THCV
CBD
CBG
CBGA
CBDA
CBDV
CBDVA

Fig. 3. LC separation of 1 μg mL^{-1} cannabinoids except ACBD at 0.1 μg mL^{-1} under optimized conditions: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).

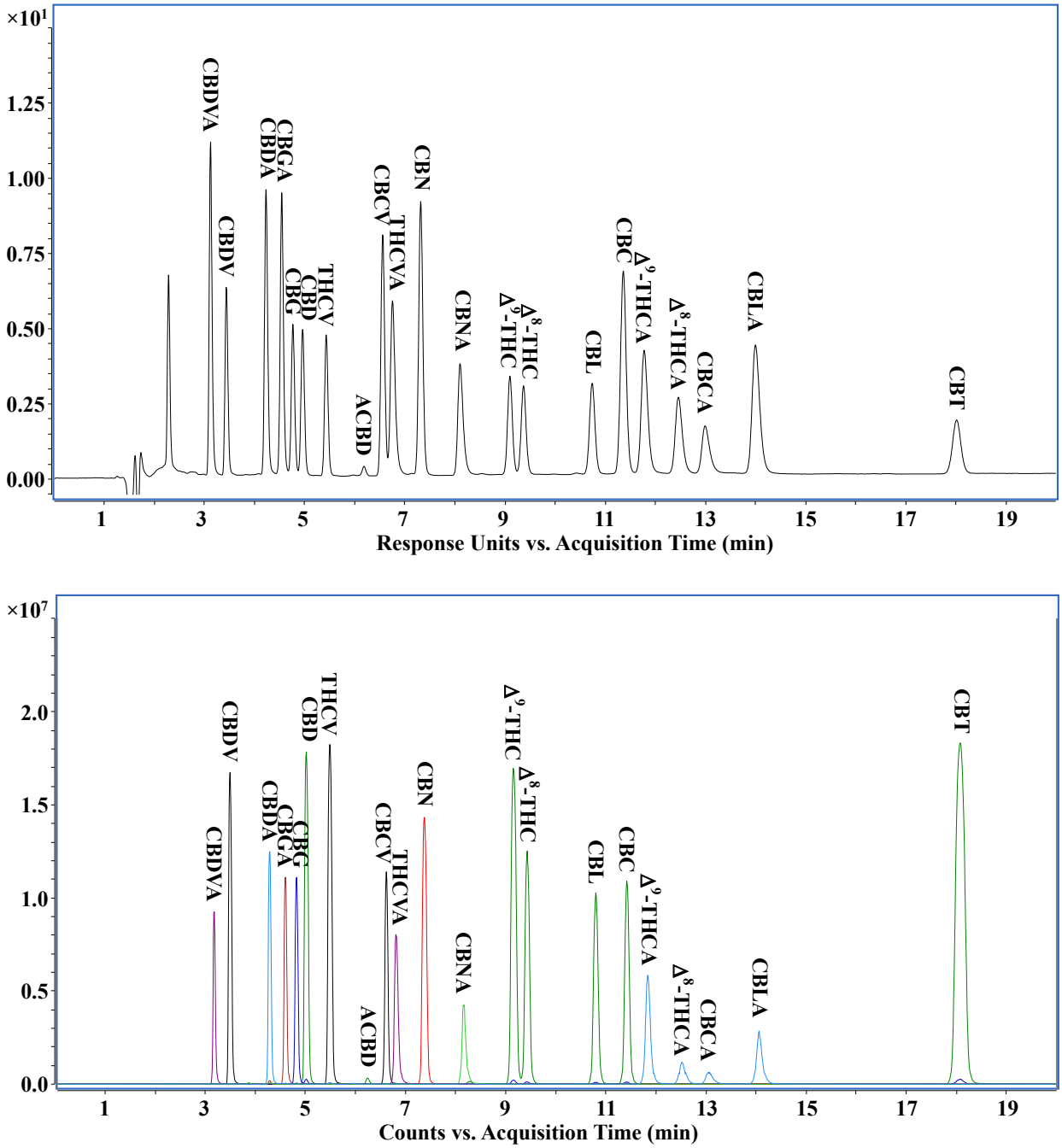


Fig. 4. LC-UV chromatograms of cannabinoids at LOQ level, i.e., 0.02 µg mL^{-1}, except ACBD at 0.5 µg mL^{-1}.

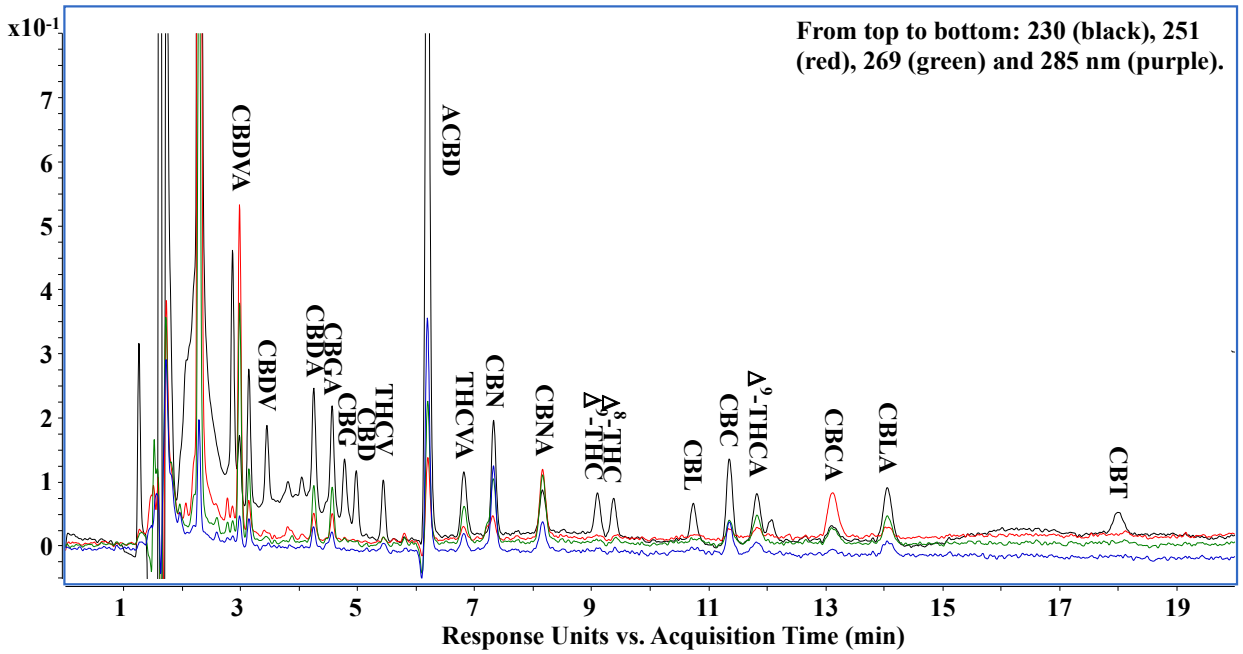


Table 1

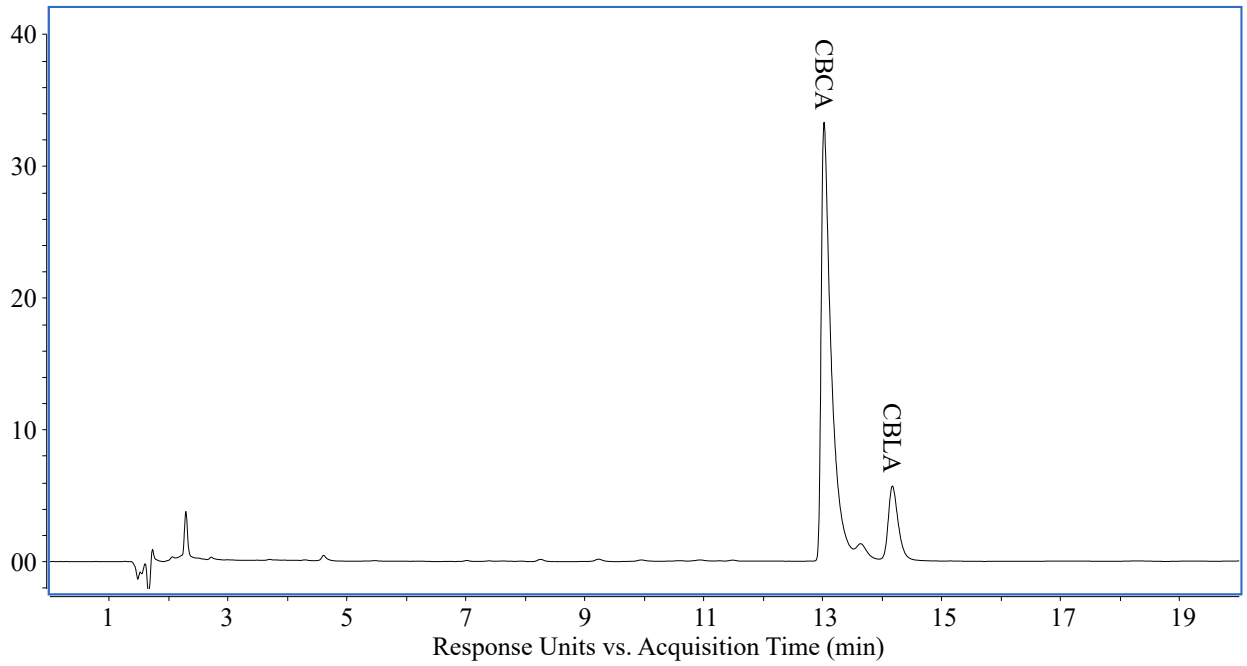
Average content (%, w/w) of cannabinoids in twenty-one samples measured in triplicates (TTHC, total THC; TCBD, total CBD).

Sample	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBC	Δ^9-THCA	CBCA	CBT	Total	TTHC	TCBD
M1	RRT0.50							0.07	0.23				0.84	0.08		1.22	0.74	
M2	RRT0.50			0.31			0.06	0.18	0.34	0.72		0.05	7.92	0.18		9.76	7.66	
M3	0.08		9.87	0.41		0.52			RRT1.33				0.28	0.38		11.53	0.24	9.17
M4	0.04		3.71	0.08		0.33							0.07	0.13		4.36	0.06	3.58
M5			3.12	0.15		0.21			RRT1.33	0.19			1.76	0.13		5.56	1.73	2.95
M6	RRT0.50			0.16				0.11	0.22	0.36			4.21	0.16		5.22	4.05	
M7	RRT0.50		12.00	0.66		0.73	0.05	0.12	RRT1.33	0.69	MP2	0.07	6.37	0.54		21.23	6.28	11.25
M8	RRT0.50		4.23	0.13		0.71		0.07	RRT1.33	0.52		0.04	2.11	0.19		7.99	2.37	4.42
MC1				0.05				0.04	0.06	0.26			1.67	0.22		2.30	1.73	
MC2				0.09				0.07	0.12	0.36			2.88	0.16		3.68	2.88	
MC3	RRT0.50			0.13			0.04	0.10	0.19	0.45			4.74	0.19		5.84	4.61	
MC4			0.08	0.11			0.06	0.07	0.08	0.46			2.39	0.12		3.38	2.56	0.07
MC5	RRT0.50		0.19	0.37			0.08	0.11	0.19	0.51			6.12	0.16		7.74	5.88	0.17
H1	0.05	RRT0.54	7.77	0.13	0.05	1.74			RRT1.33	0.21		0.15	0.12	0.33		10.55	0.31	8.56
H2	0.06	RRT0.54	14.47	0.19	0.06	2.44			RRT1.33	0.32		0.59	0.22	1.56	0.11	20.07	0.52	15.13
H3		RRT0.54	7.44	0.07		3.52			RRT1.33	0.31		0.17	0.12	0.26	0.06	11.93	0.41	10.04
H4			6.80	0.06		1.00			RRT1.33	0.13		0.07	0.14	0.25		8.46	0.25	6.97
H5			3.02	10.66	0.54	0.50				0.15		0.21	0.12	0.33	0.06	15.59	0.26	3.15
HC1			1.77	3.96	0.91	2.70				0.14		0.27		0.10	0.11	9.96	0.14	4.26
Δ^8-H1		MP1		7.81	0.72					0.36	4.67	0.18		0.15		13.89	0.36	
Δ^8-H2		RRT0.54	3.85			26.97		0.17	RRT1.33	5.16	12.13	RRT1.82	0.07	0.15	0.18	48.81	5.22	30.34

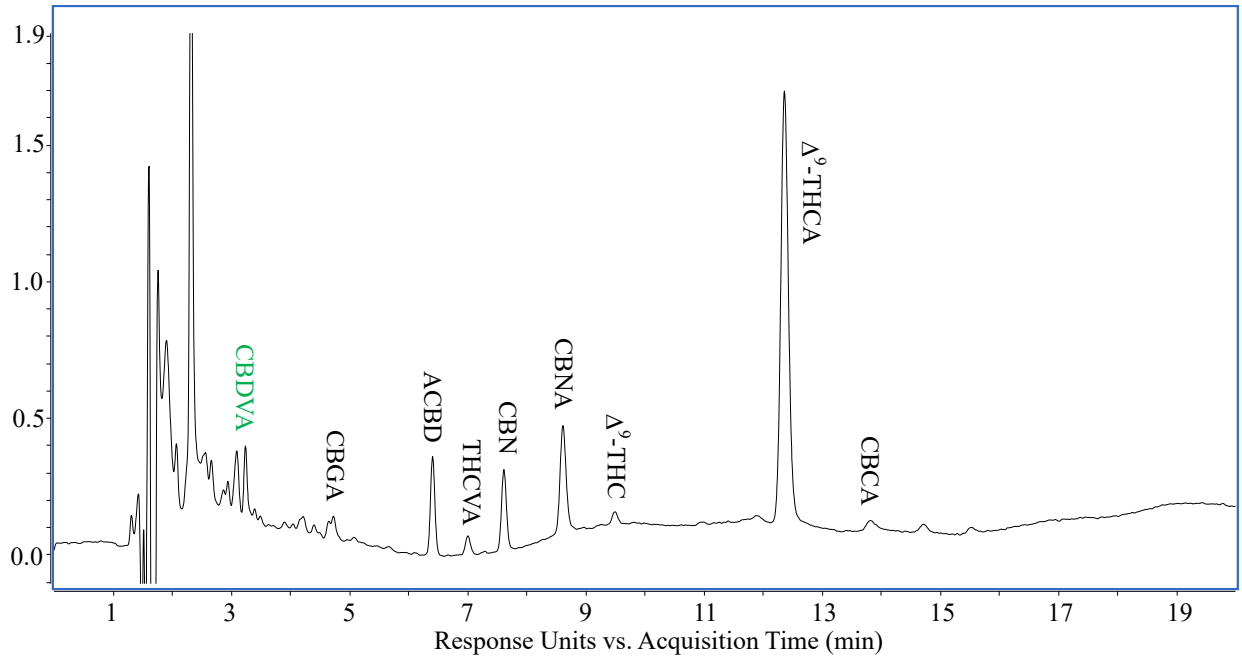
THCV and CBL were only detected in Δ^8-H2, both at 0.06% (w/w), while CBLA was only detected in H2 at 0.06% (w/w).

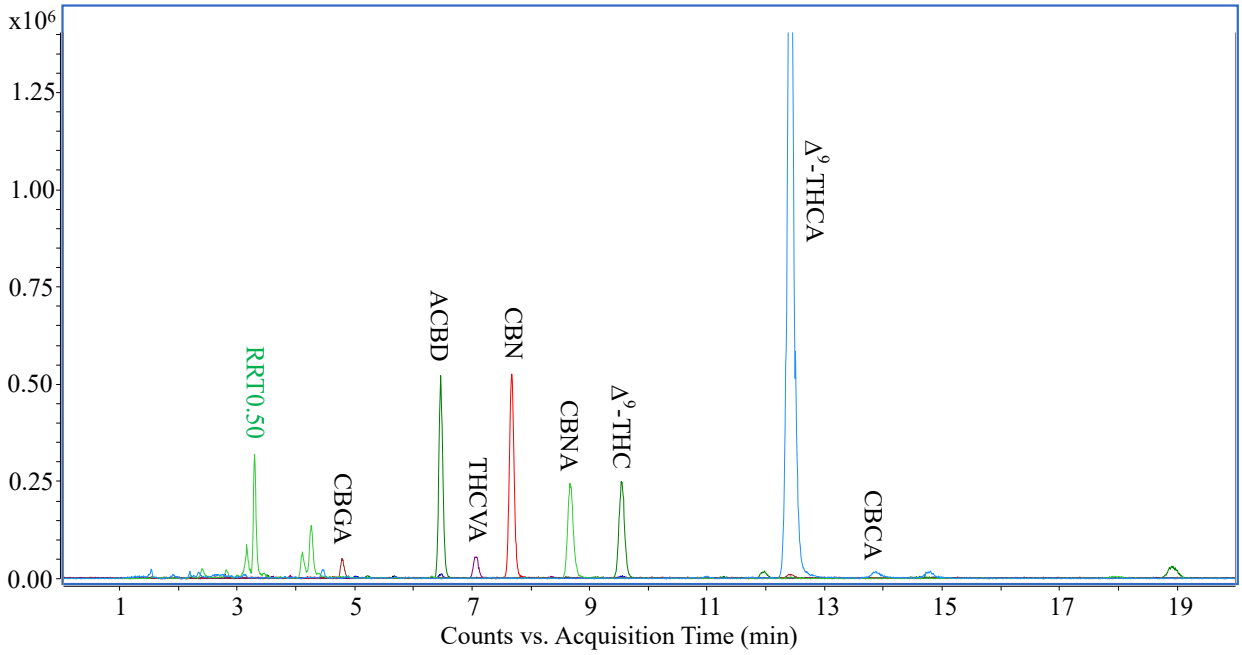
Please refer to the texts for the abbreviations reported in green, light red and dark red.

Supplementary Figure S1. LC-UV chromatogram at 230 nm of 25 µg/mL CBCA.

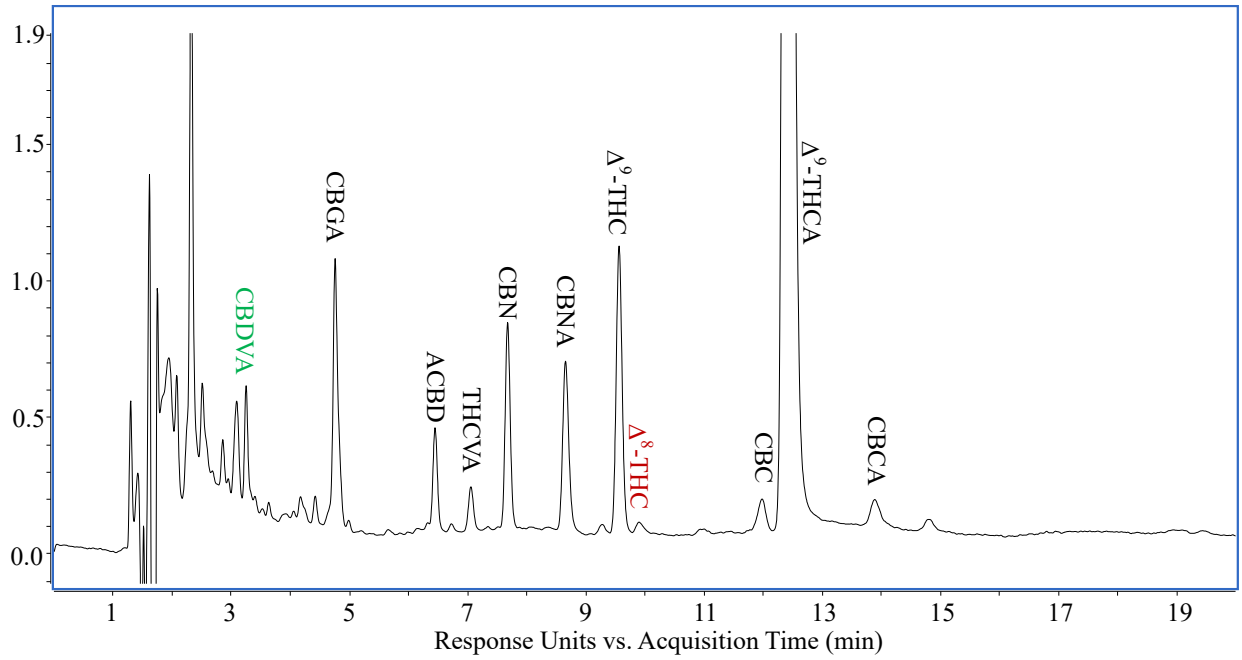


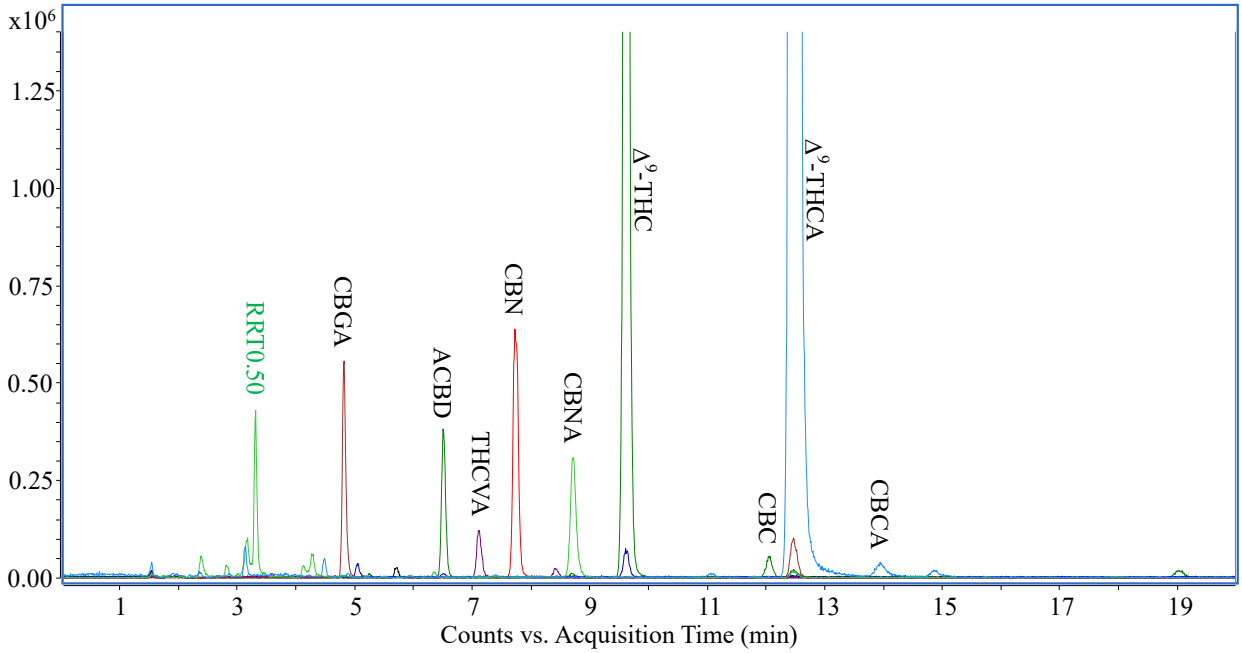
Supplementary Figure S2. M1: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



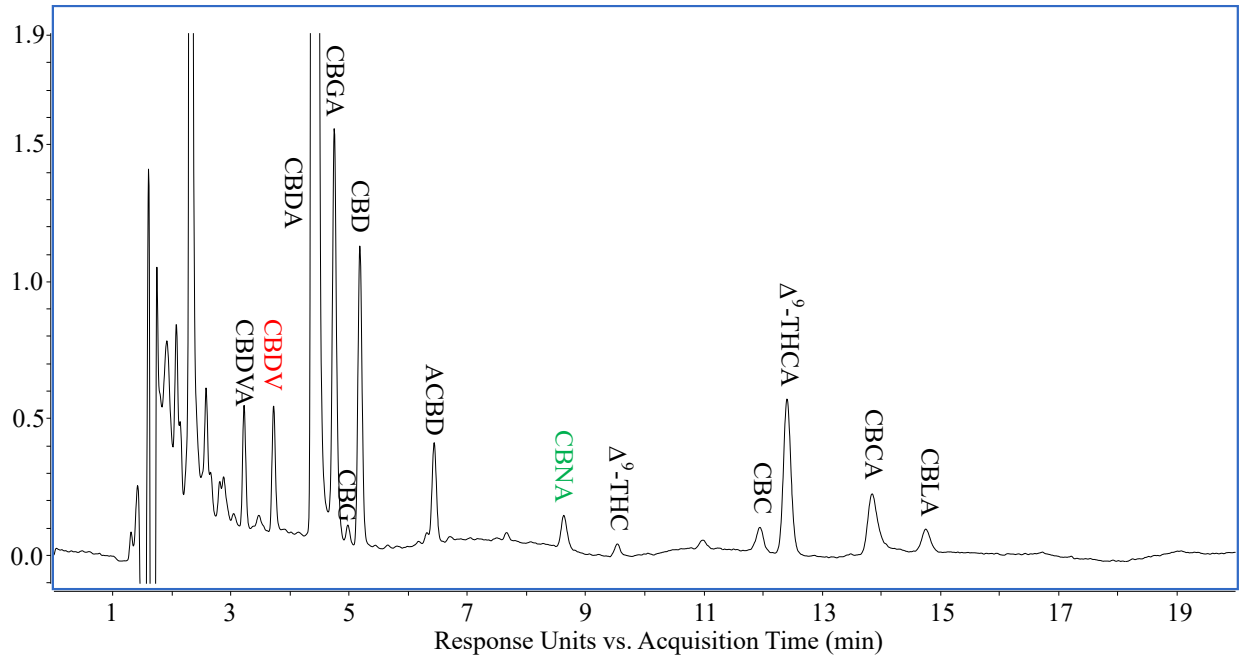


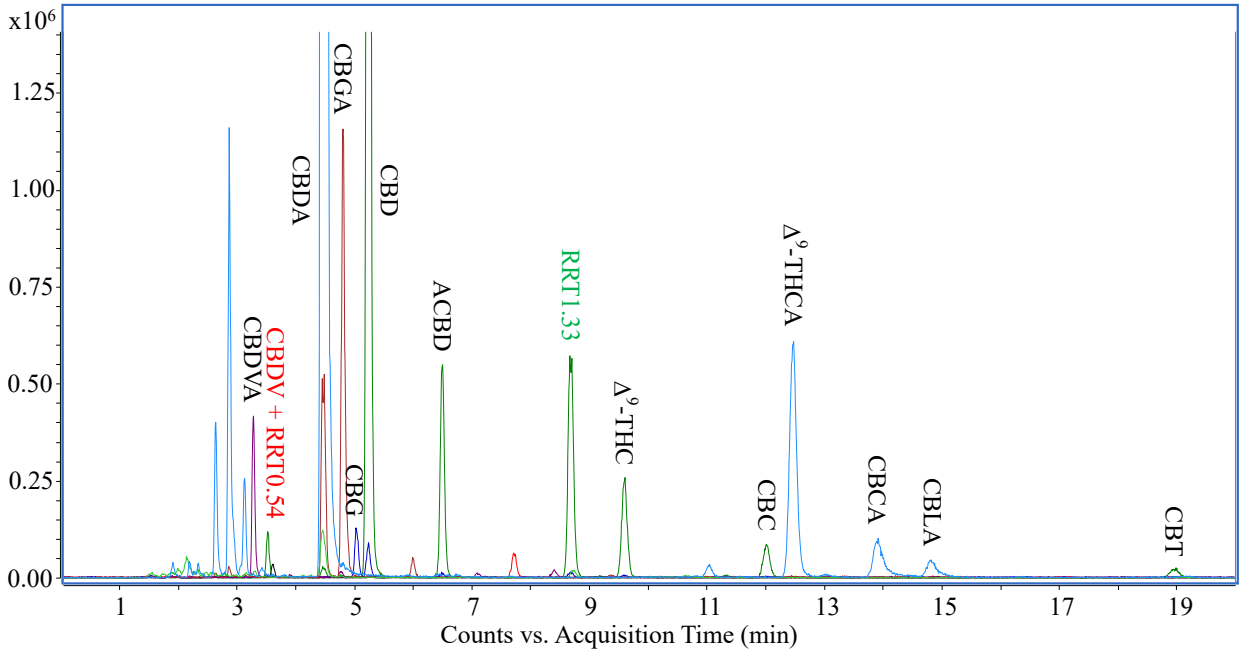
Supplementary Figure S3. M2: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



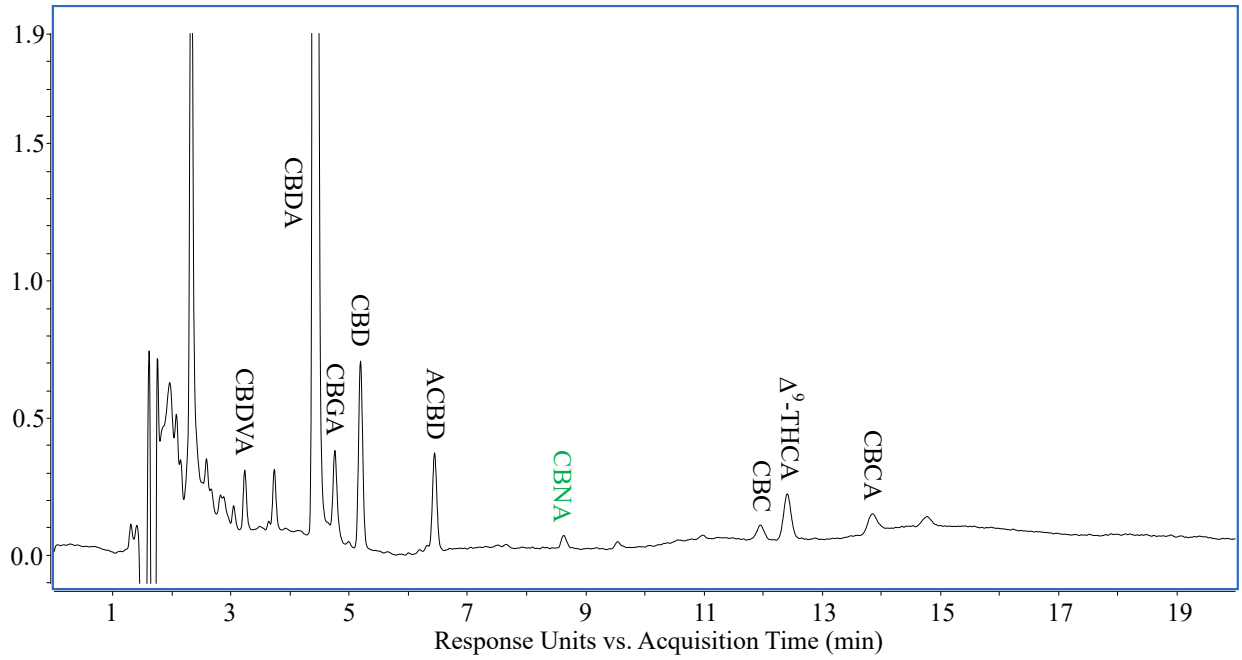


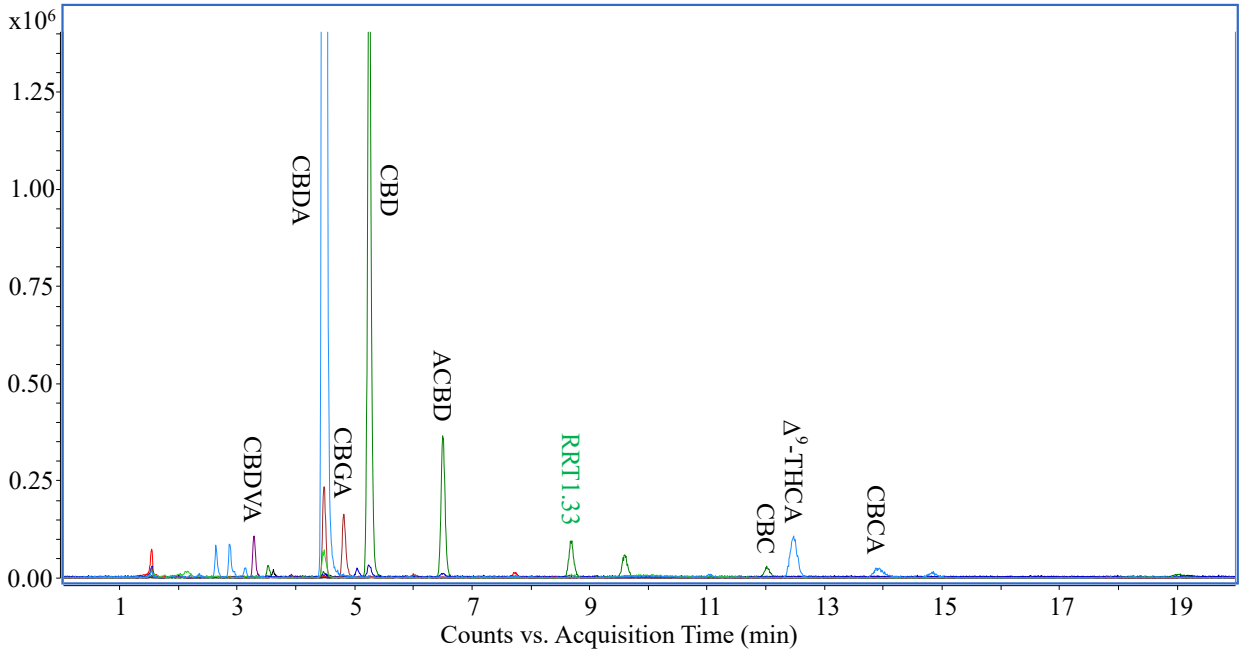
Supplementary Figure S4. M3: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



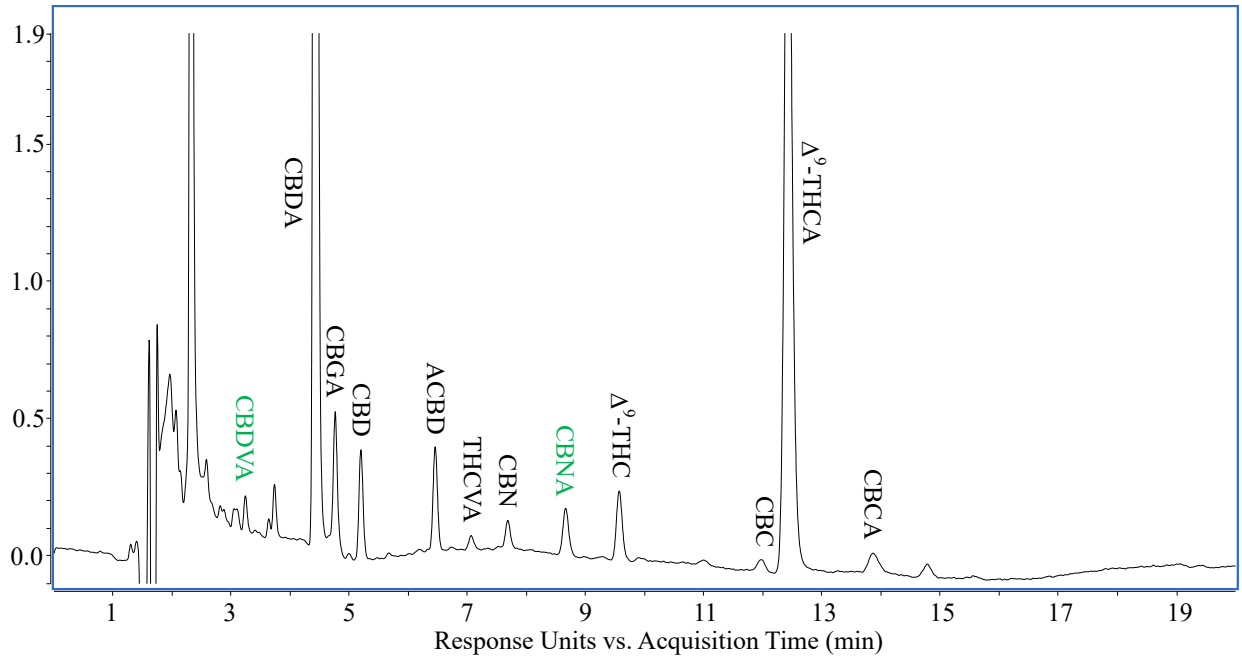


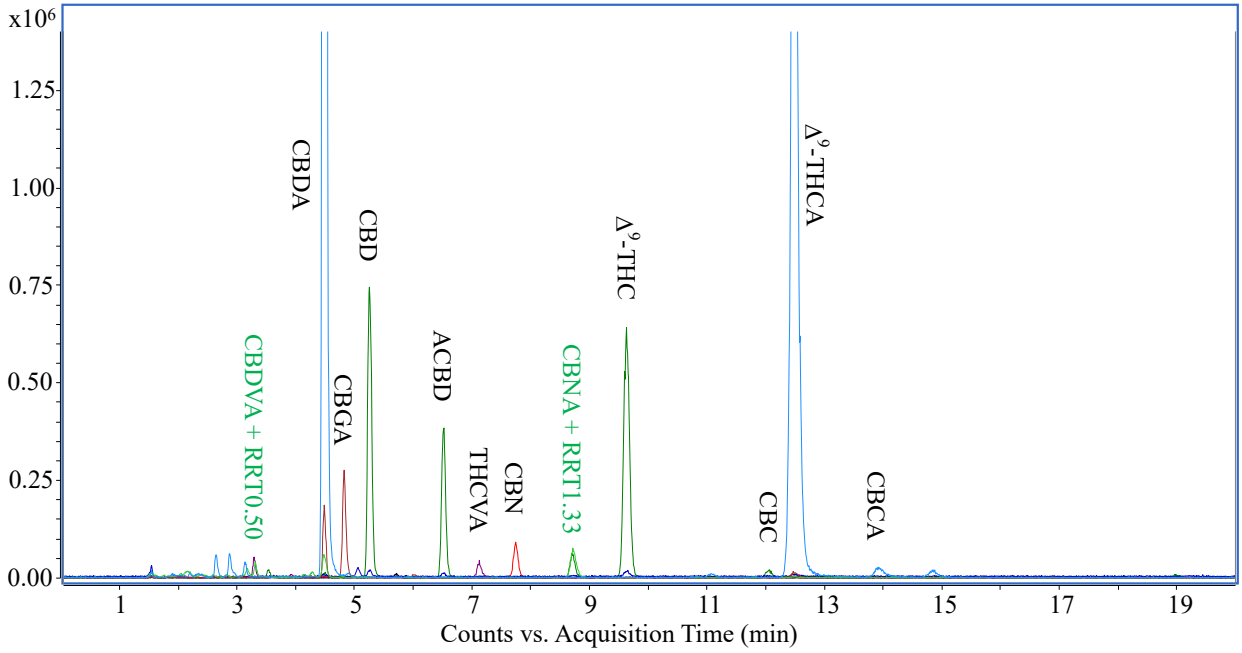
Supplementary Figure S5. M4: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



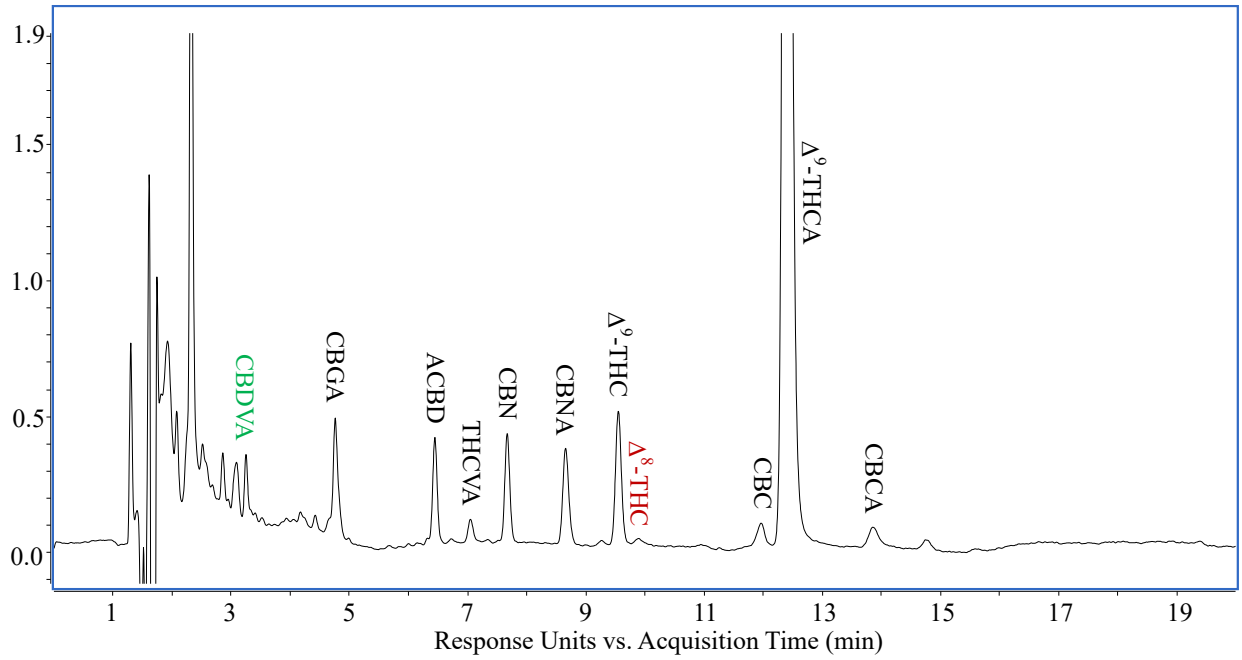


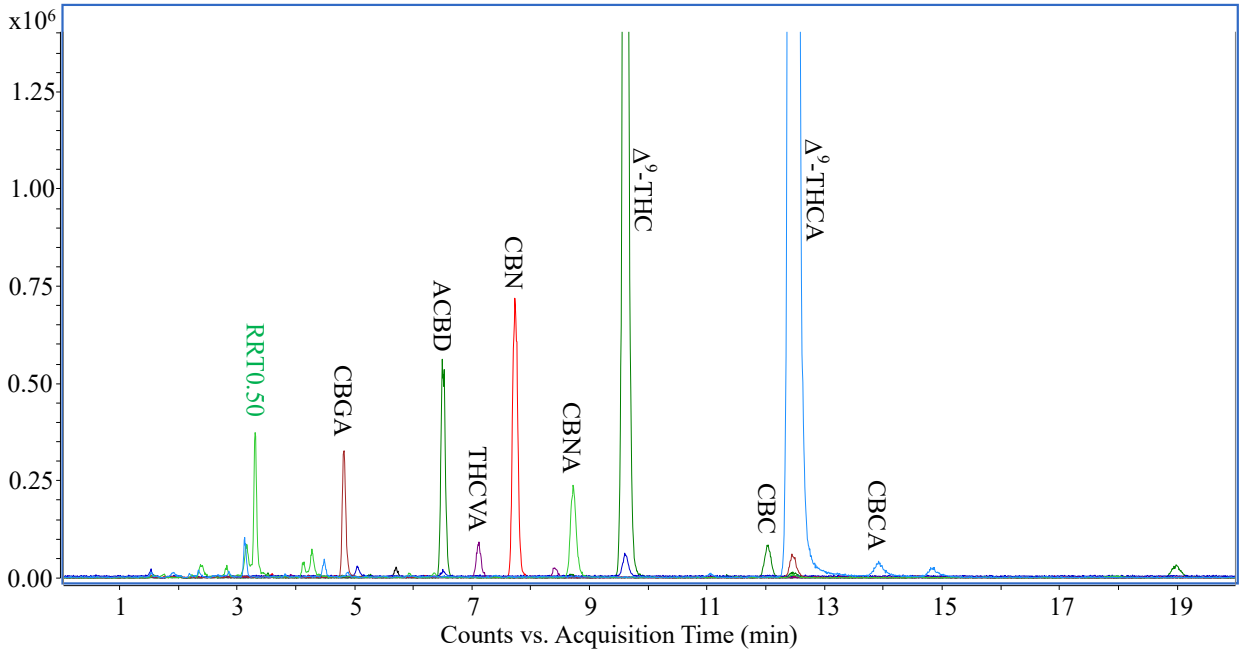
Supplementary Figure S6. M5: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



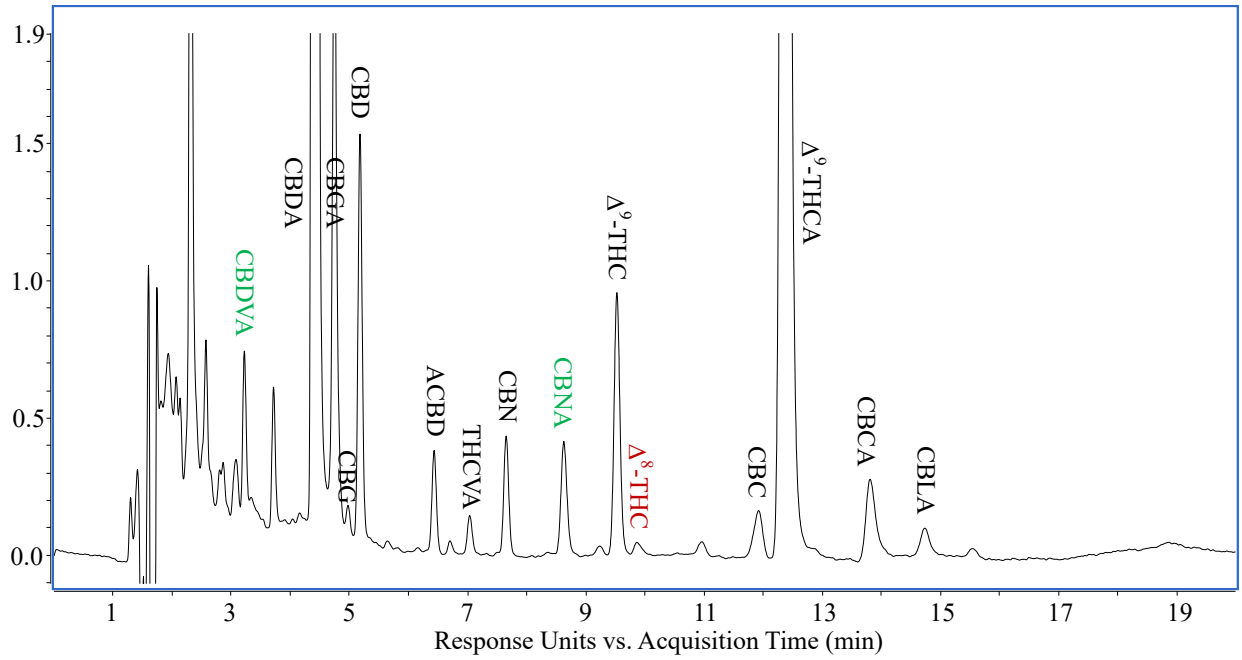


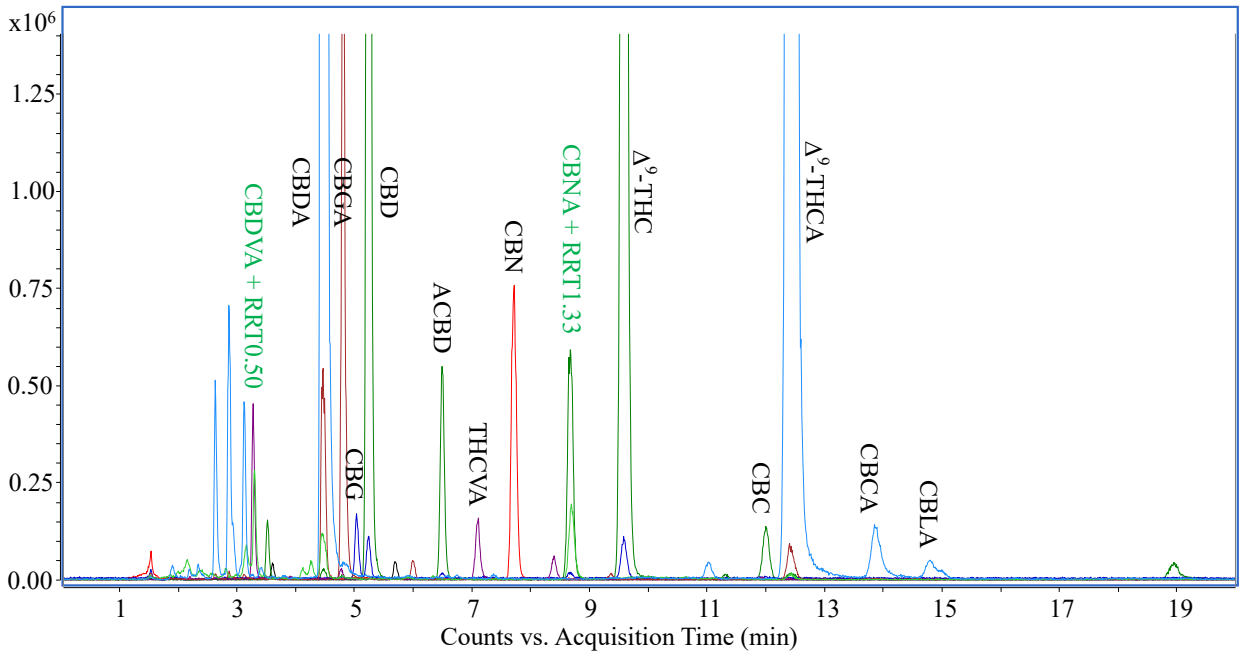
Supplementary Figure S7. M6: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



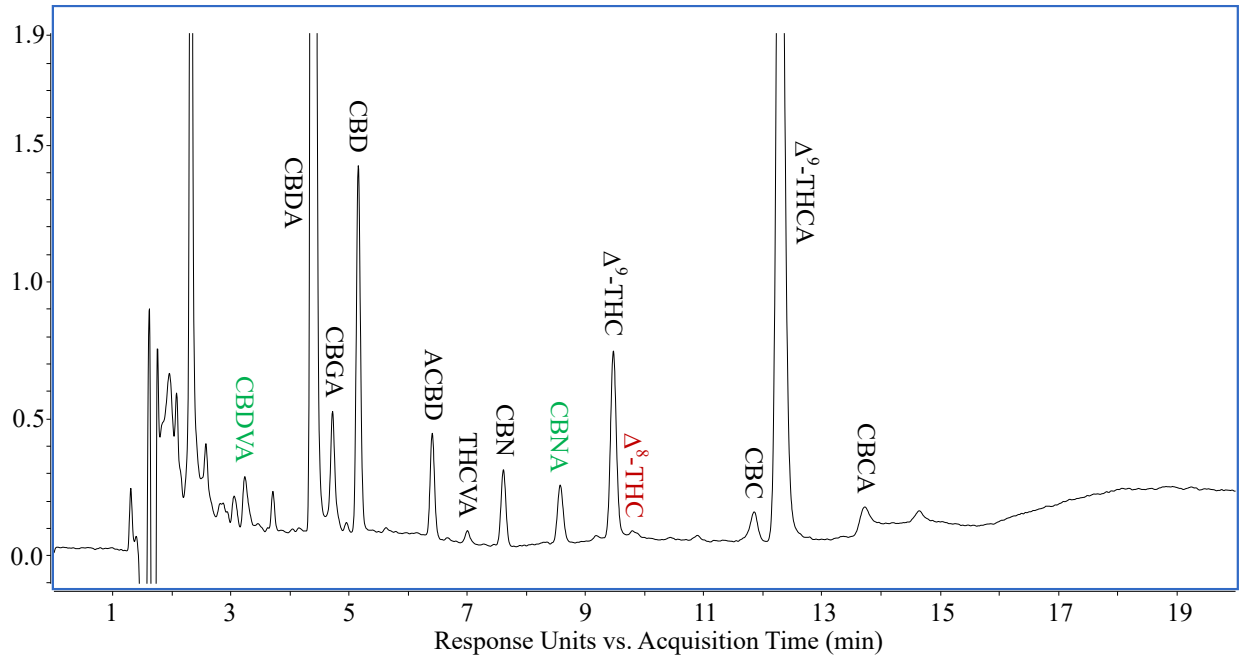


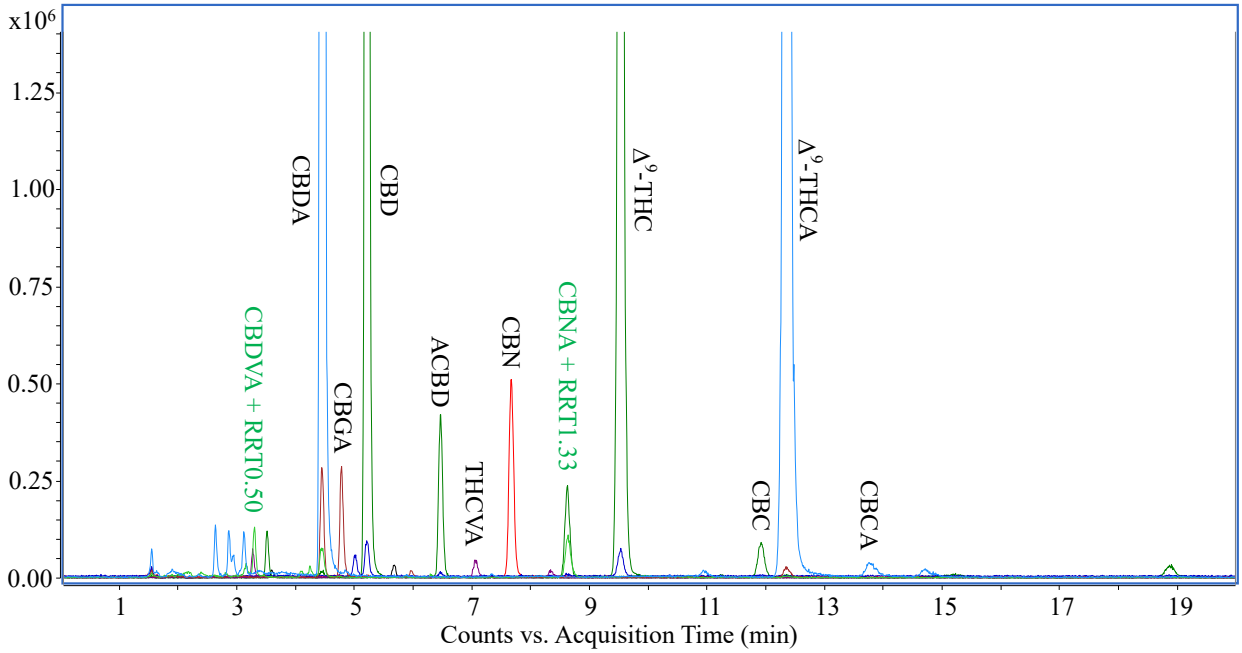
Supplementary Figure S8. M7: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



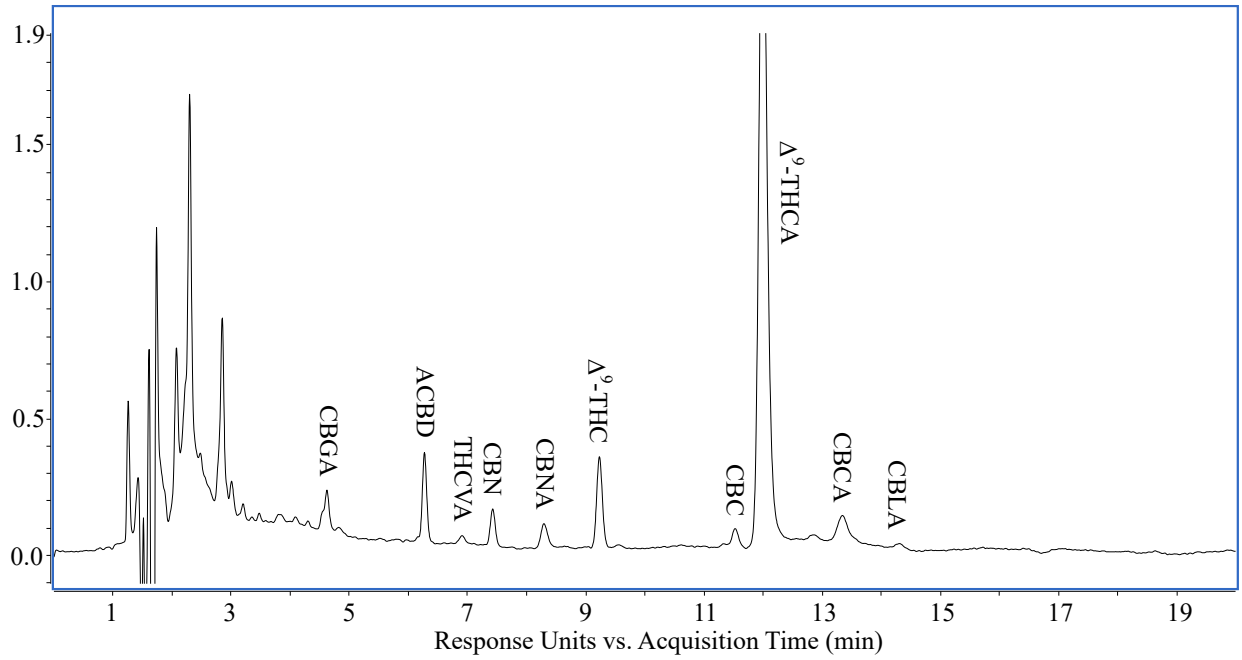


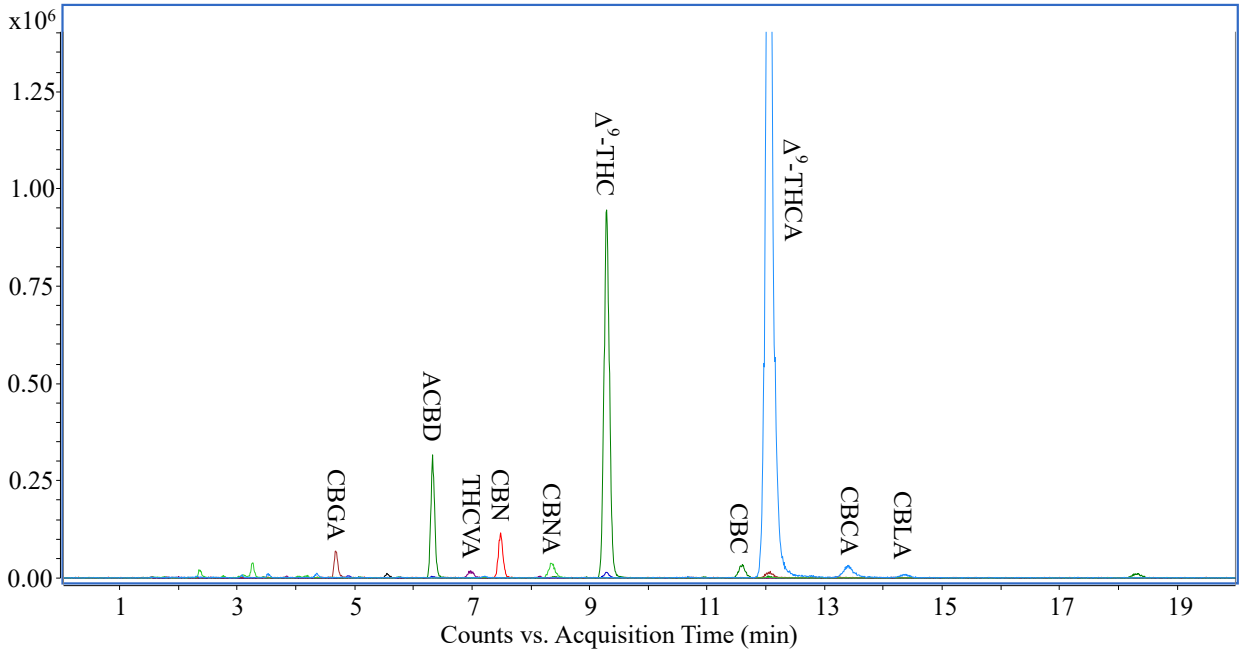
Supplementary Figure S9. M8: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



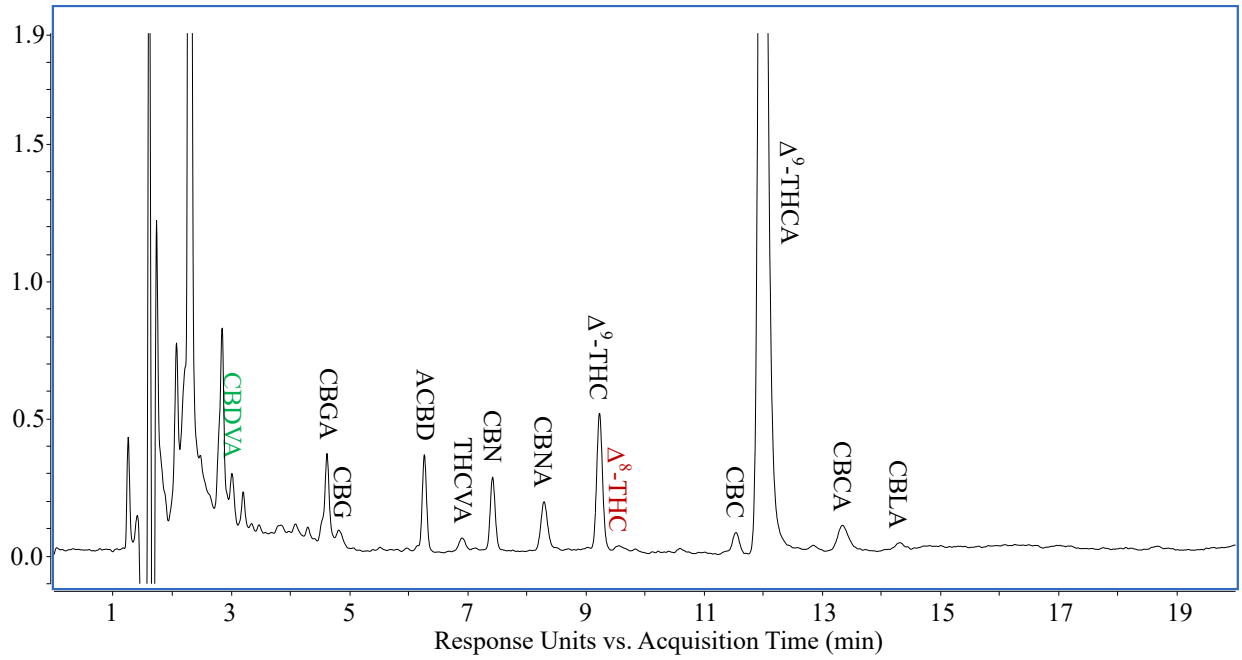


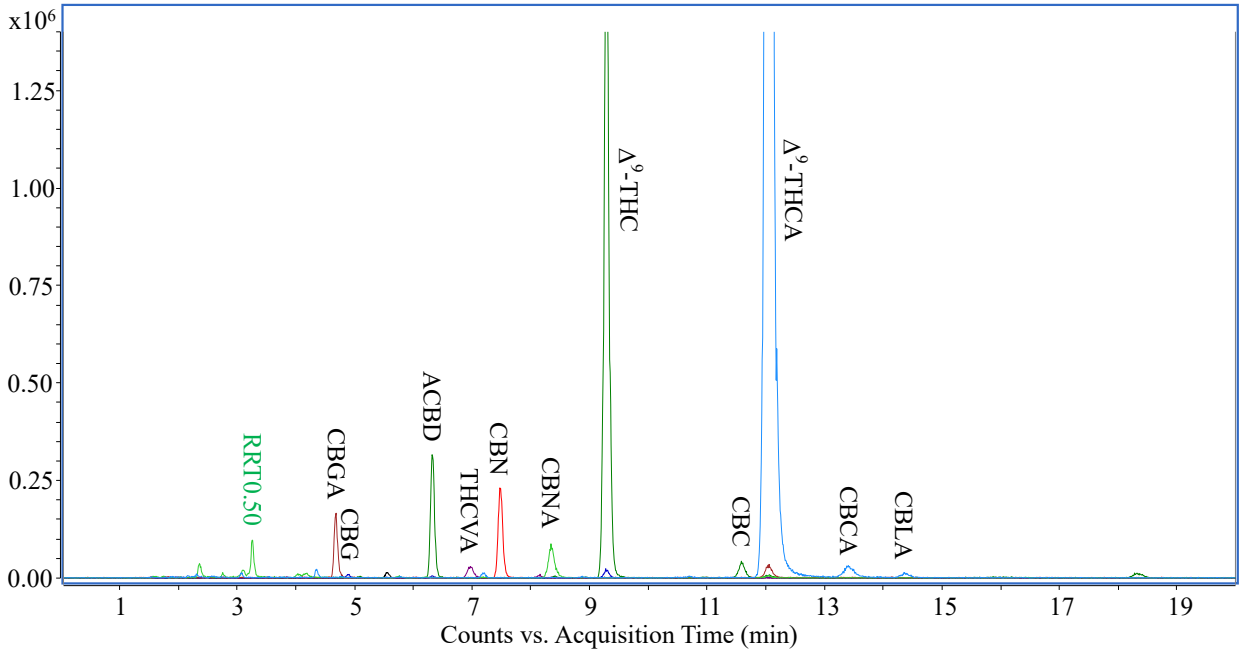
Supplementary Figure S10. MC1: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



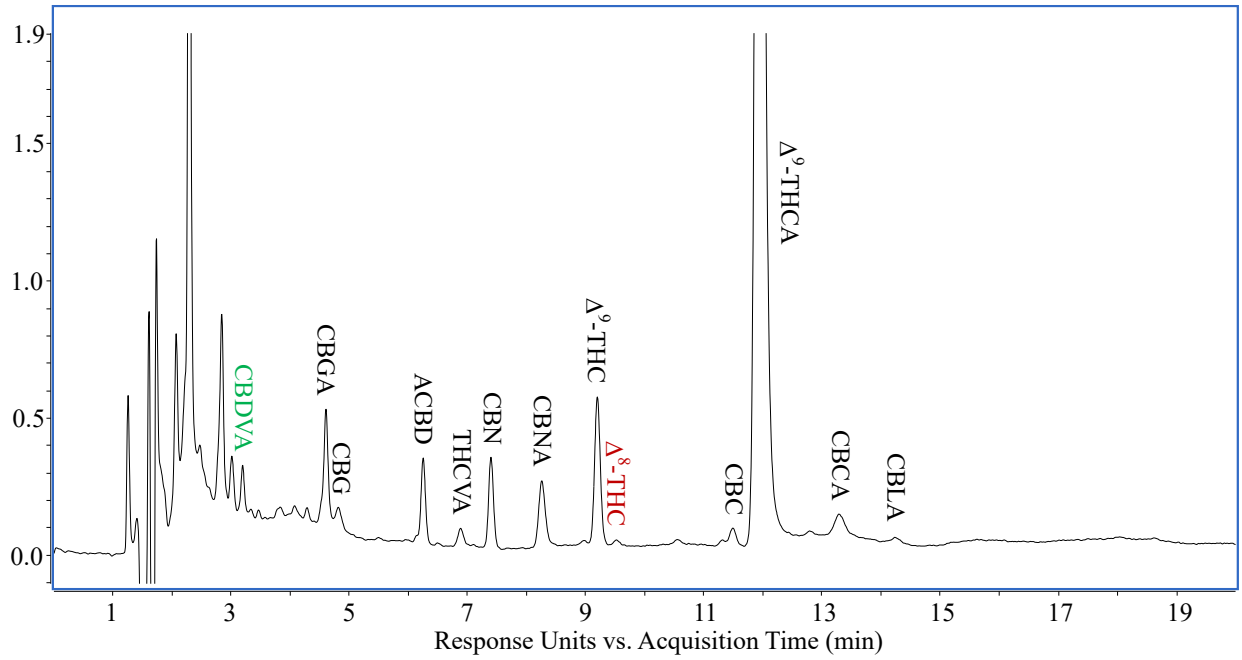


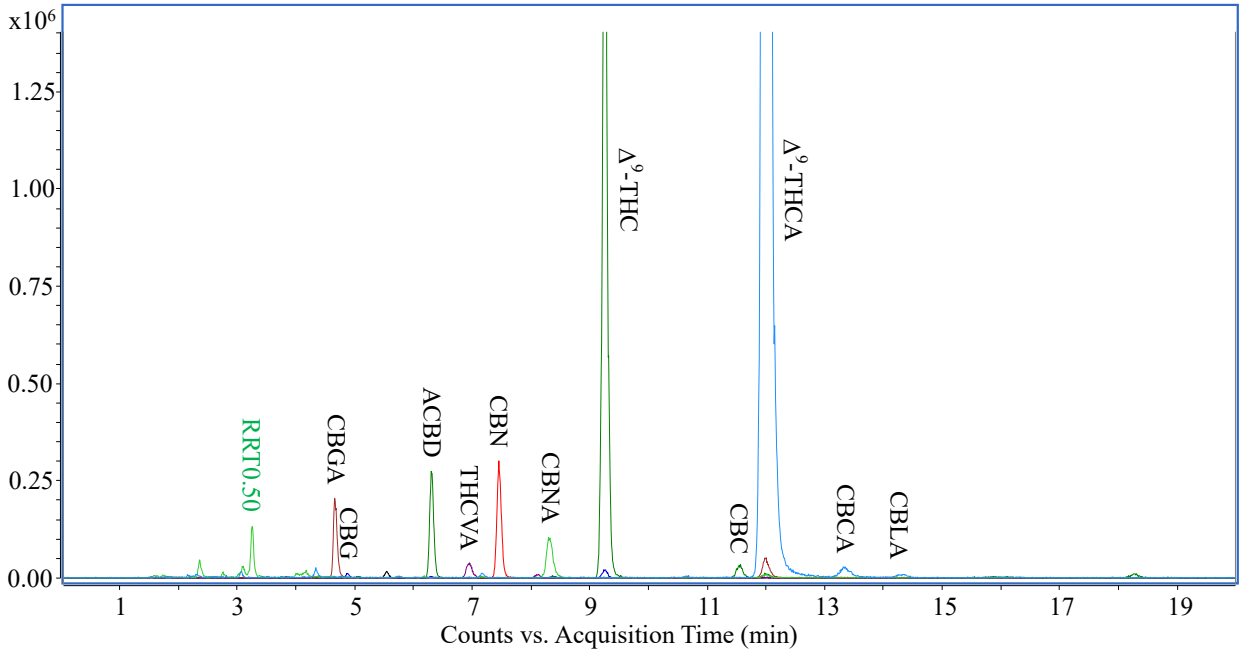
Supplementary Figure S11. MC2: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



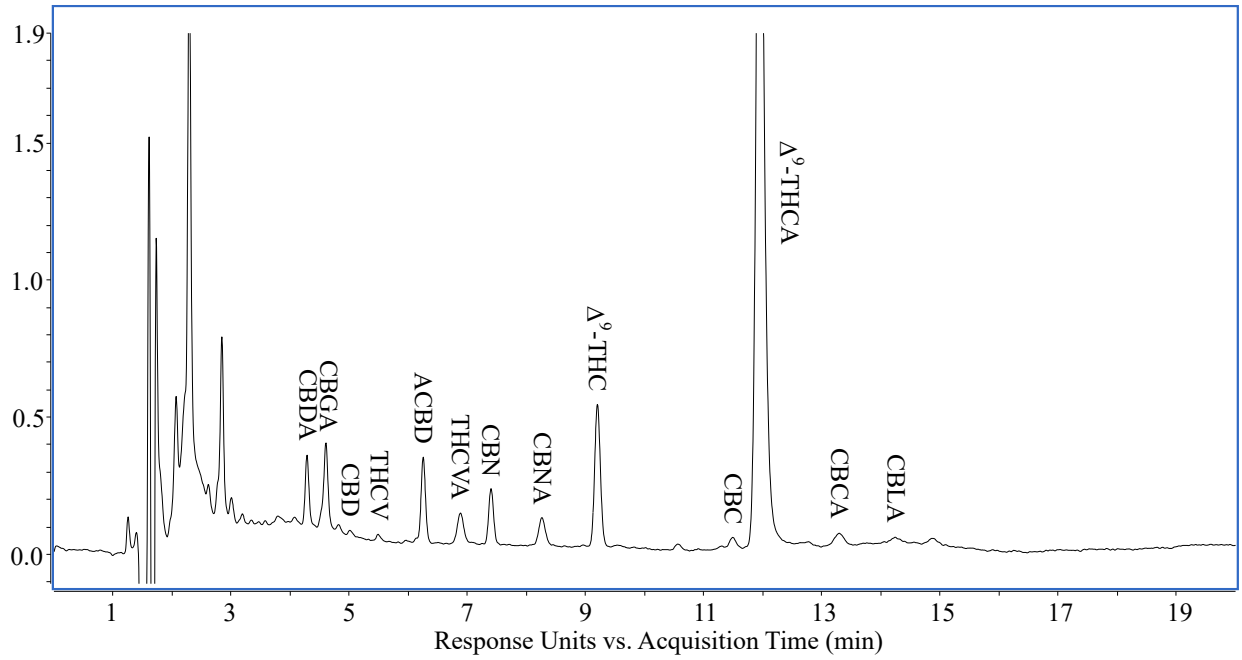


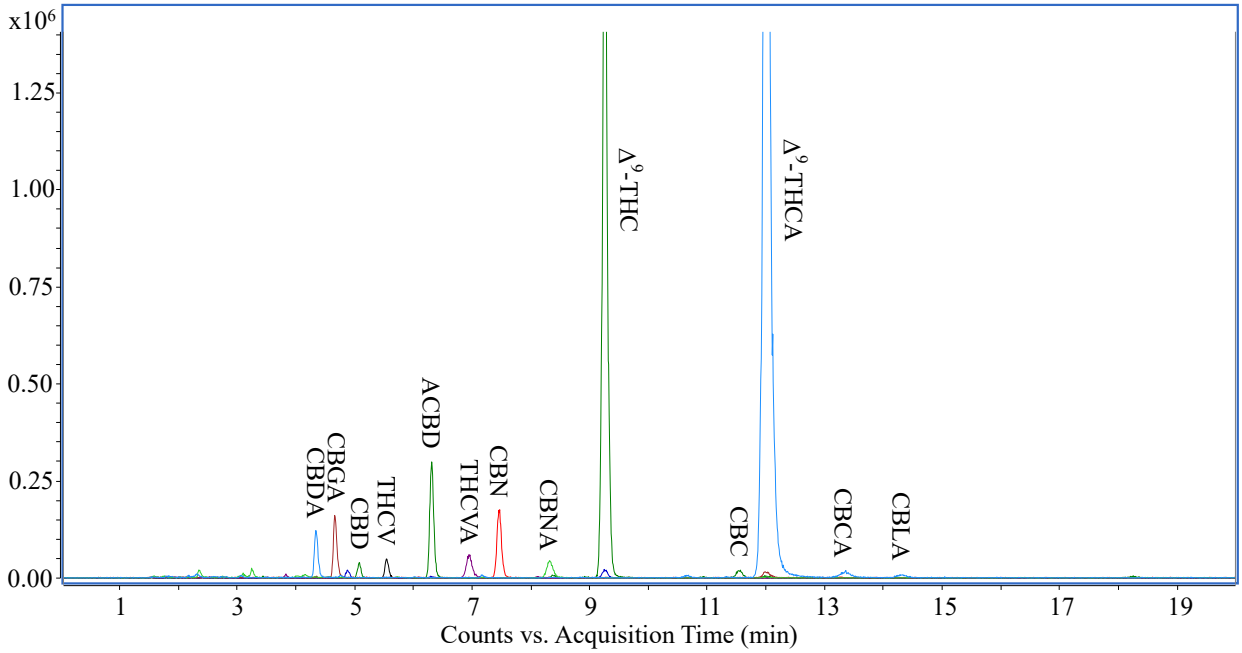
Supplementary Figure S12. MC3: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



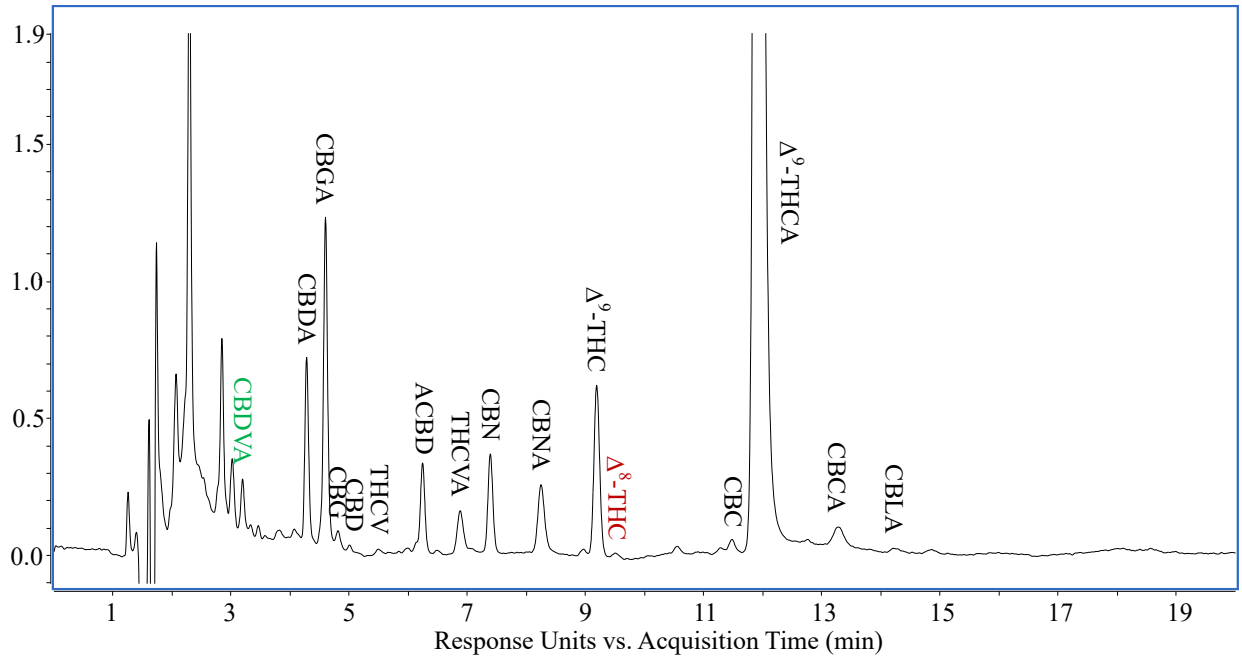


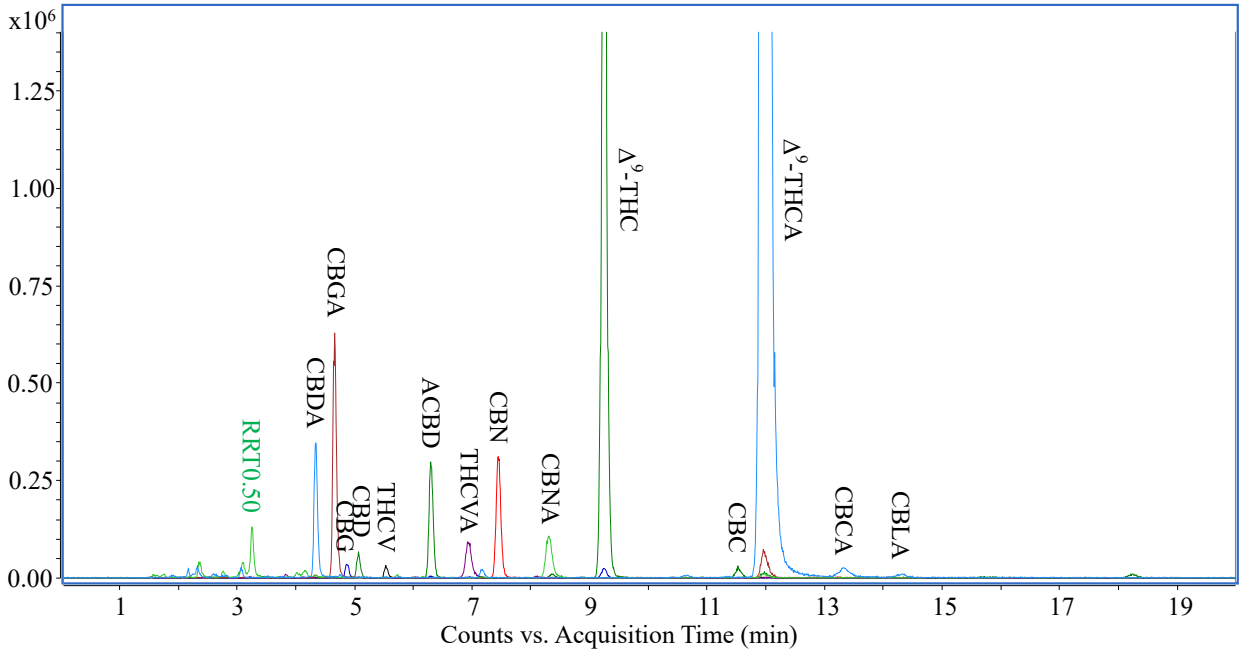
Supplementary Figure S13. MC4: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



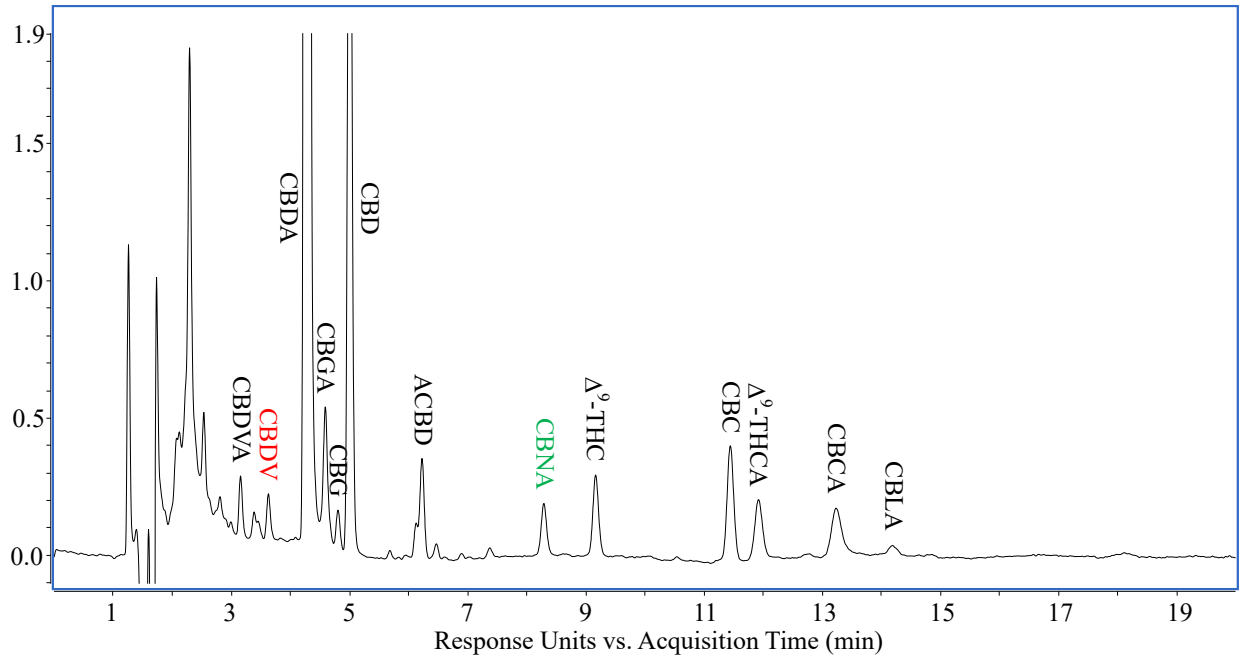


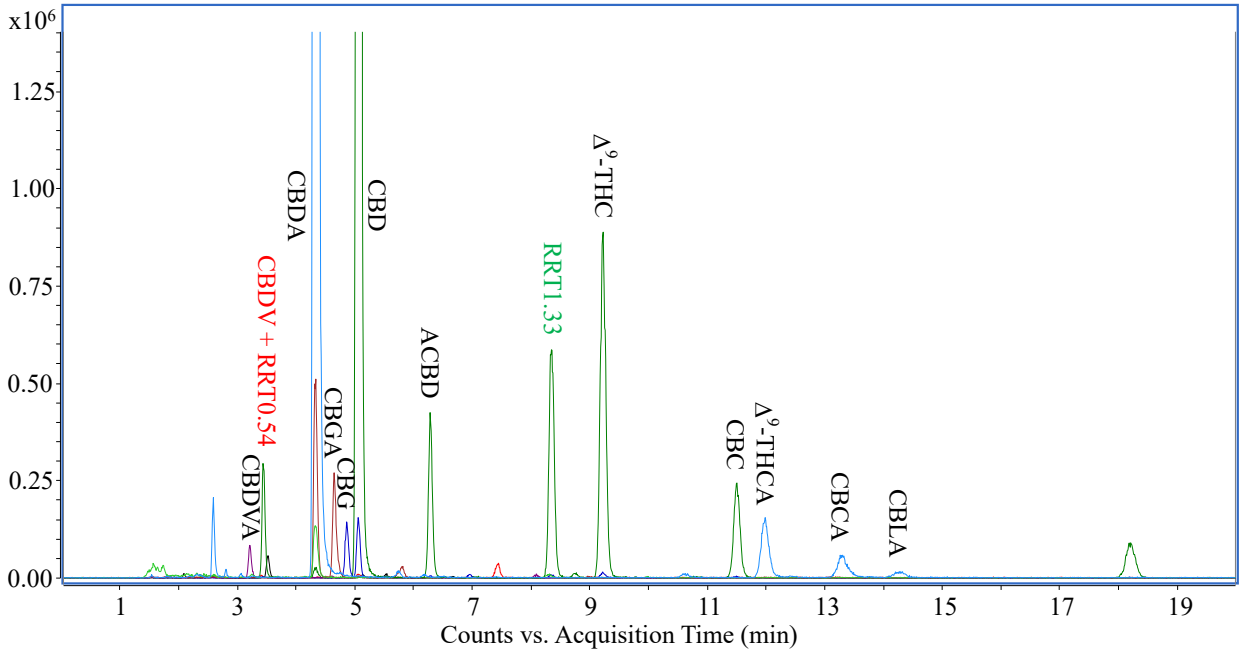
Supplementary Figure S14. MC5: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



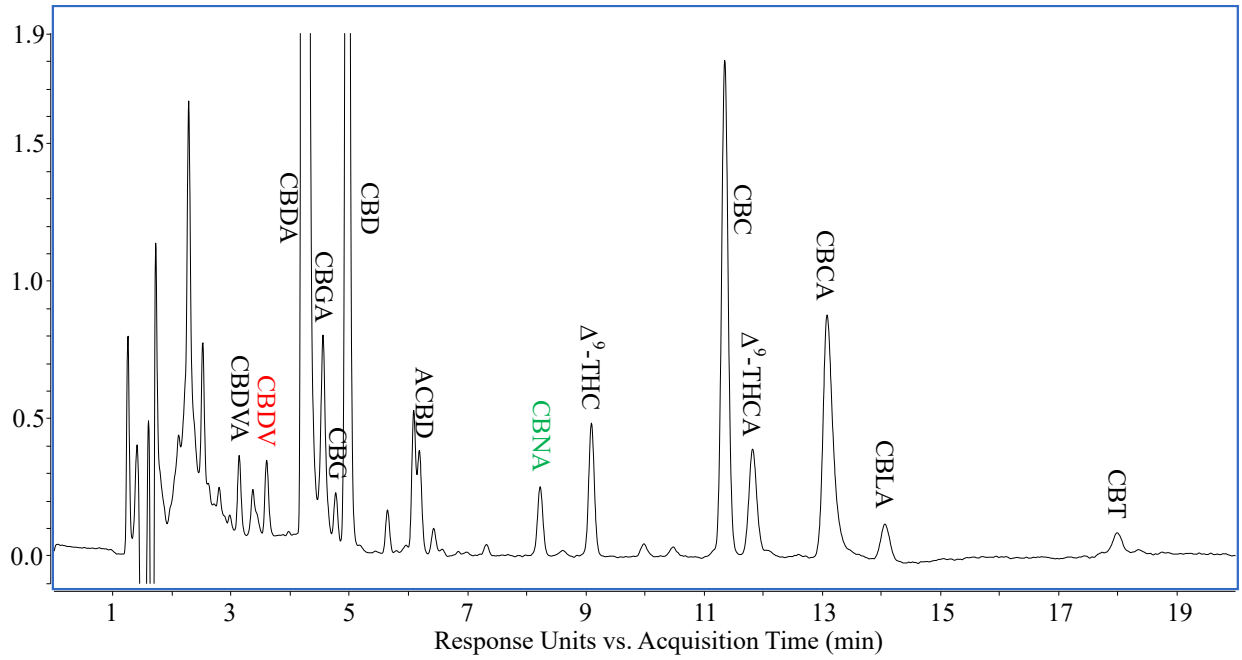


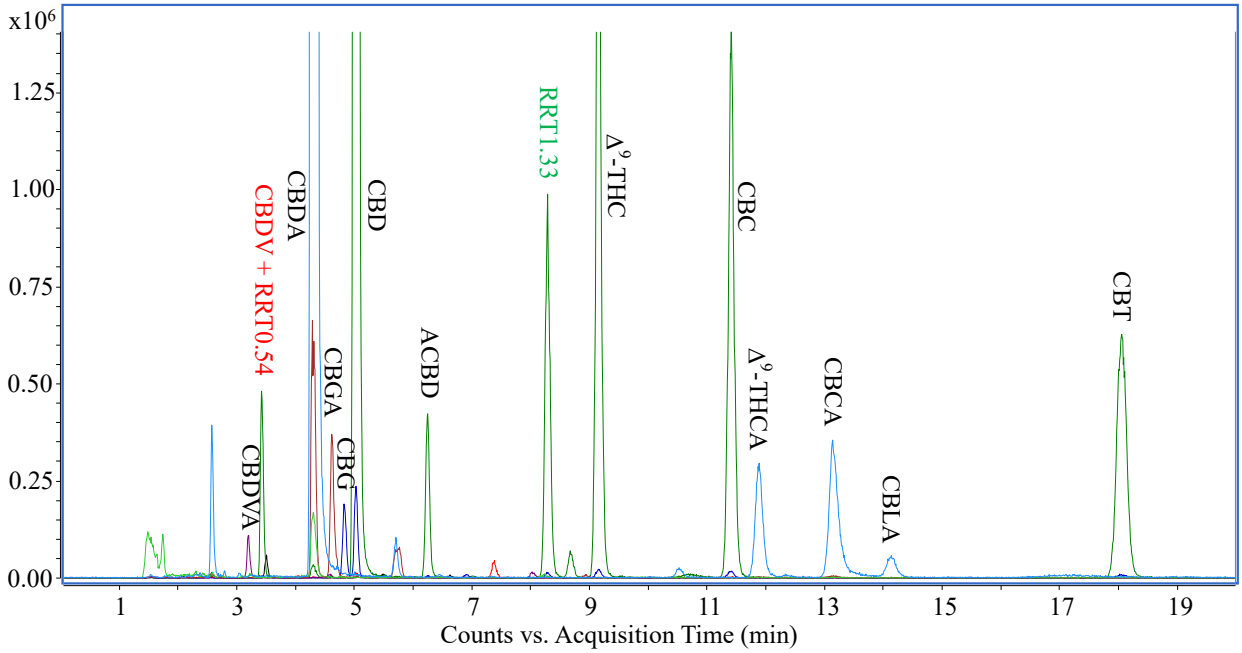
Supplementary Figure S15. H1: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



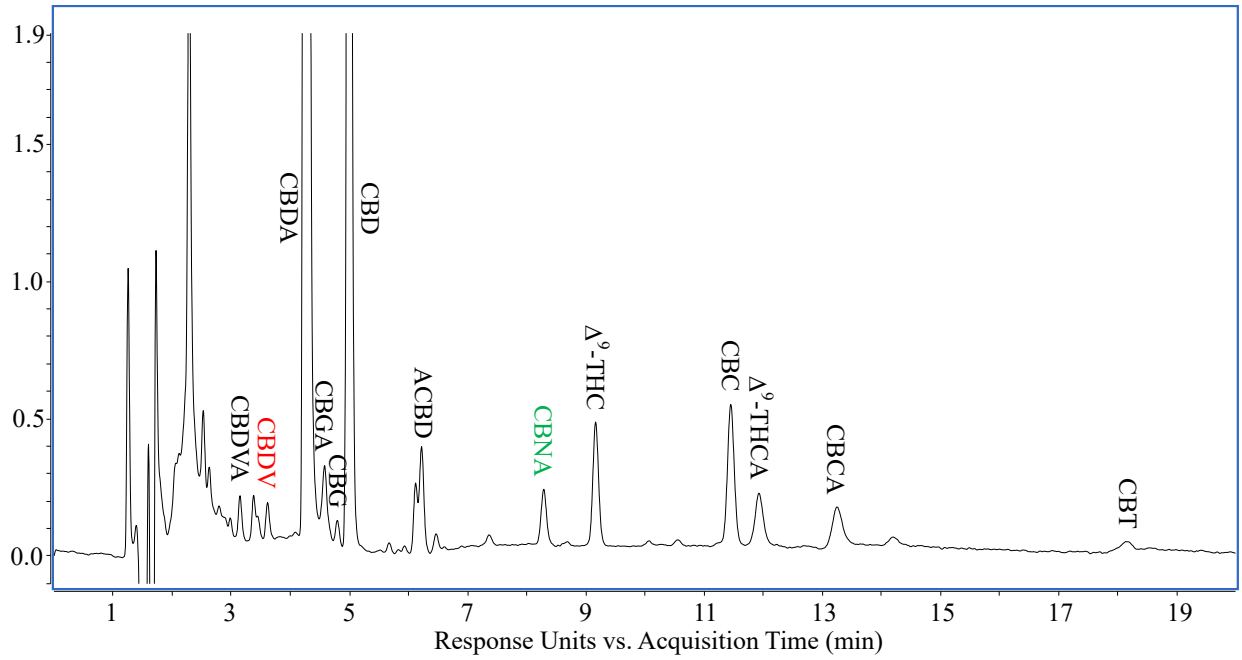


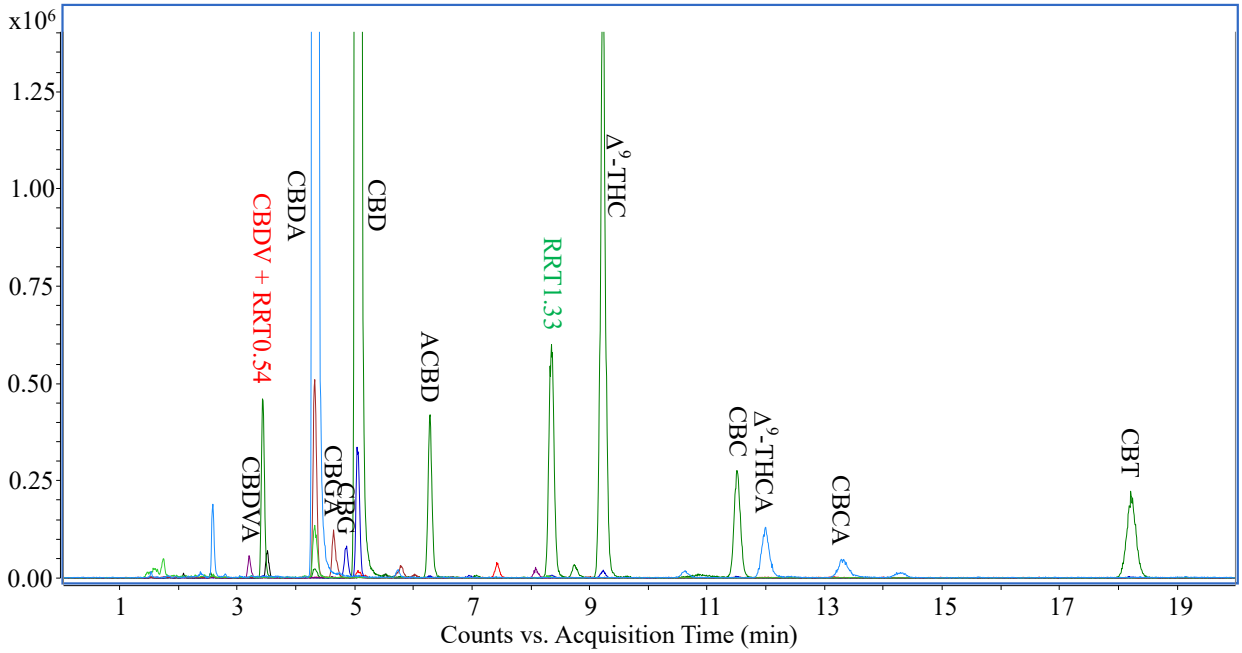
Supplementary Figure S16. H2: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



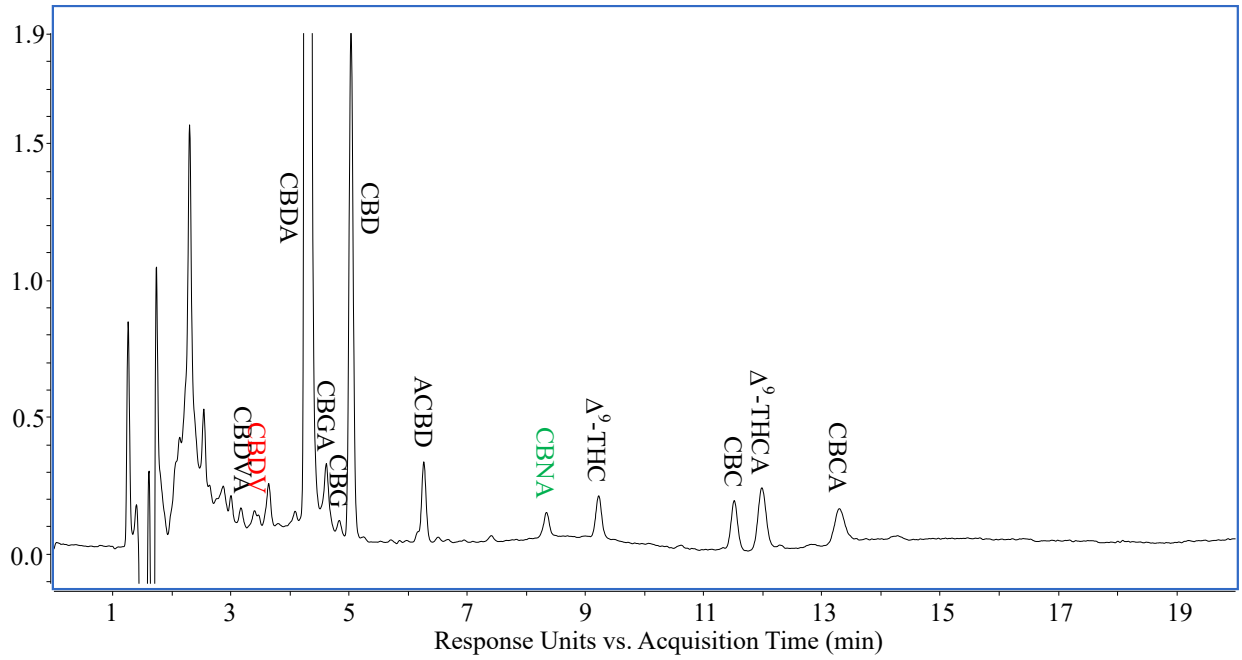


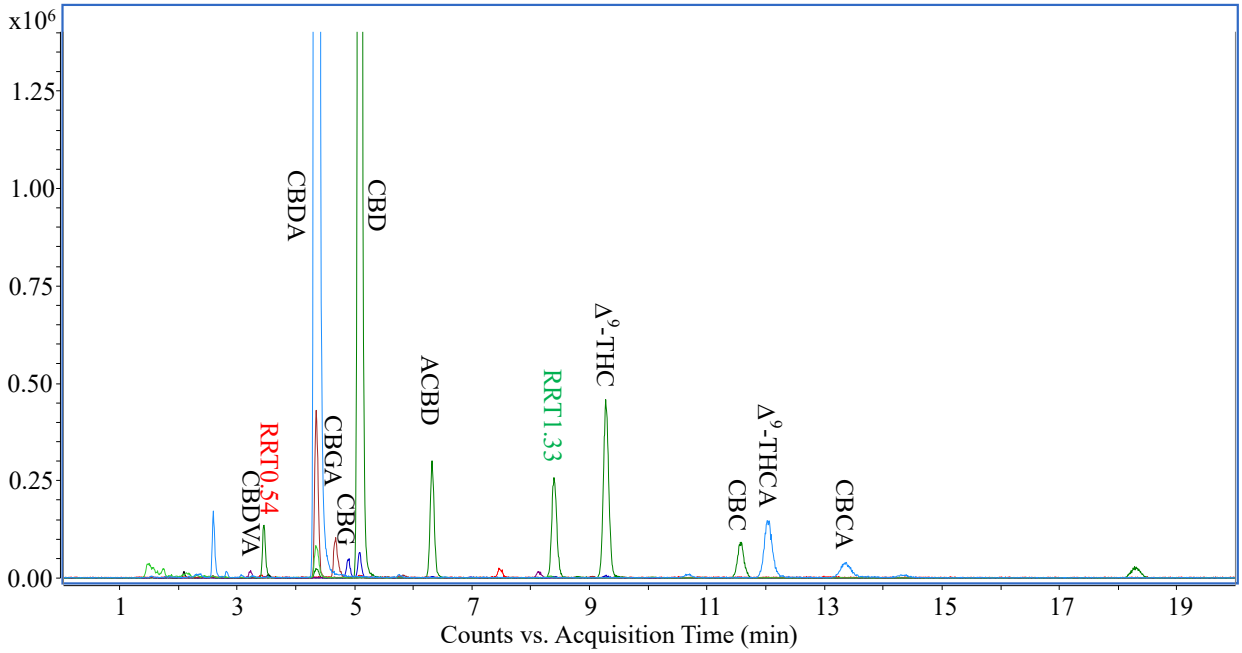
Supplementary Figure S17. H3: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



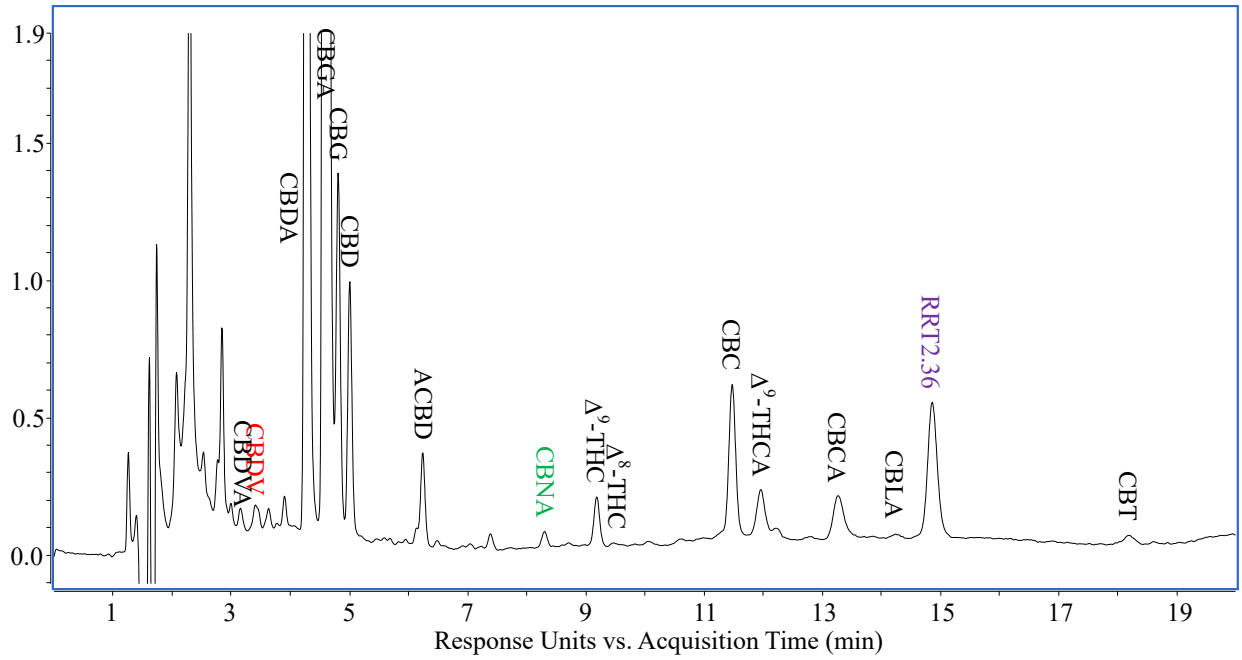


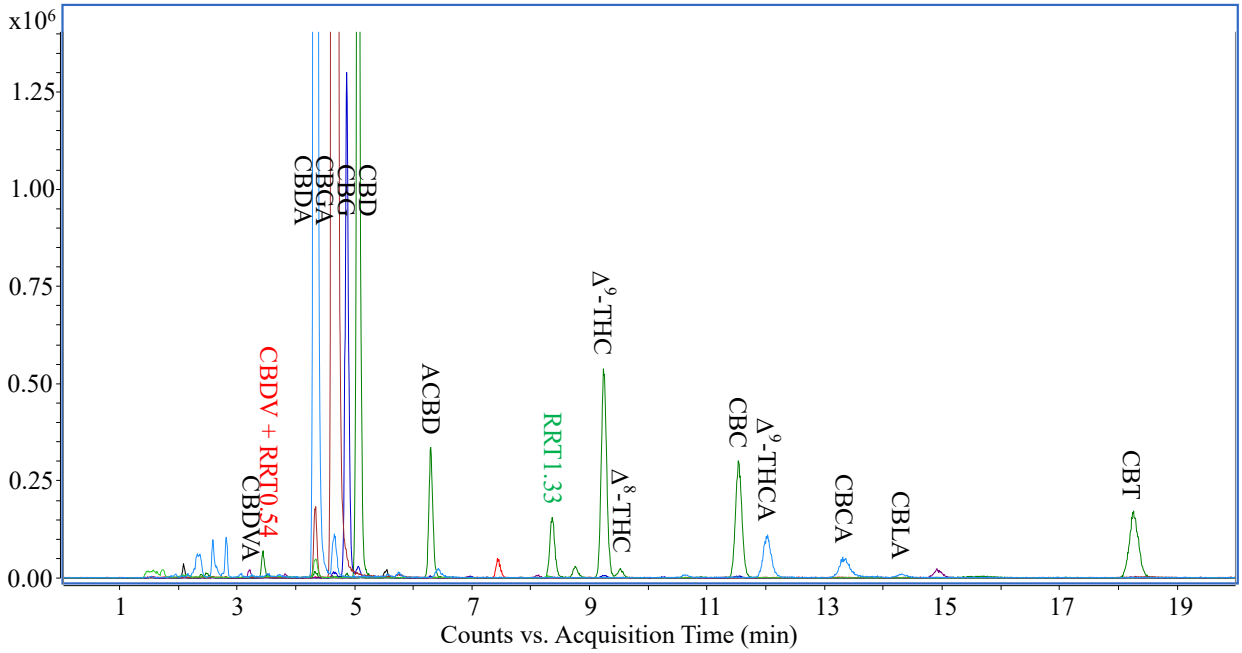
Supplementary Figure S18. H4: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



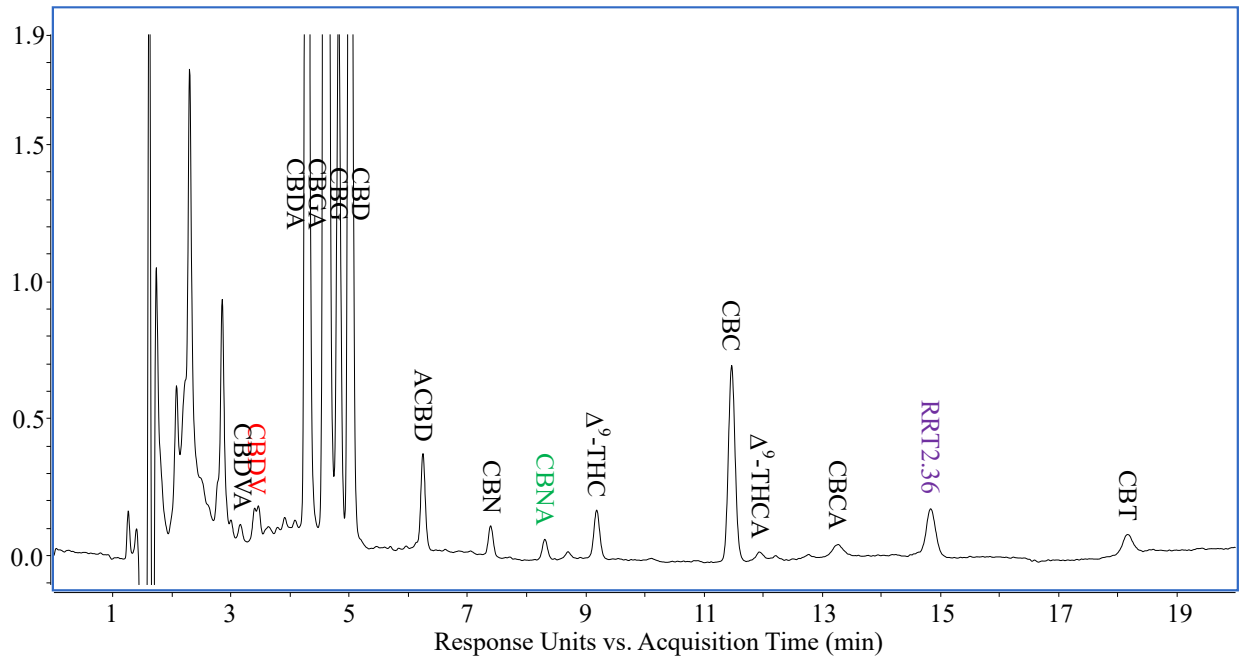


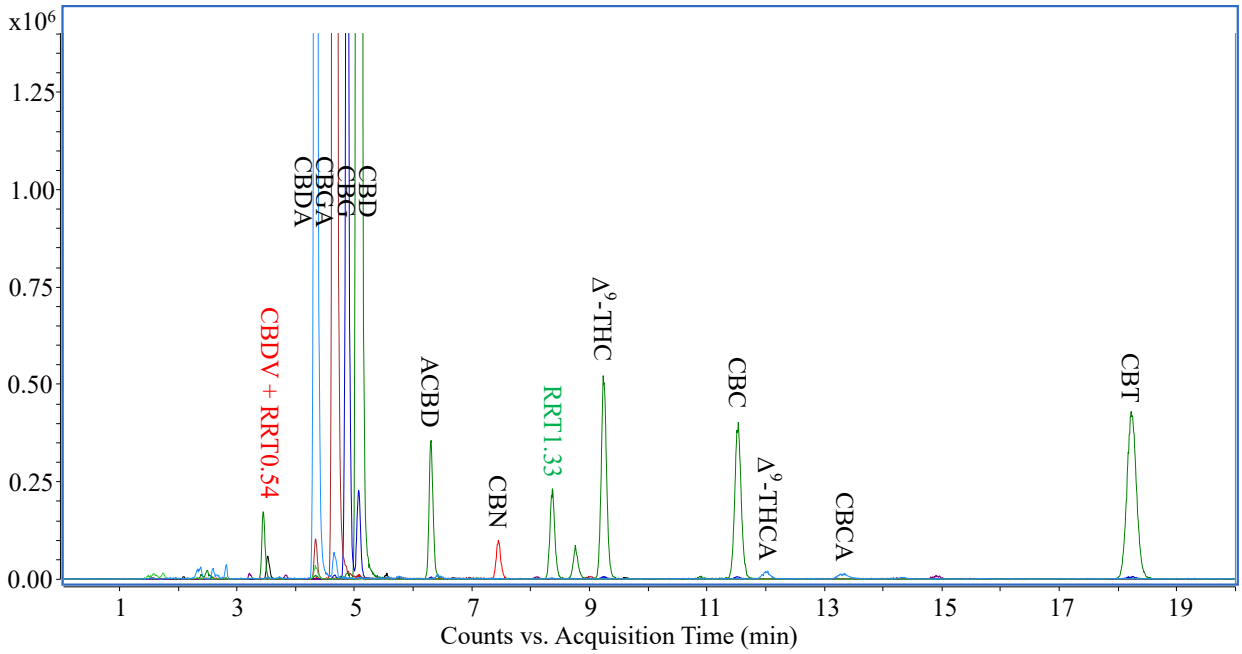
Supplementary Figure S19. H5: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).



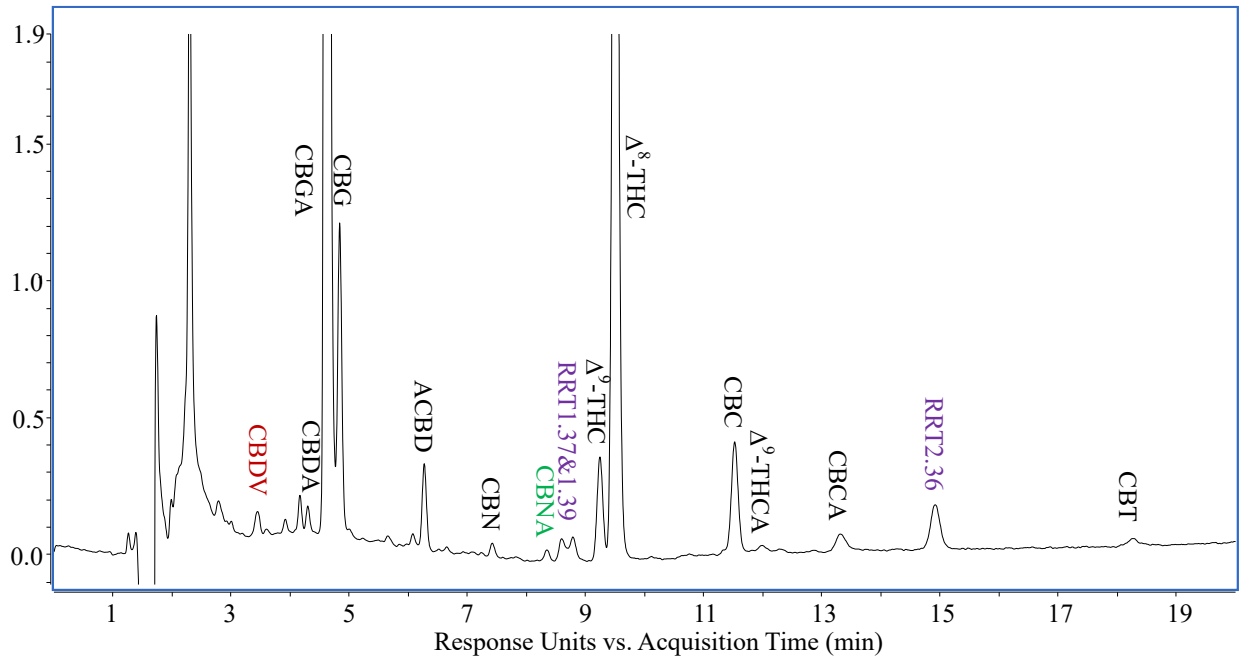


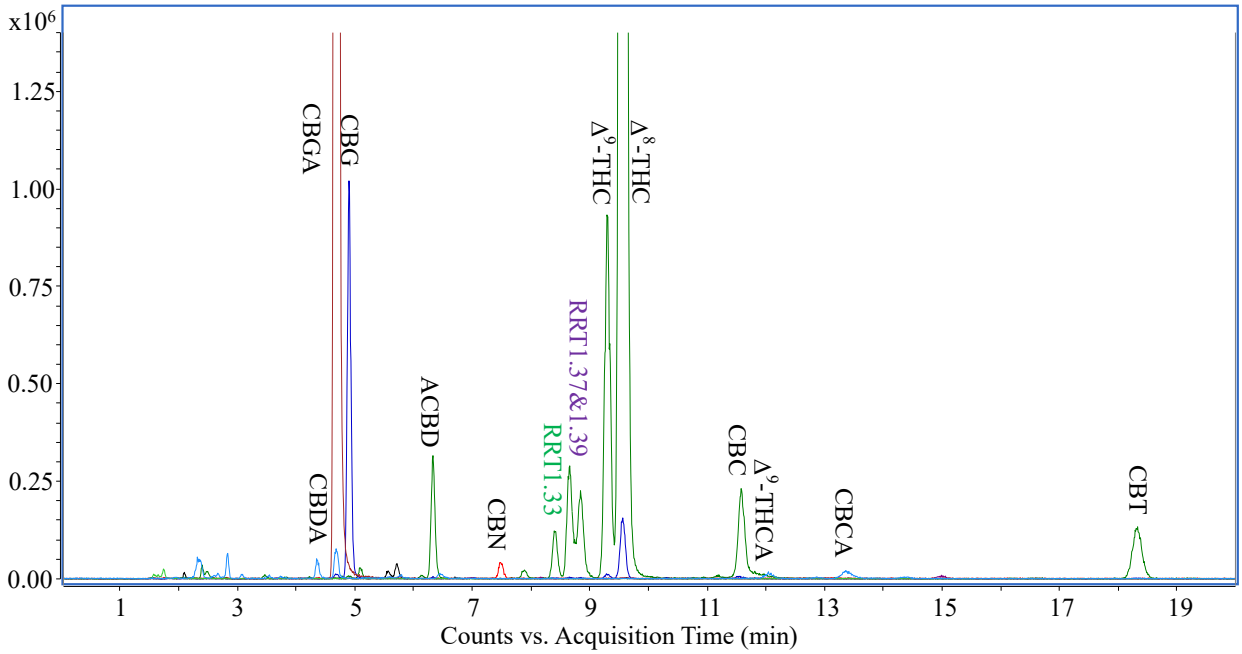
Supplementary Figure S20. HC1: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).


1.9
1.5
1.0
0.5
0.0
CBDA
CBGA
CBG
CBD
CBDV
CBDVA
ACBD
CBN
CBNA
Δ9-THC
CBC
Δ9-THCA
CBCA
RRT2.36
CBT
1
3
5
7
9
11
13
15
17
19
Response Units vs. Acquisition Time (min)

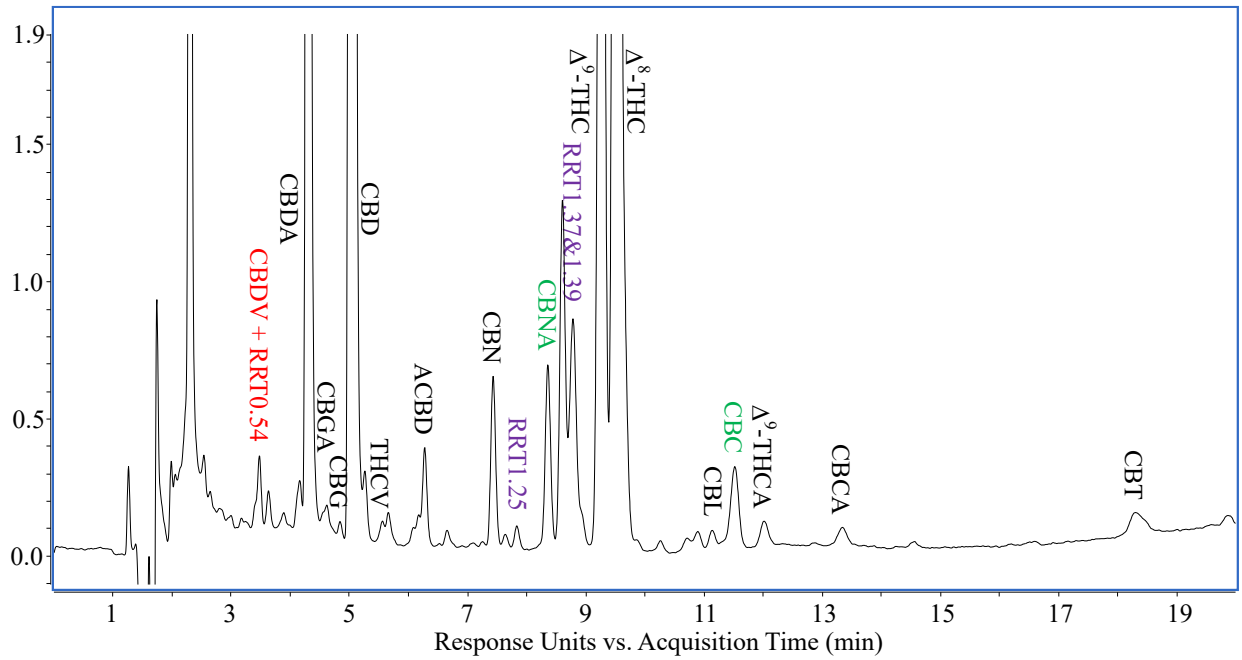

x10^6
1.25
1.00
0.75
0.50
0.25
0.00
CBDA
CBGA
CBG
CBD
CBDV + RRT0.54
ACBD
CBN
RRT1.33
Δ9-THC
CBC
Δ9-THCA
CBCA
CBT
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

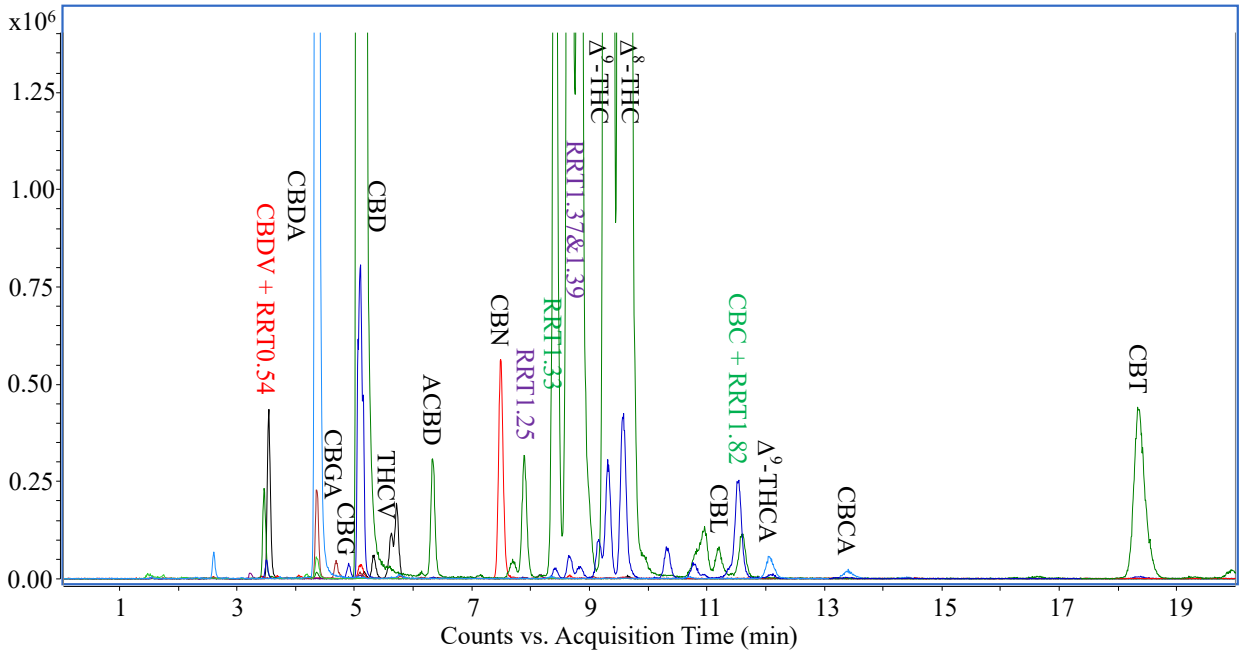
Supplementary Figure S21. Δ^8-H1: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).





Supplementary Figure S22. Δ^8-H2: (A) LC-UV chromatogram at 230 nm and (B) LC-ESI/TOFMS extracted ion chromatograms using their $[M+H]^+$ ions except $[M+H-H_2O]^+$ ion of CBGA with ±20 ppm (peaks were both color coded and labelled for easy distinguish).





Supplementary Table S1. Calibration curves.

Validation parameters		CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Δ^9-THCA	CBCA	CBLA	CBT
R^2	Day 1	0.9983	0.9986	0.9991	0.9993	0.9989	0.9984	0.9992	0.9990	0.9980	0.9981	0.9975	0.9985	0.9980	0.9983	0.9966	0.9988	0.9983	0.9980
	Day 2	0.9984	0.9985	0.9987	0.9989	0.9984	0.9977	0.9988	0.9978	0.9989	0.9979	0.9982	0.9969	0.9965	0.9948	0.9987	0.9949	0.9975	0.9979
	Day 3	0.9983	0.9989	0.9987	0.9990	0.9990	0.9992	0.9989	0.9987	0.9987	0.9991	0.9980	0.9972	0.9988	0.9991	0.9989	0.9985	0.9975	0.9975
LLOQ	Day 1	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Day 2	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Day 3	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
ULOQ	Day 1	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
	Day 2	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
	Day 3	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00

Supplementary Table S2. Precision of the QC samples: RSD values were computed using triplicate measurements for both intraday and interday.

Concentration	RSD (%)	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Δ^9-THCA	CBCA	CBLA	CBT
0.02	Day 1	1.9	0.1	1.7	1.2	1.6	2.7	1.9	2.9	3.1	2.1	1.6	2.7	4.2	2.5	2.3	2.0	4.2	6.1
	Day 2	2.5	4.9	0.6	3.0	4.6	2.0	3.4	2.8	1.2	3.8	1.5	3.8	1.6	1.6	1.9	3.2	5.3	0.9
	Day 3	8.6	2.5	3.1	3.1	0.7	1.4	3.0	7.0	2.9	1.2	5.3	3.5	2.6	1.6	4.9	4.6	2.3	8.0
	Interday	5.4	2.7	3.2	1.7	8.1	5.6	2.2	3.3	3.9	5.8	4.7	6.9	6.0	2.3	9.4	5.4	8.7	8.2
0.5	Day 1	0.1	0.2	0.3	0.4	0.3	1.0	0.6	0.9	0.3	1.1	0.6	0.4	0.6	0.1	1.6	5.4	1.3	2.4
	Day 2	0.5	0.5	0.5	0.7	0.3	0.4	0.4	0.8	0.3	1.3	3.0	2.0	0.7	0.3	1.9	2.1	0.7	2.5
	Day 3	1.3	0.2	0.4	1.7	0.1	0.3	0.4	2.1	1.5	0.3	2.1	0.7	0.9	0.5	0.3	0.5	1.8	1.3
	Interday	2.2	1.3	0.5	1.3	5.3	4.4	0.4	0.2	0.5	1.0	0.8	2.4	1.1	1.7	4.3	4.0	1.7	1.2
12.5	Day 1	0.1	0.0	0.1	0.1	0.7	1.8	0.4	0.1	3.3	1.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	1.2
	Day 2	0.5	0.6	0.5	0.6	1.3	0.4	0.6	0.6	1.9	0.6	3.9	0.5	0.4	0.4	0.5	0.5	0.8	1.6
	Day 3	0.4	0.7	0.4	0.4	0.2	0.4	0.4	0.4	0.4	0.4	0.6	0.5	0.6	0.5	2.2	0.3	1.0	0.7
	Interday	3.9	0.8	3.6	3.6	4.7	3.3	4.1	3.7	4.2	4.3	3.4	3.3	4.2	2.7	3.0	2.8	6.1	4.3

Supplementary Table S3. Accuracy of the QC samples: average recovery values were computed using triplicate measurements for both intraday and interday.

Concentration	Accuracy (%)	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Δ^9-THCA	CBCA	CBLA	CBT
0.02	Day 1	105.1	102.6	103.3	98.1	97.3	98.6	97.7	100.5	99.6	109.4	96.5	95.9	89.6	108.3	91.1	90.2	90.0	90.9
	Day 2	98.3	103.0	99.5	101.1	92.9	96.1	102.0	96.4	100.9	109.8	105.5	110.0	88.4	103.6	109.2	95.1	106.1	107.2
	Day 3	94.6	107.7	96.9	100.9	108.6	106.9	101.0	94.3	107.0	98.9	104.0	104.5	98.5	104.5	96.7	100.5	93.9	100.6
	Interday	99.4	104.4	99.9	100.1	99.6	100.5	100.2	97.1	102.5	106.0	102.0	103.4	92.2	105.5	99.0	95.2	96.7	99.5
0.5	Day 1	106.2	104.9	103.3	105.4	102.7	100.2	104.1	105.0	104.5	103.5	103.4	99.0	105.6	102.5	112.3	96.0	107.0	104.3
	Day 2	102.9	104.0	102.5	103.1	94.5	108.8	103.3	104.5	104.0	104.2	101.9	101.9	103.6	105.9	104.7	104.0	103.9	102.1
	Day 3	101.8	106.7	102.3	103.0	104.5	102.1	104.1	104.8	104.9	102.2	102.1	103.8	105.8	103.2	104.1	100.5	103.9	104.2
	Interday	103.6	105.2	102.7	103.8	100.6	103.7	103.8	104.8	104.5	103.3	102.5	101.6	105.0	103.9	107.0	100.2	104.9	103.5
12.5	Day 1	100.3	97.6	98.5	100.4	96.2	97.5	97.9	98.6	97.7	97.1	97.2	100.0	97.4	97.4	104.9	102.3	96.5	98.8
	Day 2	102.3	99.3	100.1	98.8	98.9	102.9	98.8	100.5	97.0	97.1	95.3	97.4	98.4	99.8	99.7	107.5	95.5	98.1
	Day 3	108.2	98.7	105.5	105.8	105.4	103.5	105.4	106.0	104.5	104.5	101.7	104.0	105.1	102.8	105.4	107.3	106.4	105.9
	Interday	103.6	98.5	101.4	101.6	100.2	101.3	100.7	101.7	99.8	99.6	98.1	100.5	100.3	100.0	103.4	105.7	99.5	101.0

Supplementary Table S4. Average recovery of 0.3% (w/w) ACBD spiked in 23 samples and the corresponding RSD value measured in triplicates.

Sample	Recovery (%)	RSD (%)
M0	102.6	2.2
M1	98.5	1.3
M2	106.1	2.5
M3	105.7	1.6
M4	103.0	2.1
M5	101.3	1.8
M6	106.0	3.3
M7	99.1	4.1
M8	98.1	2.6
MC0	101.6	1.5
MC1	96.1	4.6
MC2	98.0	4.8
MC3	93.6	1.9
MC4	95.3	7.0
MC5	103.3	4.0
H1	99.6	3.6
H2	101.2	2.1
H3	101.9	3.1
H4	101.9	3.7
H5	102.6	1.1
HC1	99.6	1.4
Δ^8-H1	99.1	2.8
Δ^8-H2	100.8	3.4

Supplementary Table S5. RSD values (%) of the corresponding contents listed in **Table 1** (TTHC, total THC; TCBD, total CBD).

Sample	CBDVA	CBDV	CBDA	CBGA	CBG	CBD	THCV	THCVA	CBN	CBNA	Δ^9-THC	Δ^8-THC	CBL	CBC	Δ^9-THCA	CBCA	CBLA	CBT	Total	TTHC	TCBD
M1									3.0	4.3					2.7	10.4			1.8	2.9	
M2				13.3				6.2	13.4	11.8	9.8			17.5	12.0	13.6			11.8	11.8	
M3	6.7		5.8	5.5		5.4									7.3	1.4			5.7	5.8	5.8
M4	4.0		4.3	6.7		3.7									12.0	7.0			4.0	12.0	4.3
M5			3.9	6.7		6.3					9.7				4.9	5.9			4.4	5.4	4.1
M6				4.5					6.3	0.9	4.9				2.2	6.7			2.7	2.5	
M7			1.4	1.6		1.8		3.7	4.4		1.7			0.9	1.8	1.7			1.5	1.8	1.4
M8			5.1	4.5		5.6			7.3		5.6			10.2	5.1	4.8			5.3	5.2	5.2
MC1				5.7					2.2	5.9	1.3				2.5	4.1			2.5	2.3	
MC2				8.7					5.5	8.5	10.4				9.5	9.5			9.2	9.6	
MC3				3.7				6.4	1.5	6.0	5.0				5.6	2.4			5.4	5.6	
MC4			7.3	12.0				4.8	5.4	10.4	8.6				16.0	11.4			13.5	14.5	6.1
MC5			4.2	4.4				5.8	4.2	1.2	3.9				3.5	1.3			3.5	3.5	4.9
H1	9.5		7.6	7.1	8.2	5.6					8.7			8.9	8.1	5.4			7.2	8.4	12.3
H2	1.1		1.4	1.2	2.7	0.7					2.7			1.6	0.8	1.8	3.3	4.9	1.3	1.7	2.0
H3			3.8	3.8		2.9					4.1			3.8	5.1	2.8		3.0	3.5	4.3	6.2
H4			3.8	3.6		5.4					3.1			4.8	4.4	3.6			4.0	3.7	4.0
H5			1.7	1.7	2.0	1.8					0.9			1.5	1.2	1.5		5.8	1.6	0.8	1.7
HC1			4.0	6.3	4.8	4.0					4.5			4.0		5.0		9.8	5.0	3.3	4.0
Δ^8-H1				11.8	7.6						10.5	11.0		7.1		3.6			11.0	10.4	19.6
Δ^8-H2			3.0			3.1	4.7		1.6		2.8	2.8	7.9		5.0	7.7		8.0	3.0	2.8	3.1