Potency testing of up to sixteen cannabinoids in hemp-infused edibles using liquid chromatography diode array detector with optional confirmation of identity by electrospray ionization time-of-flight mass spectrometry

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Fig. 1. Effects of the content of formic acid in the aqueous solvent on the retention time of cannabinoids. Default content of acetonitrile and column oven temperature were 73% (v/v) and 30ºC. The separated mixture contained 1 μg/mL individual cannabinoid except for ACBD at 0.5 μg/mL.

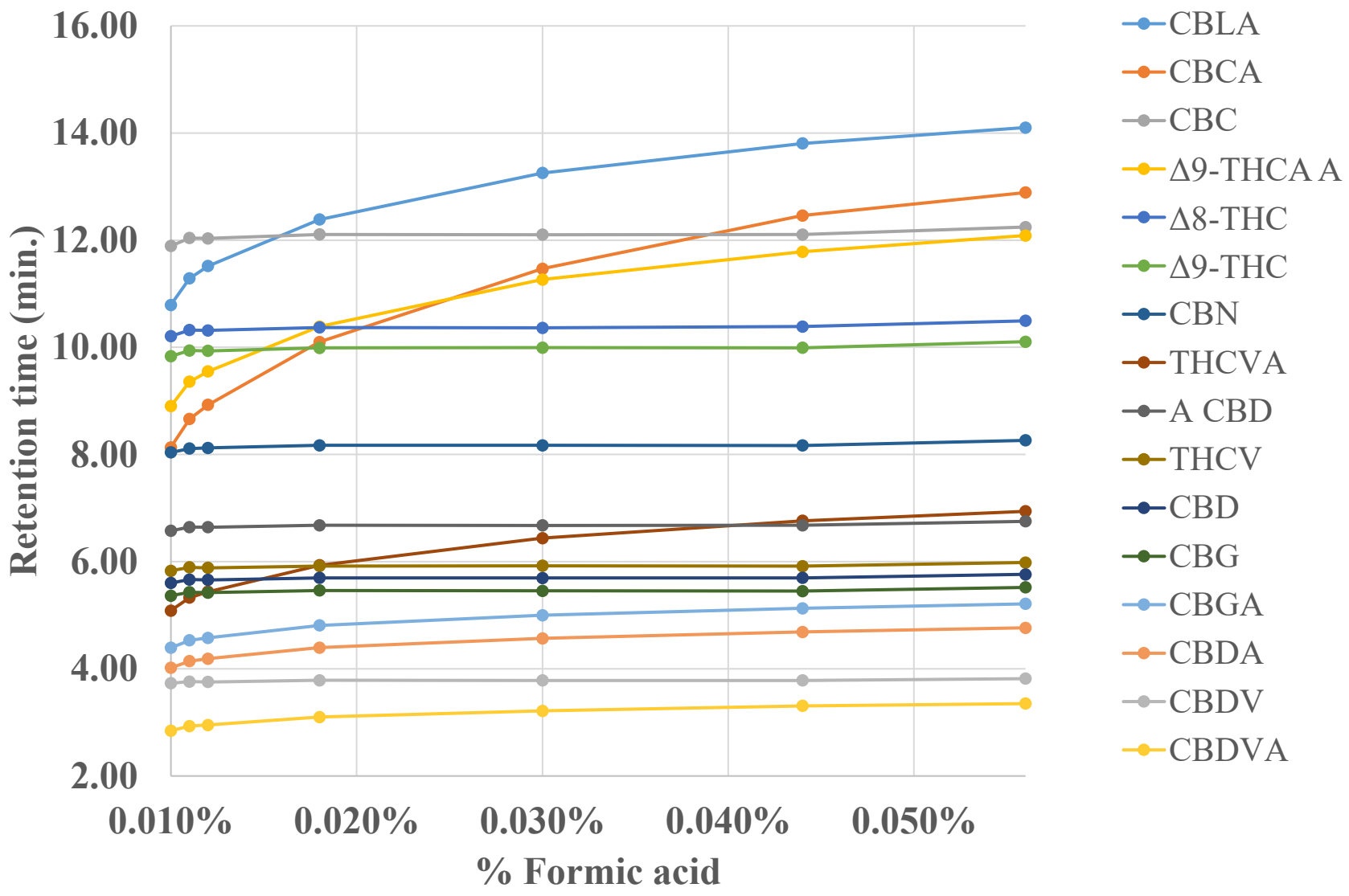
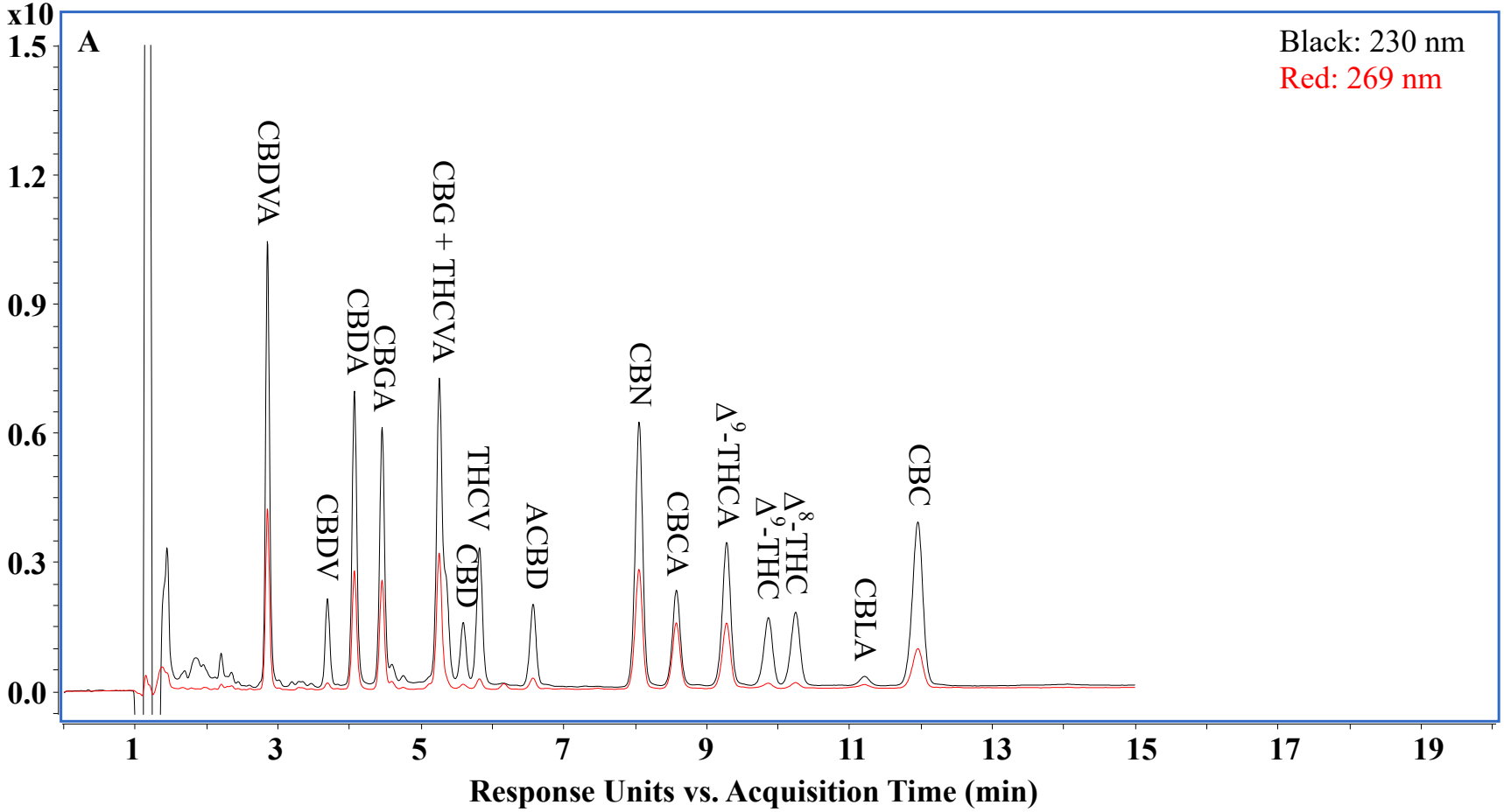
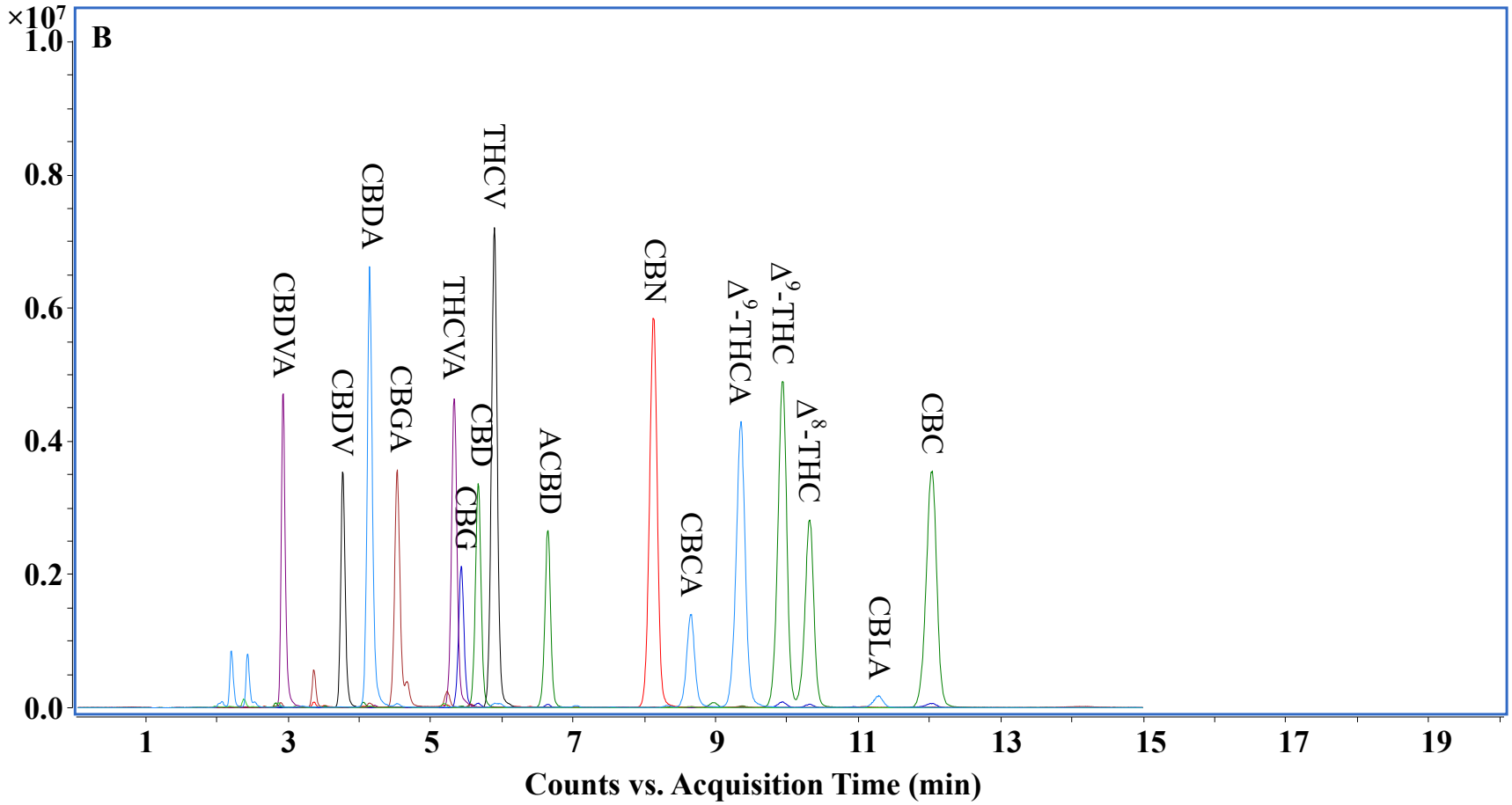
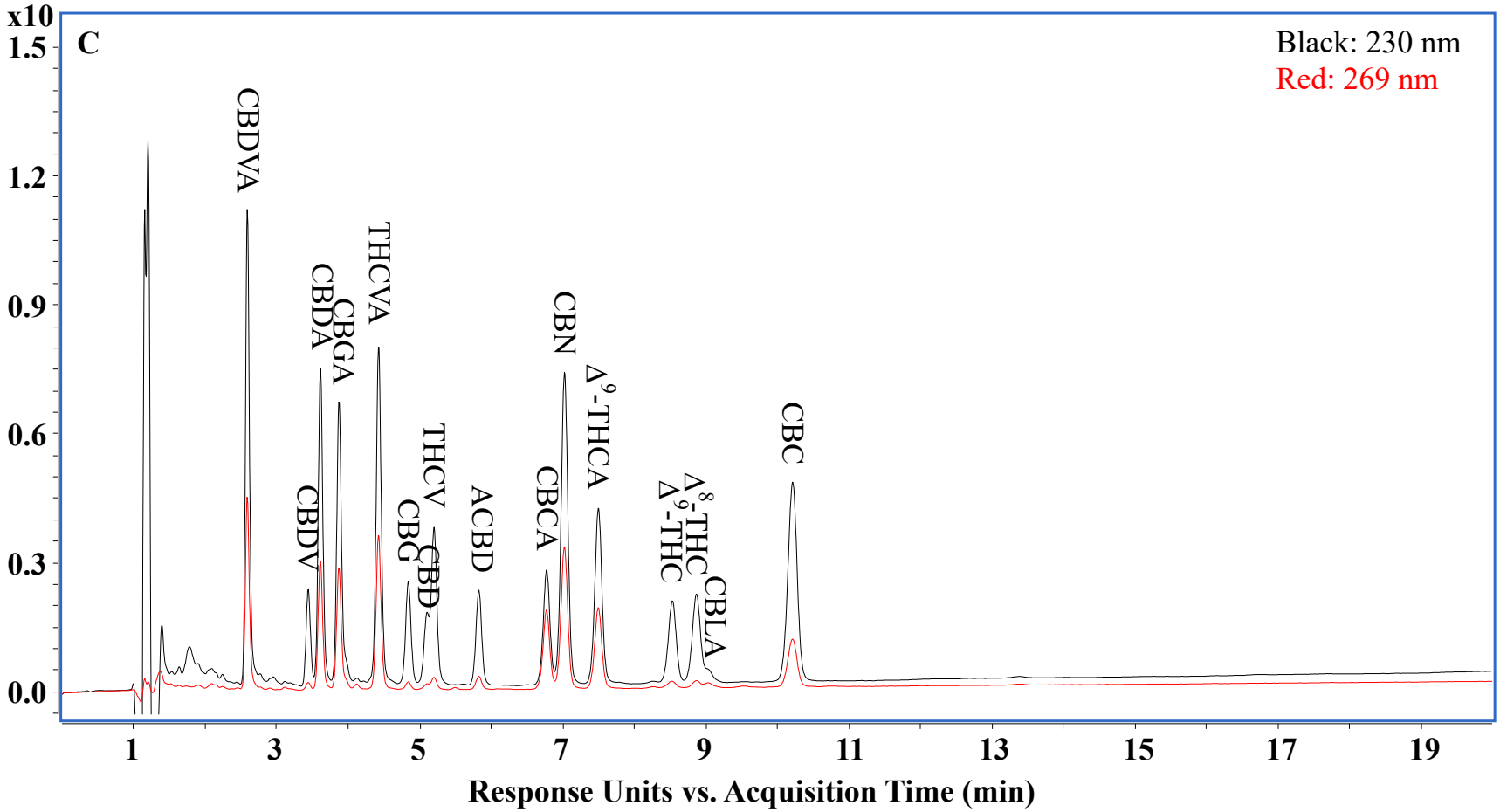

16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
Retention time (min.)
0.010%
0.020%
0.030%
0.040%
0.050%
% Formic acid
CBLA
CBCA
CBC
Δ9-THCA A
Δ8-THC
Δ9-THC
CBN
THCVA
A CBD
THCV
CBD
CBG
CBGA
CBDA
CBDV
CBDVA

Fig. 2. LC-UV chromatograms (A and C) and LC-EICs (B and D) of cannabinoids (both color coded and labelled in LC-EICs) at 1 µg/mL except for ACBD at 0.5 µg/mL using an aqueous solvent containing 2 mM ammonium formate and 0.011% (v/v) formic acid and a mobile phase containing 73% (v/v) acetonitrile at 30ºC (A and B) and 45ºC (C and D).




×10^7
1.0
0.8
0.6
0.4
0.2
0.0
B
CBDVA
CBDV
CBDA
CBGA
THCVA
CBG
CBD
THCV
ACBD
CBN
CBCA
Δ9-THCA
Δ9-THC
Δ8-THC
CBLA
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)


x10
1.5
1.2
0.9
0.6
0.3
0.0
C
Black: 230 nm
Red: 269 nm
CBDVA
CBDV
CBDA
CBGA
THCVA
CBG
THCV
CBD
ACBD
CBCA
CBN
Δ9-THCA
Δ9-THC
Δ8-THC
CBLA
CBC
1
3
5
7
9
11
13
15
17
19
Response Units vs. Acquisition Time (min)

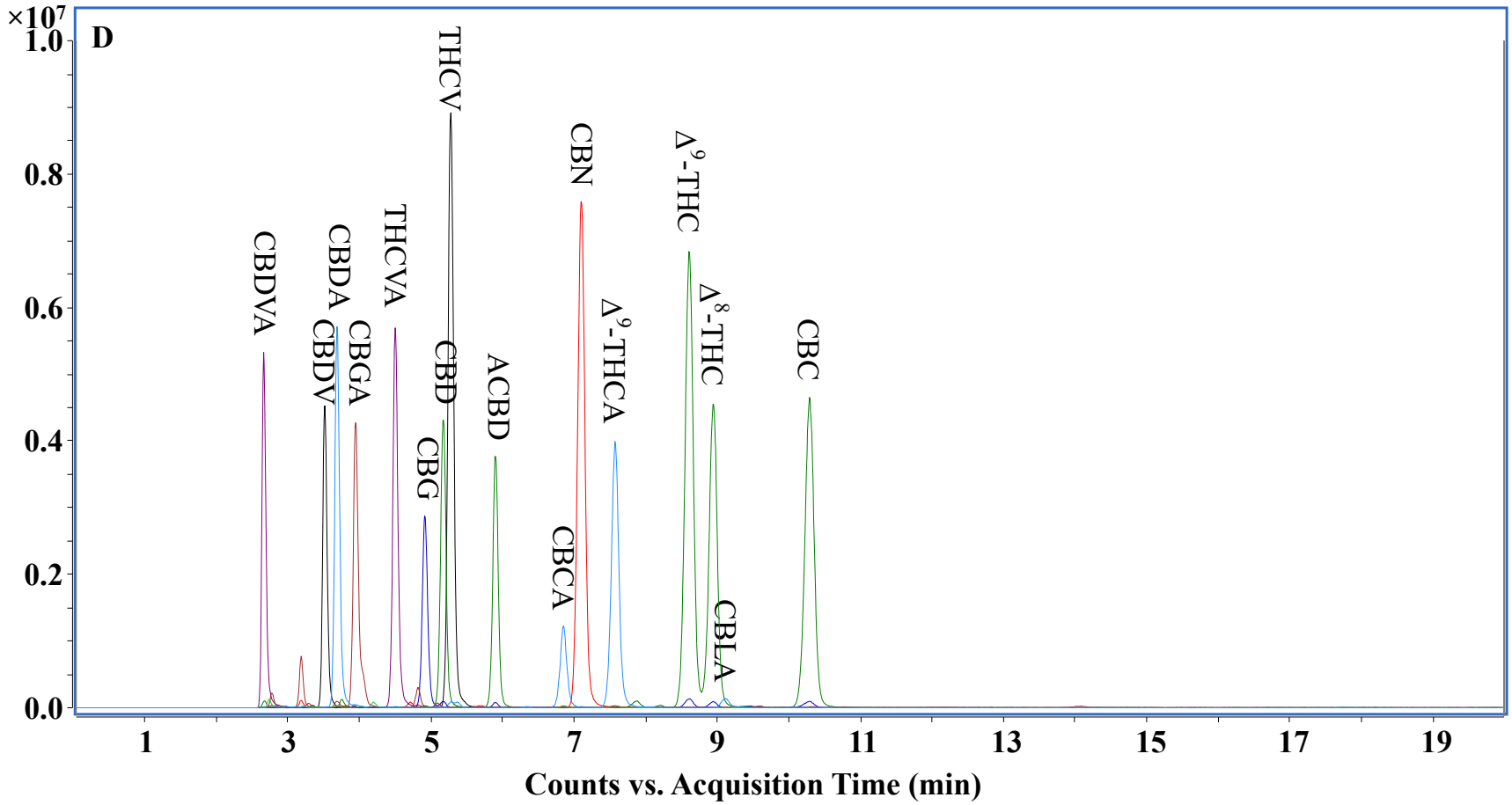

×10^7
D
1.0
0.8
0.6
0.4
0.2
0.0
CBDVA
CBDV
CBDA
CBGA
THCVA
CBG
THCV
CBD
ACBD
CBCA
CBN
Δ9-THCA
Δ9-THC
Δ8-THC
CBLA
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

Fig. 3. LC-UV chromatogram of cannabinoids at LOQ level, i.e., 0.02 µg/mL, except for ACBD at 0.5 µg/mL detected at 230 nm under optimized LC conditions: an aqueous solvent containing 2 mM ammonium formate and 0.011% formic acid (v/v) and a mobile phase containing 73% (v/v) acetonitrile at 30ºC.

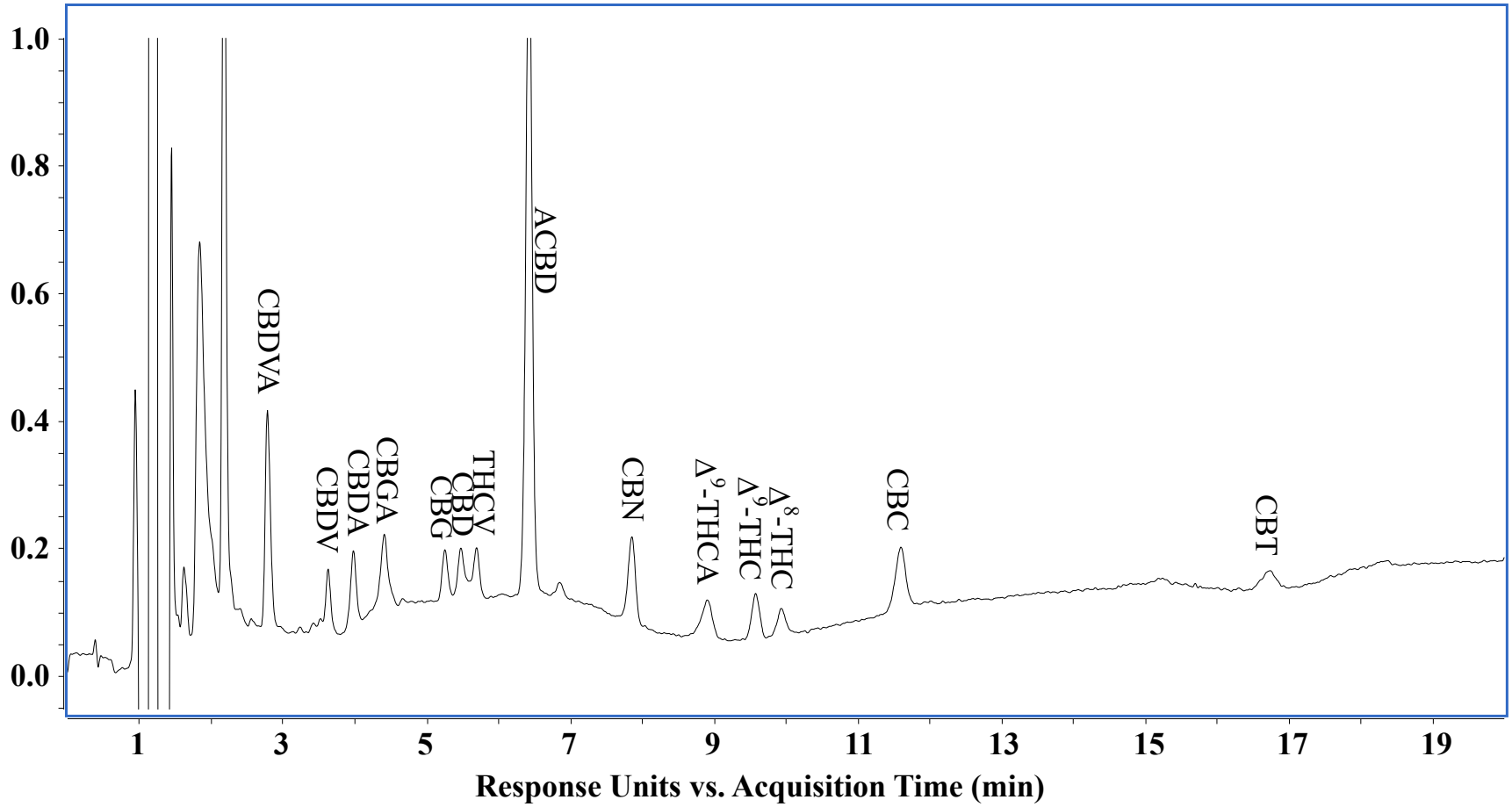
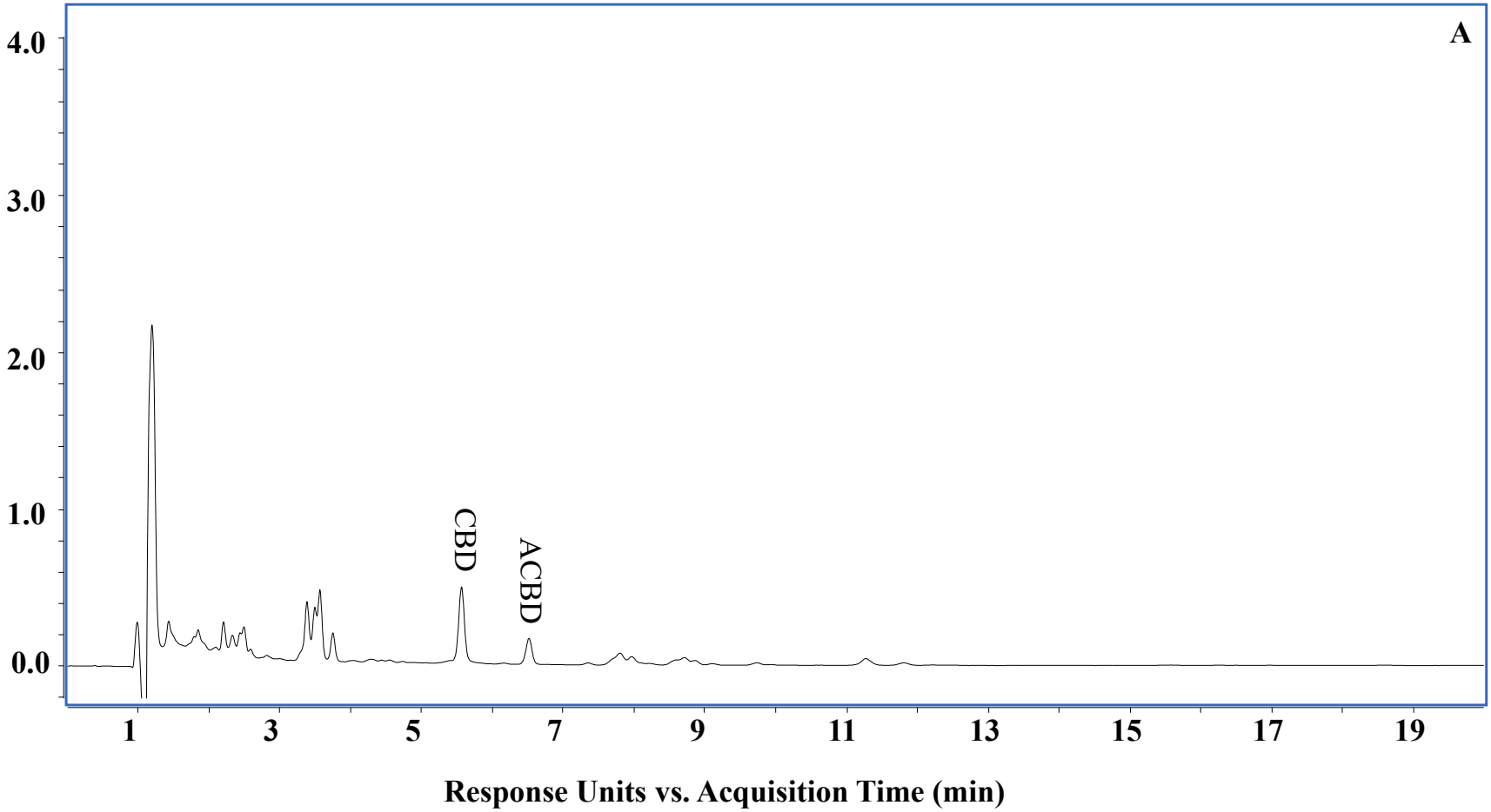
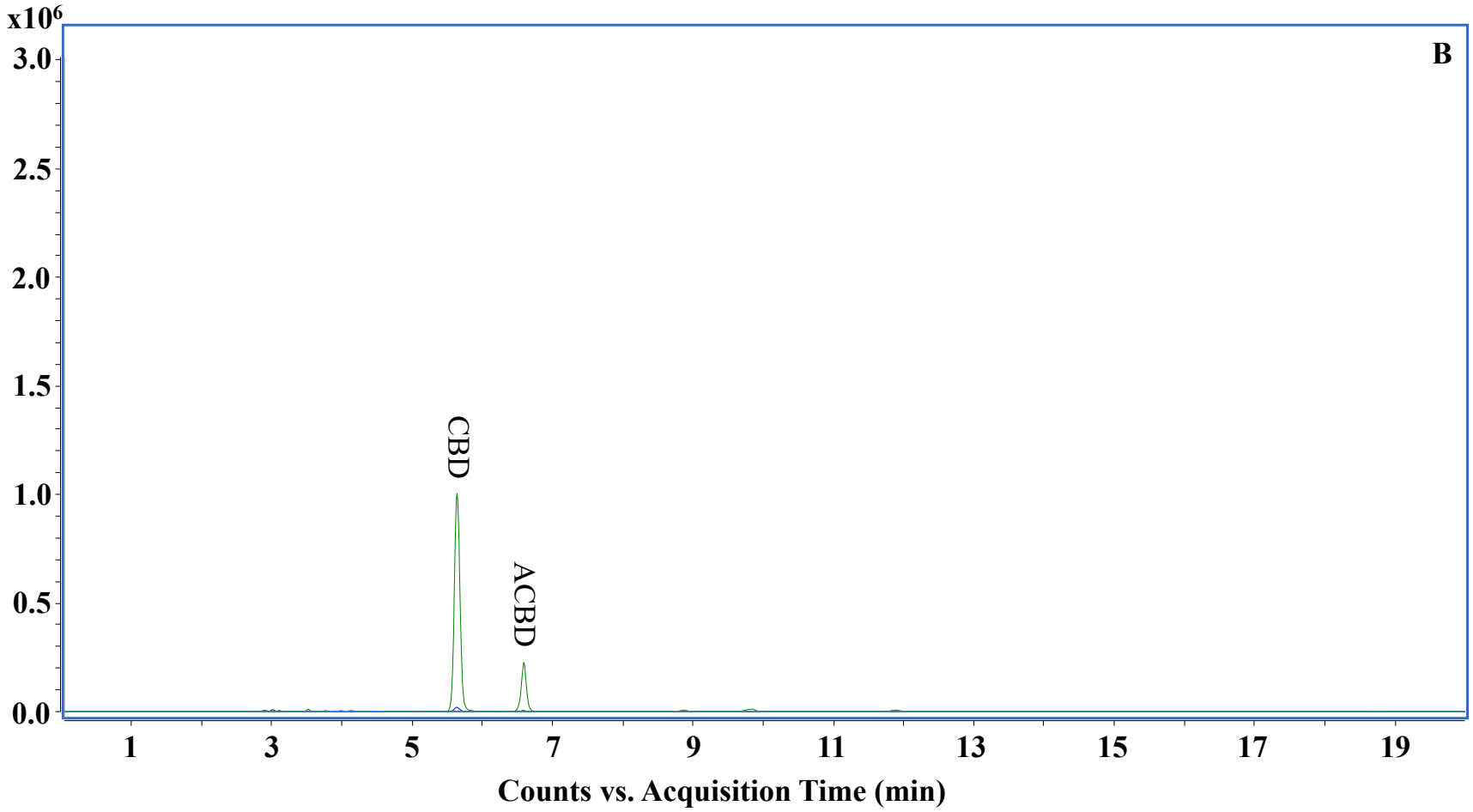
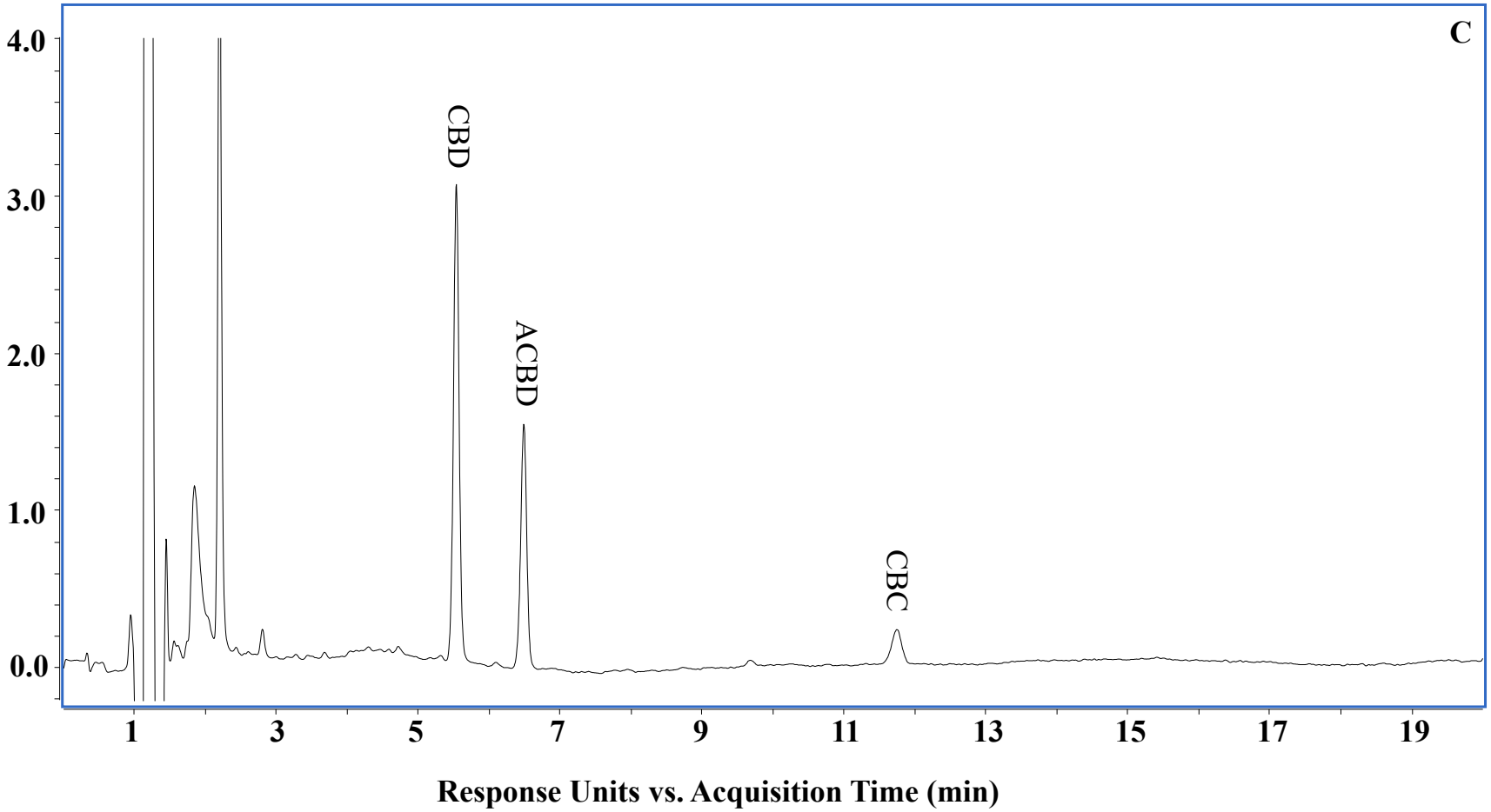
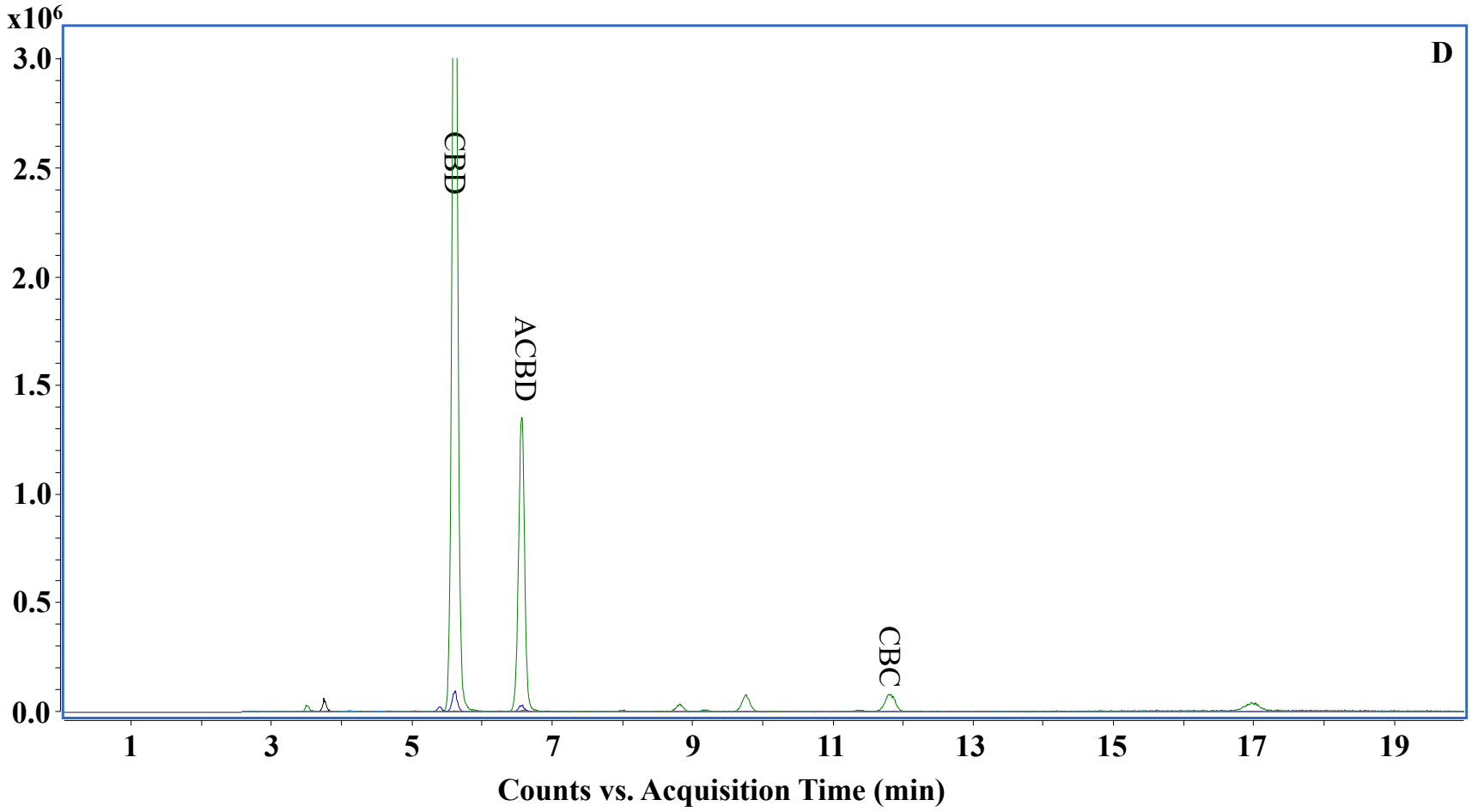


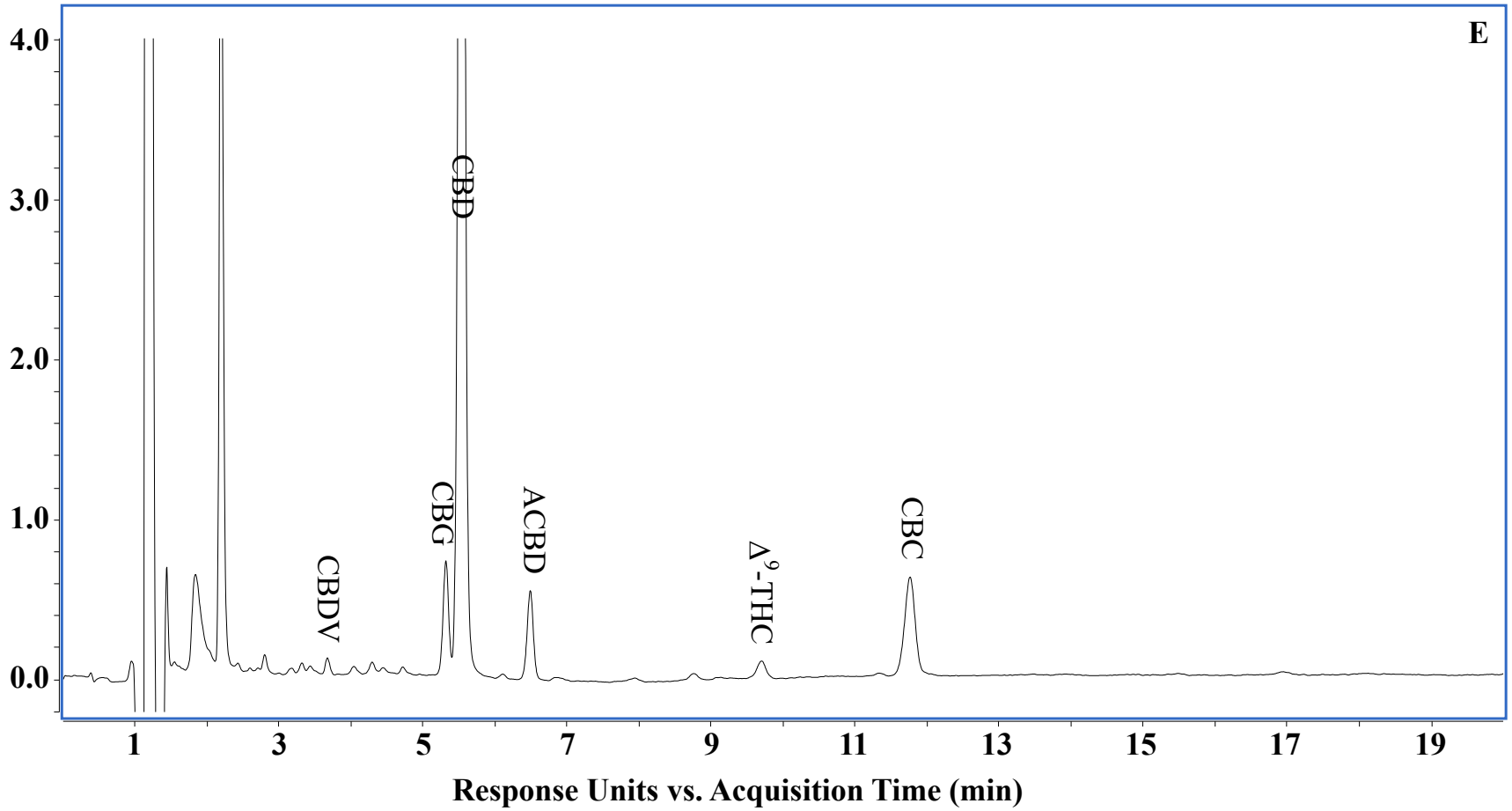
Fig. 4. LC-UV chromatograms (A, C, E) and LC-EICs (B, D, F) (cannabinoids are both color coded and labelled in LC-EICs) of 2.50 mg/mL grapefruit sparkling coconut water spiked with 0.003% (w/w) ACBD, 250 µg/mL CBD gourmet gummies (sweet watermelon flavor) spiked with 0.3% (w/w) ACBD, and 250 µg/mL CBD cat food kitty nugs spiked with 0.1% (w/w) ACBD.




x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
B
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)


C
CBD
ACBD
CBC
4.0
3.0
2.0
1.0
0.0
1
3
5
7
9
11
13
15
17
19
Response Units vs. Acquisition Time (min)


x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
D
CBD
ACBD
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)


E
4.0
3.0
2.0
1.0
0.0
CBDV
CBG
CBD
ACBD
Δ9-THC
CBC
1
3
5
7
9
11
13
15
17
19
Response Units vs. Acquisition Time (min)

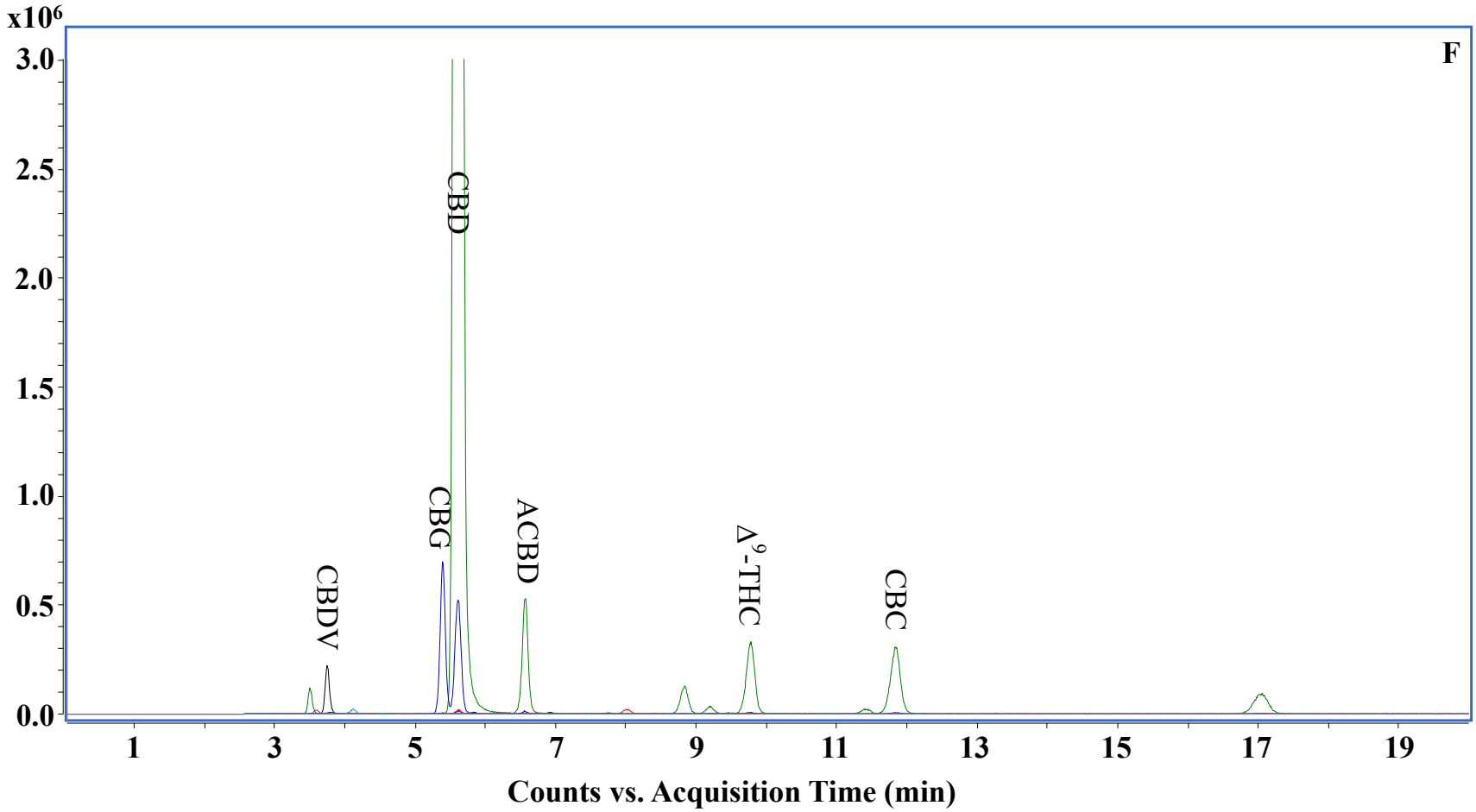

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
F
CBDV
CBG
CBD
ACBD
Δ9-THC
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

Table 1

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

Edibles[1]	ACBD[2]	CBDV	CBG	CBD	CBN	Δ^9-THC	CBC	CBT	Total[3]
E1	0.003×98%			0.0058					0.0058
E2	0.003×97%			0.0040					0.0040
E3	0.3×90%			0.068					0.068
E4	0.3×96%		1.413	0.489					1.903
E5	0.3×93%			0.010	0.393				0.404
E6	0.3×96%	0.011		1.174		0.009	0.010	0.010	1.214
E7	0.3×94%	0.008	0.027	1.157		0.020	0.008	0.036	1.256
E8	0.3×100%			0.033					0.033
E9	0.3×102%			0.524					0.524
E10	0.3×100%			0.393			0.019		0.412
E11	0.3×103%		0.058	0.048					0.106
E12	0.3×101%			0.043					0.043
E13	0.1×91%			0.024					0.024
E14	0.1×97%			0.211			0.014		0.225
E15	0.1×95%			0.125					0.125
E16	0.1×99%			0.029					0.029
E17	0.1×95%	0.009	0.080	1.440		0.018	0.056		1.603
E18	0.1×97%			0.026					0.026
E19	0.1×91%			0.097					0.097
E20	0.3×108%	0.025	0.008	6.068	0.009	0.172	0.012	0.133	6.429

[1]Please refer to section 2.1. Chemicals and reagents for the identity of each edible; [2]Spiking content × recovery %; [3]Excluding ACBD.

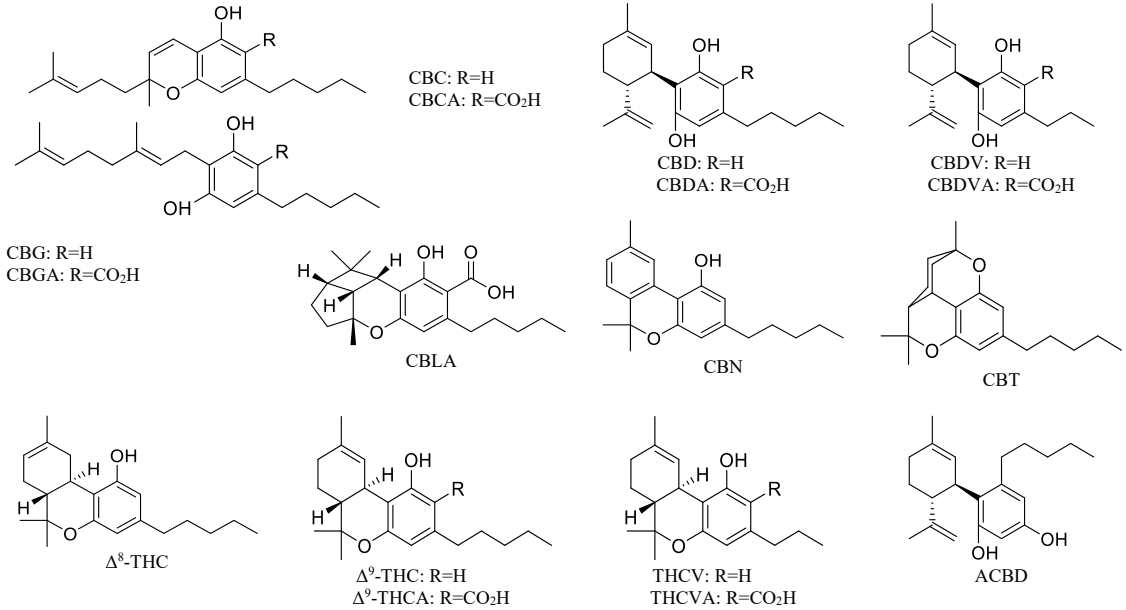
Table 2

RSD values (%) of cannabinoids in hemp-infused edibles measured in triplicates.

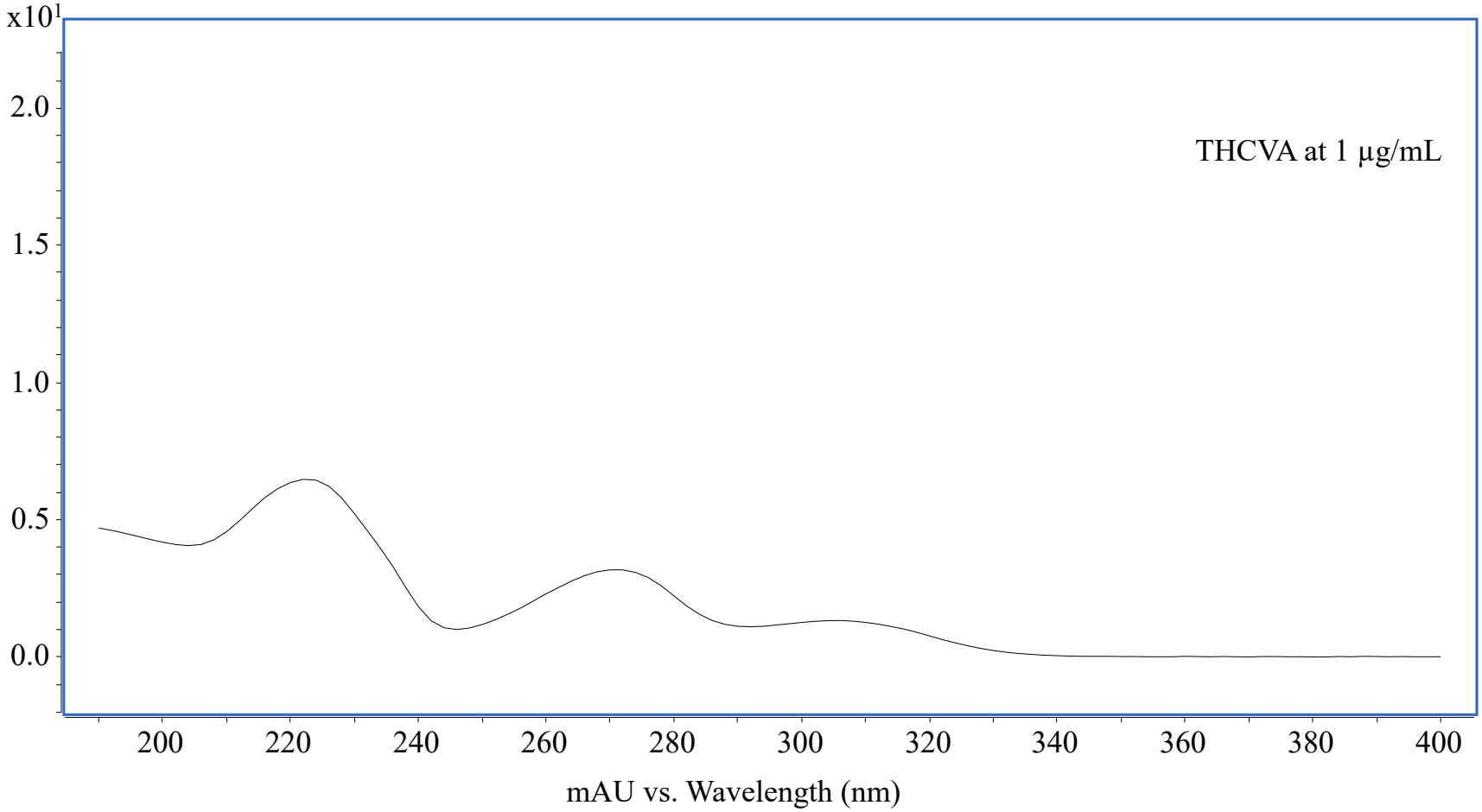
Edibles[1]	ACBD	CBDV	CBG	CBD	CBN	Δ^9-THC	CBC	CBT	Total[2]
E1	1.1			1.9					1.9
E2	0.5			5.4					5.4
E3	3.5			7.4					7.4
E4	3.3		5.8	5.7					5.8
E5	2.0			5.9	2.5				2.5
E6	2.6	4.1		2.4		4.5	3.7	14.7	2.5
E7	4.3	2.9	5.3	5.3		5.9	6.5	7.4	5.3
E8	1.7			2.9					2.9
E9	0.8			2.9					2.9
E10	2.0			4.8			3.0		4.5
E11	6.5		8.2	4.0					6.2
E12	3.3			9.5					9.5
E13	0.8			1.0					1.0
E14	1.4			0.8			3.4		0.5
E15	1.2			1.4					1.4
E16	0.7			2.9					2.9
E17	1.6	0.5	3.3	1.3		1.8	1.1		1.3
E18	5.1			7.4					7.4
E19	1.5			1.8					1.8
E20	2.4	5.0	2.0	4.3	1.6	2.0	3.9	3.1	4.1

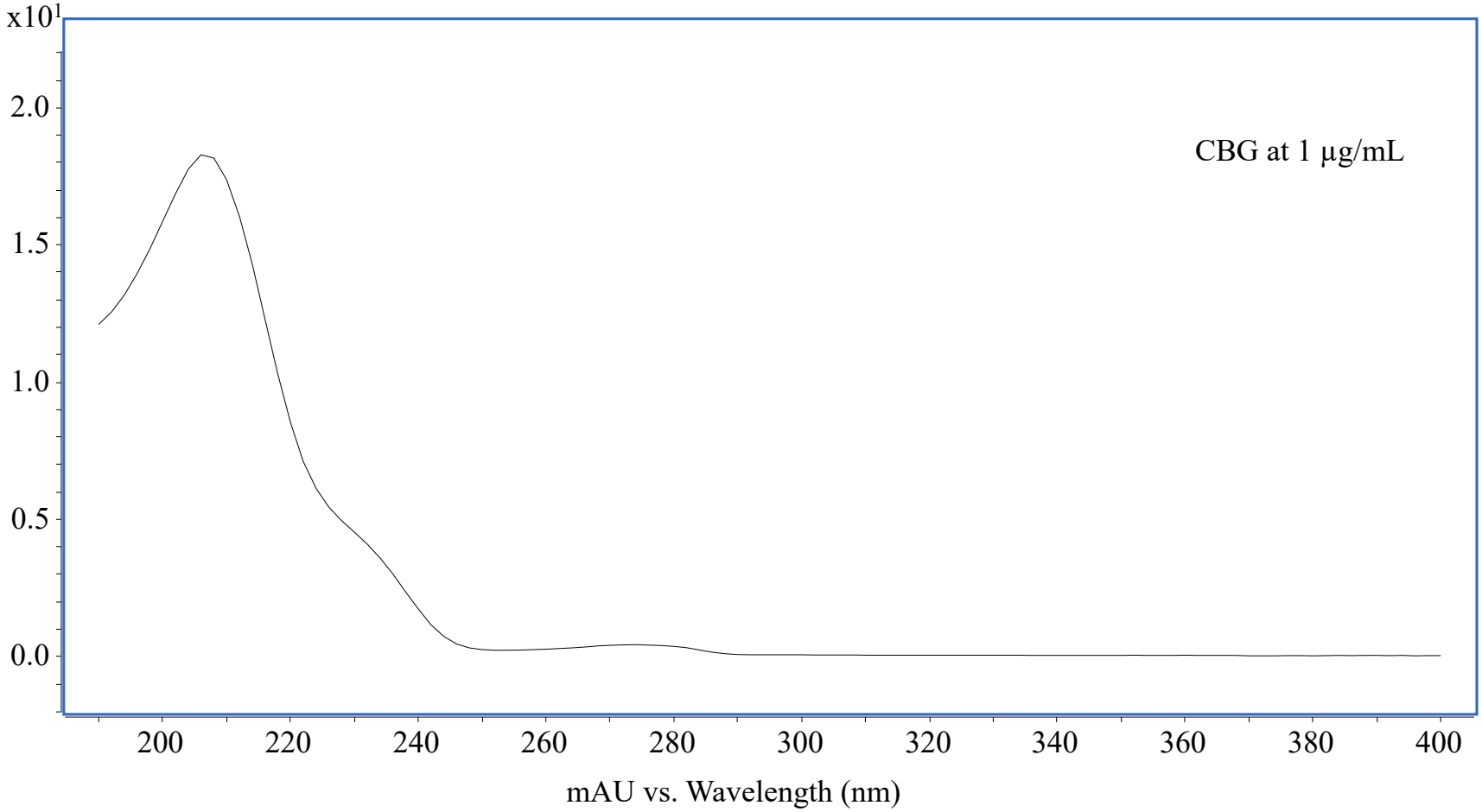
[1]Please refer to section 2.1. Chemicals and reagents for the identity of each edible; [2]Excluding ACBD.

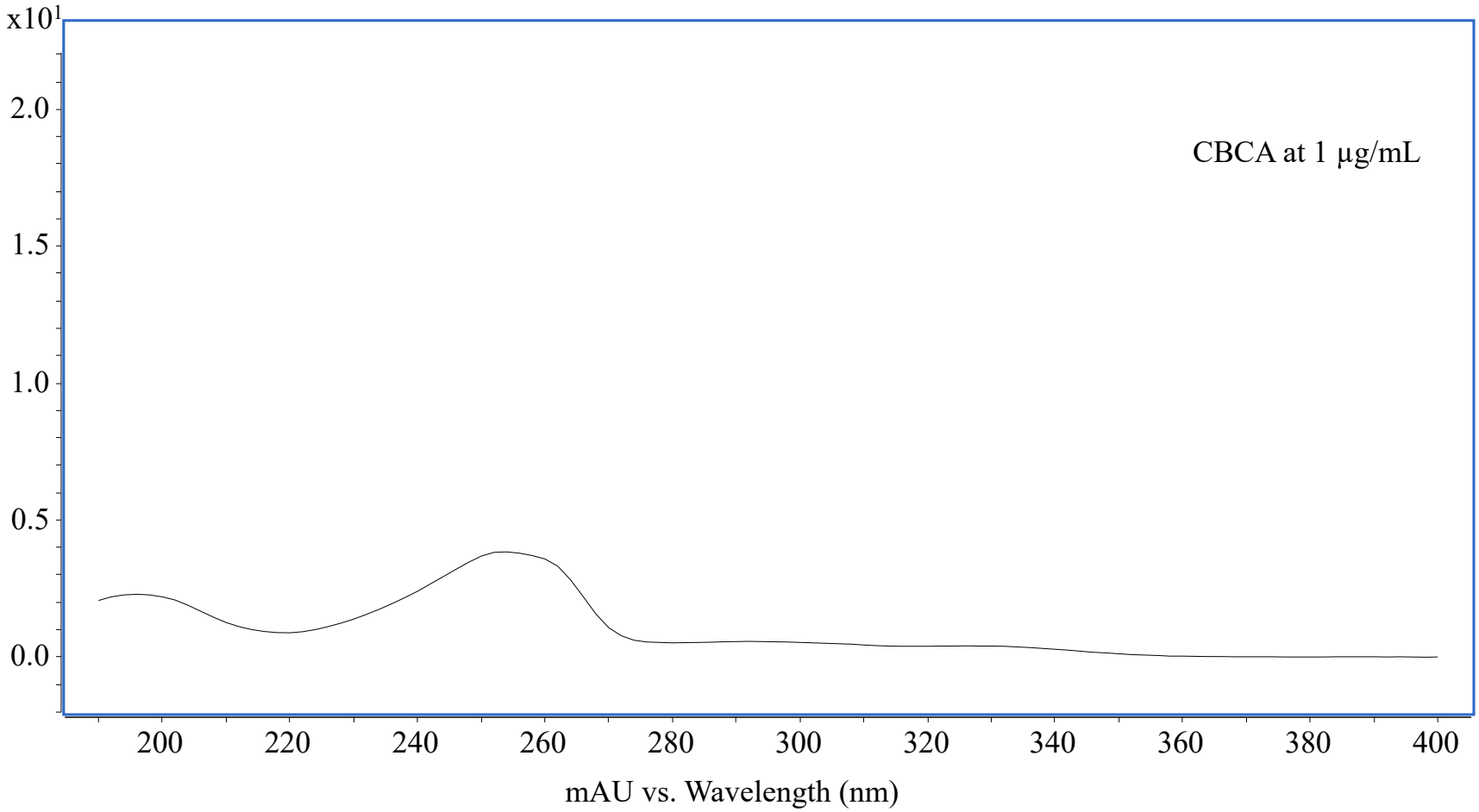
Supplementary Fig. S1. Chemical structure of seventeen cannabinoids used in this study.

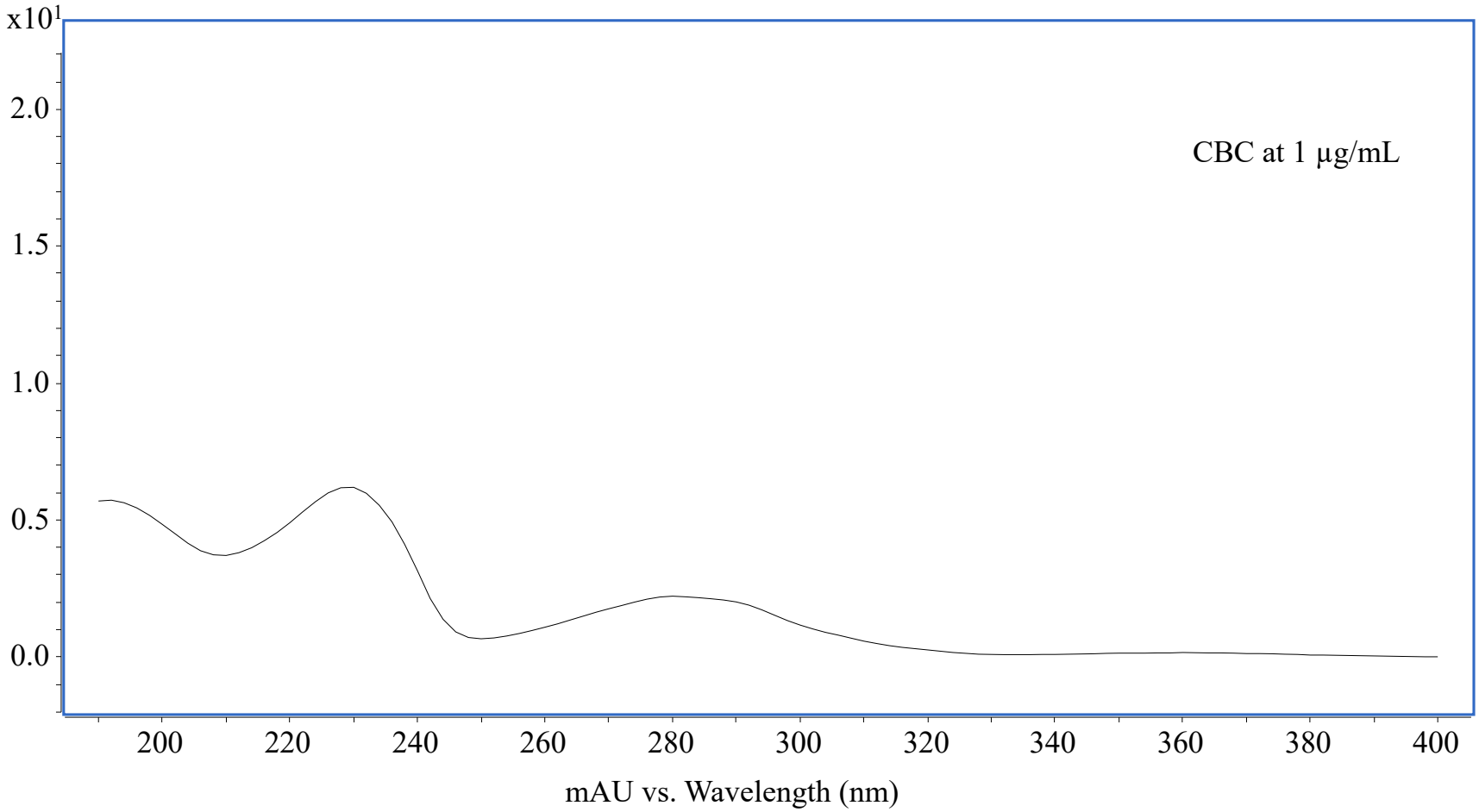


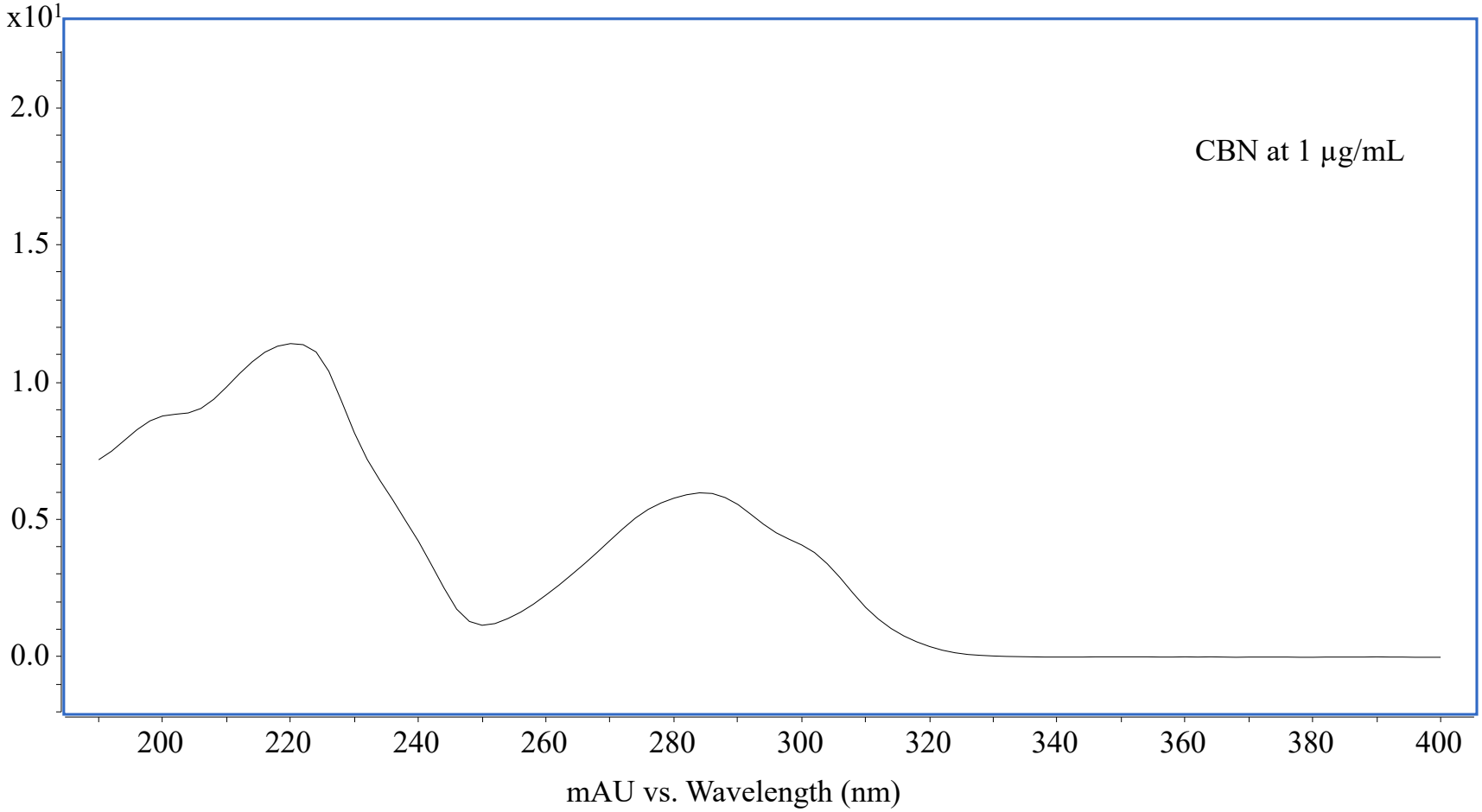
Supplementary Fig. S2. From top to bottom, UV absorption spectra of 1 µg/mL THCVA (A), CBG (B), CBCA (C), CBC (D), CBN (E), and CBT(F).

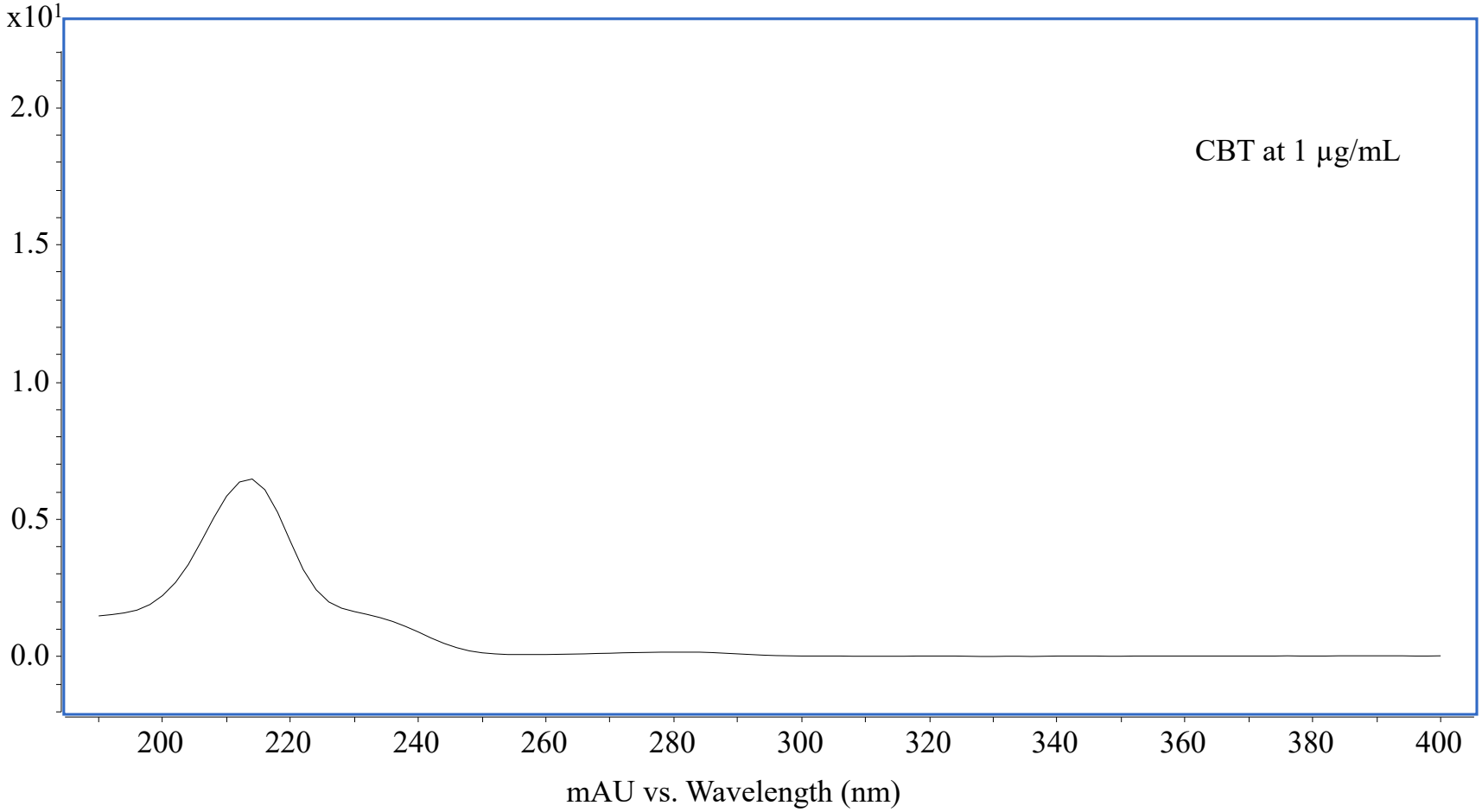



x10^1
2.0
1.5
1.0
0.5
0.0
CBG at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

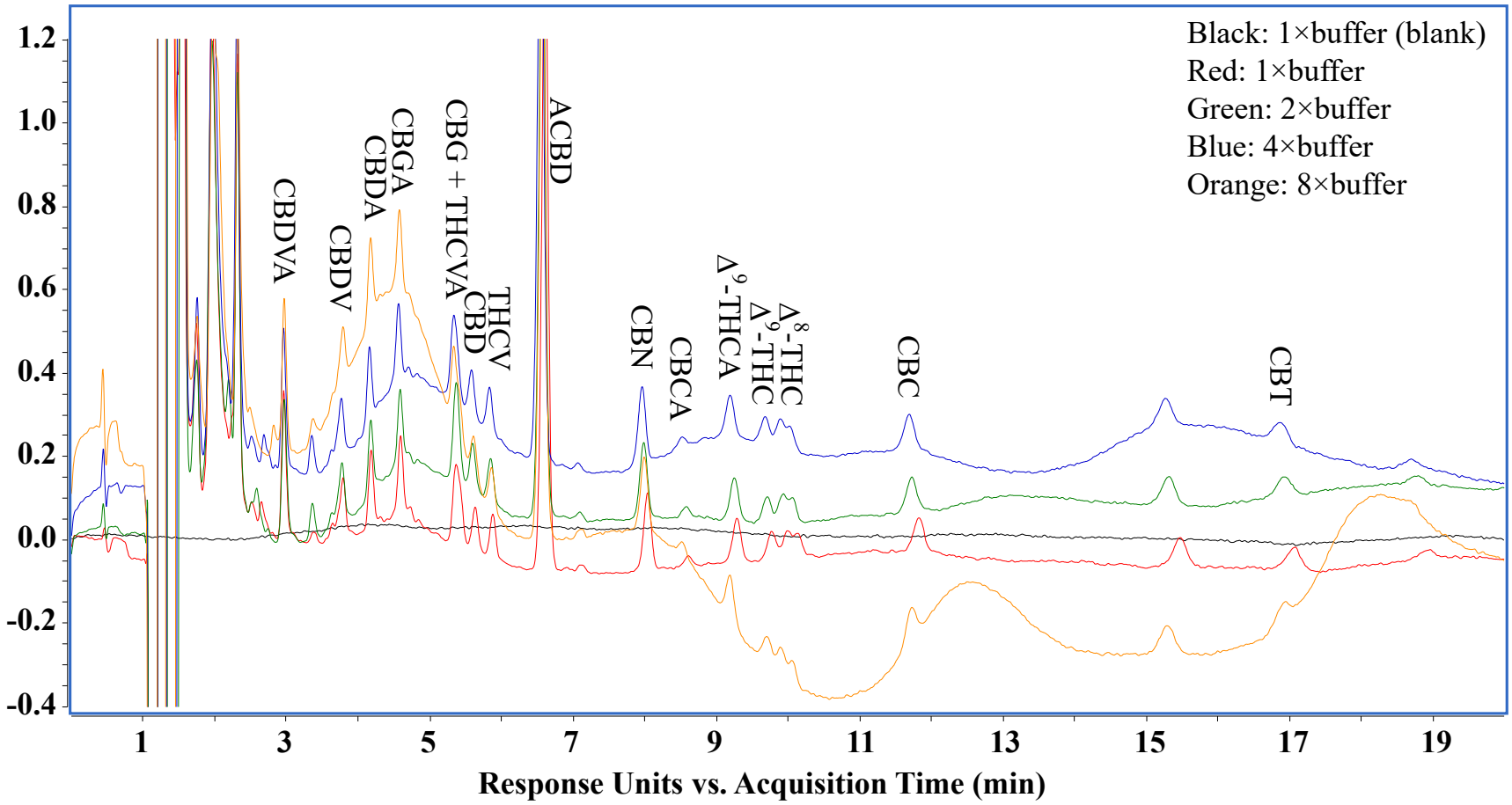

x10^1
2.0
1.5
1.0
0.5
0.0
CBCA at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)


x10^1
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)

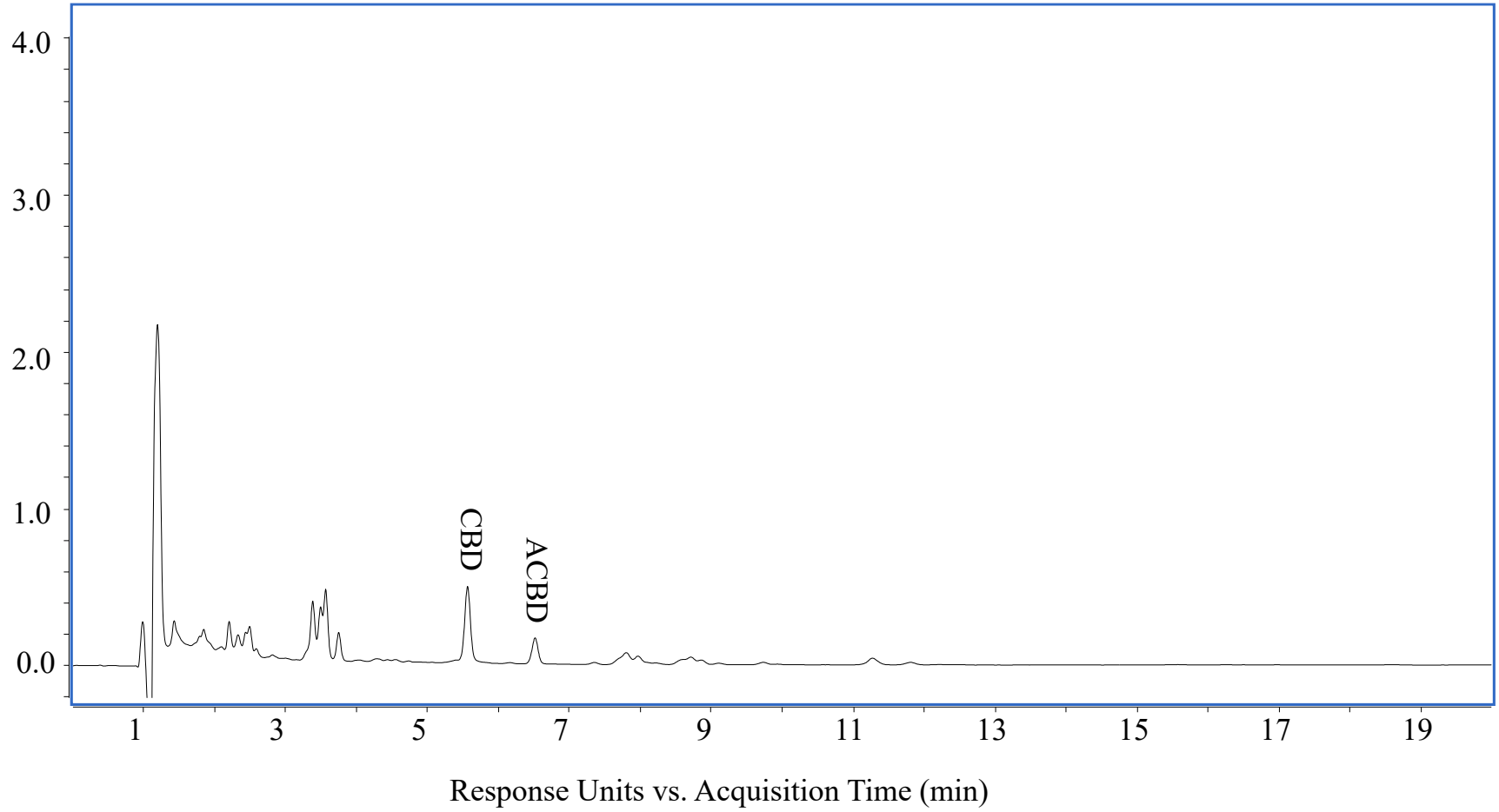

x10^1
2.0
1.5
1.0
0.5
0.0
CBN at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

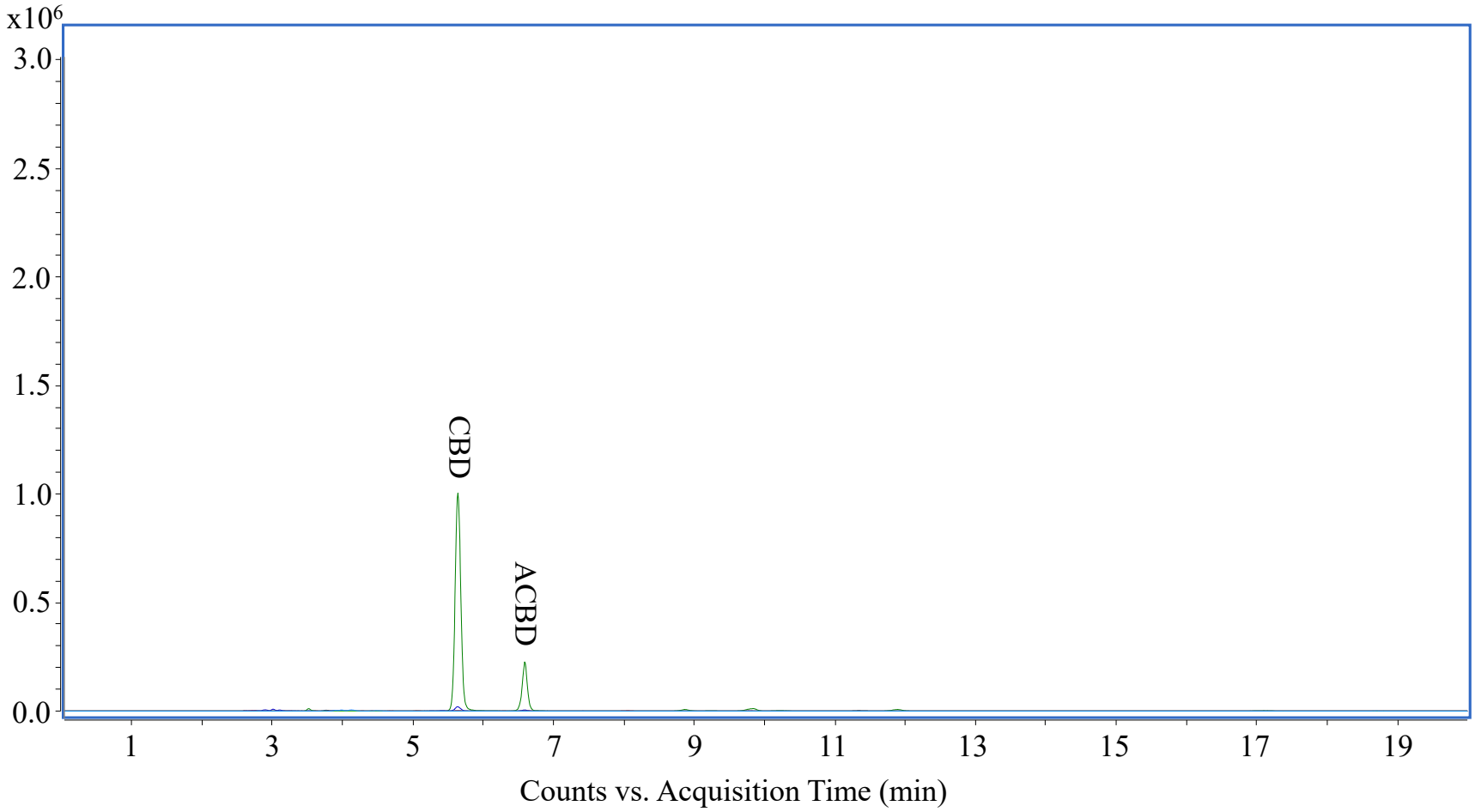

x10^1
2.0
1.5
1.0
0.5
0.0
CBT at 1 µg/mL
200
220
240
260
280
300
320
340
360
380
400
mAU vs. Wavelength (nm)

Supplementary Fig. S3. LC-UV chromatograms of cannabinoids at LOQ level, i.e., 0.02 µg/mL, except for ACBD at 0.5 µg/mL under optimized LC conditions: an aqueous solvent containing 2 mM ammonium formate and 0.011% (v/v) formic acid and a mobile phase containing 73% (v/v) acetonitrile at 30ºC.

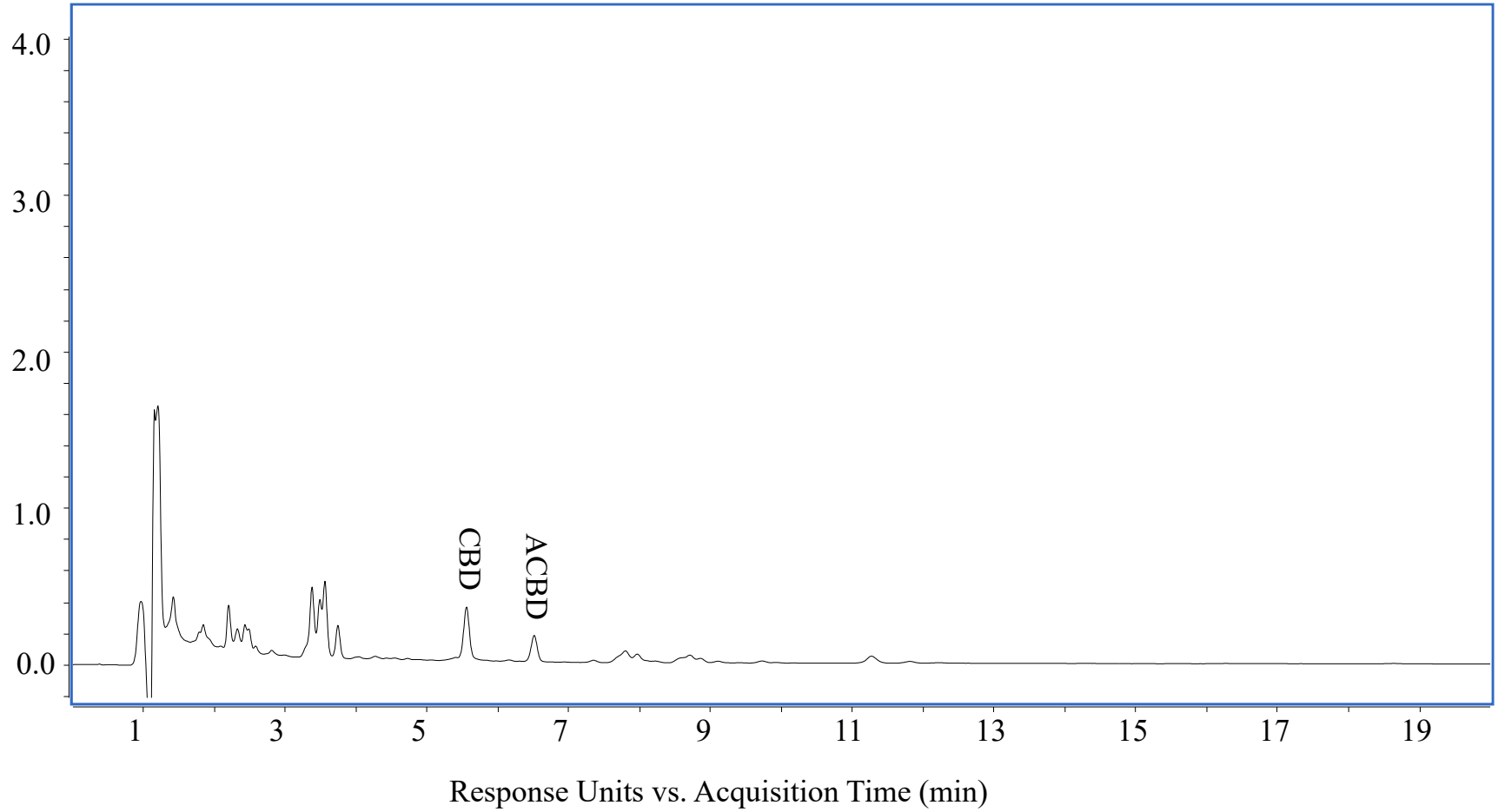


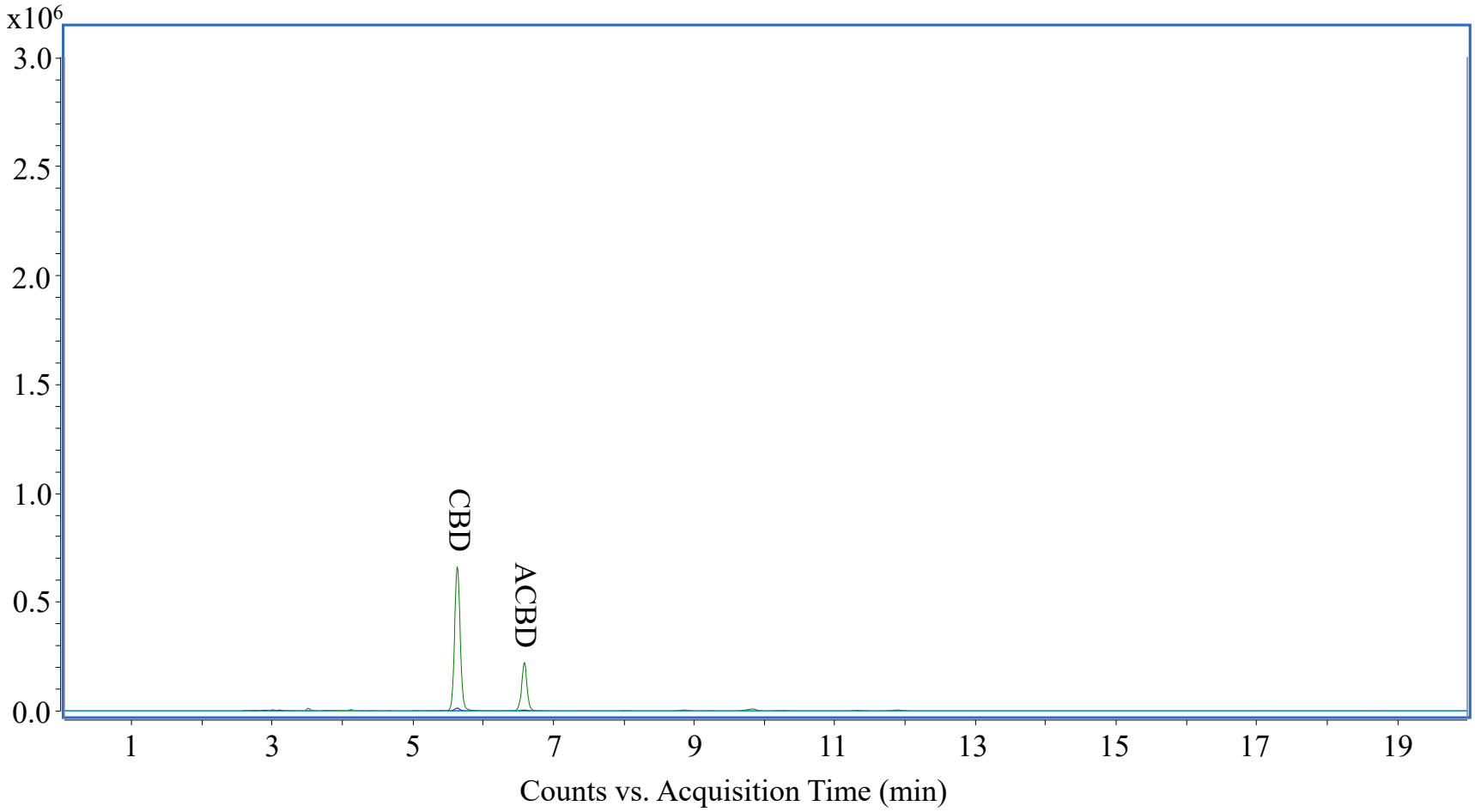
Supplementary Fig. S4. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 2.50 mg/mL grapefruit sparkling coconut water spiked with 0.003% (w/w) ACBD.



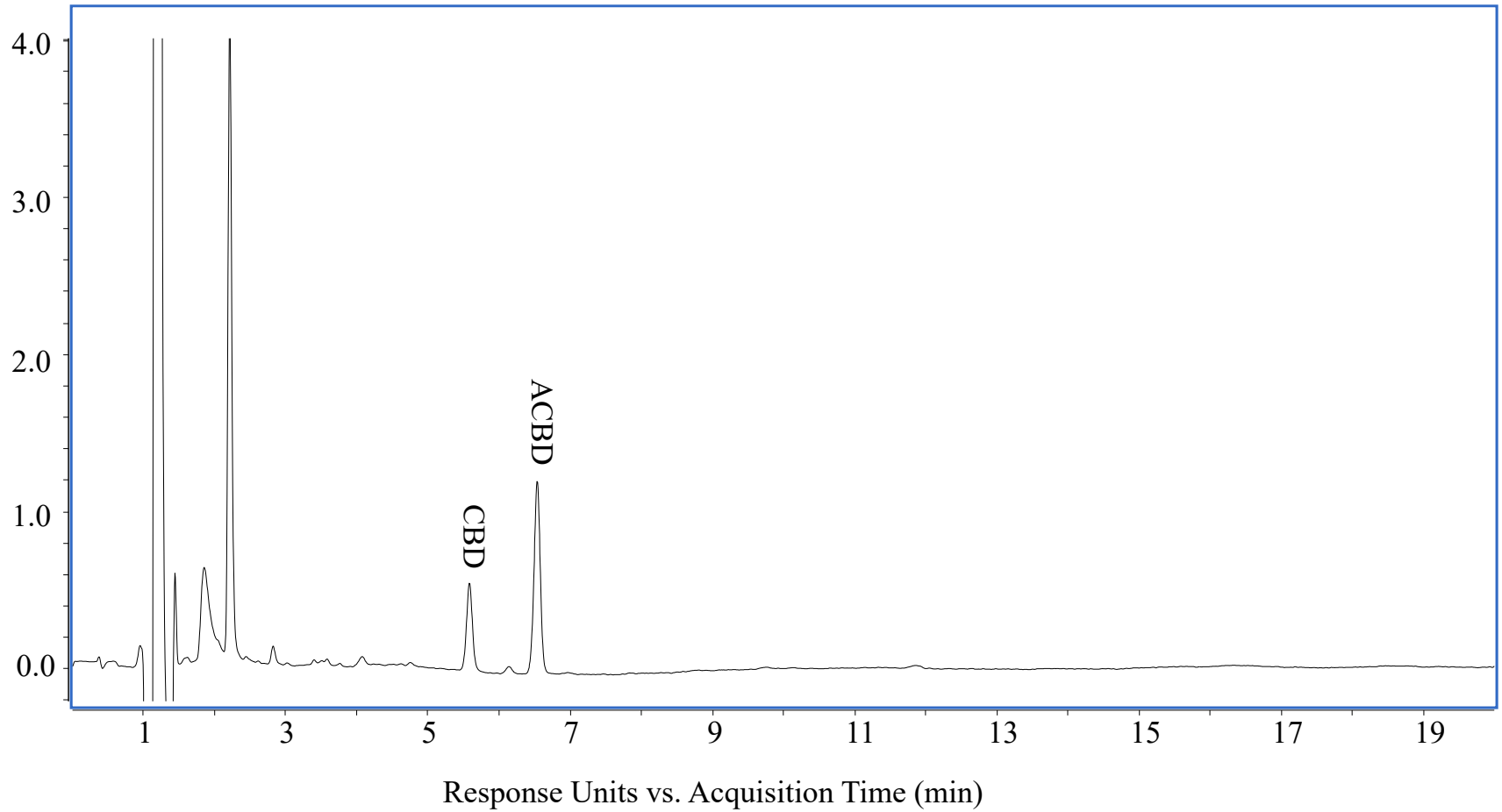

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

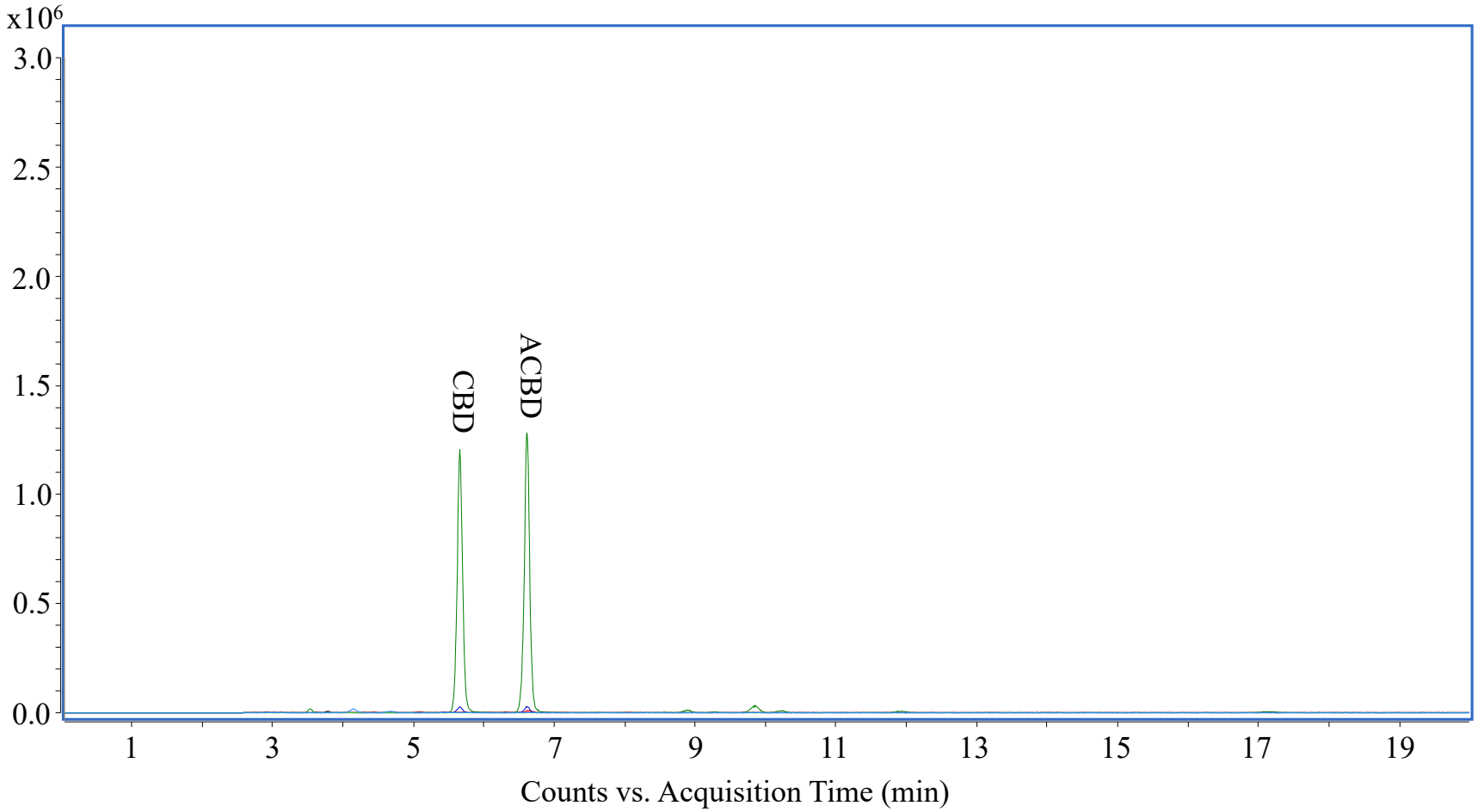
Supplementary Fig. S5. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 2.50 mg/mL lemon sparkling black tea spiked with 0.003% (w/w) ACBD.



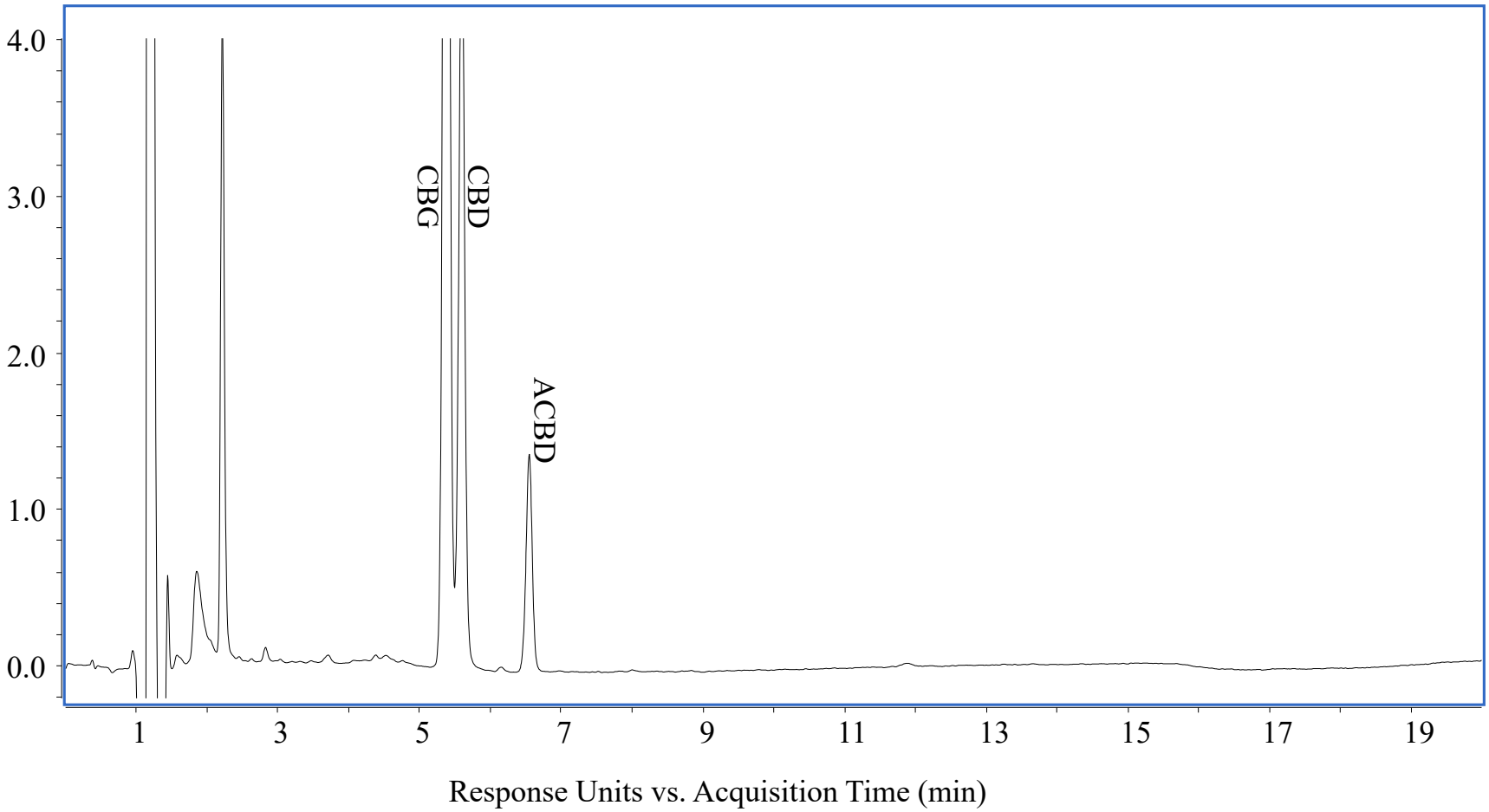

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

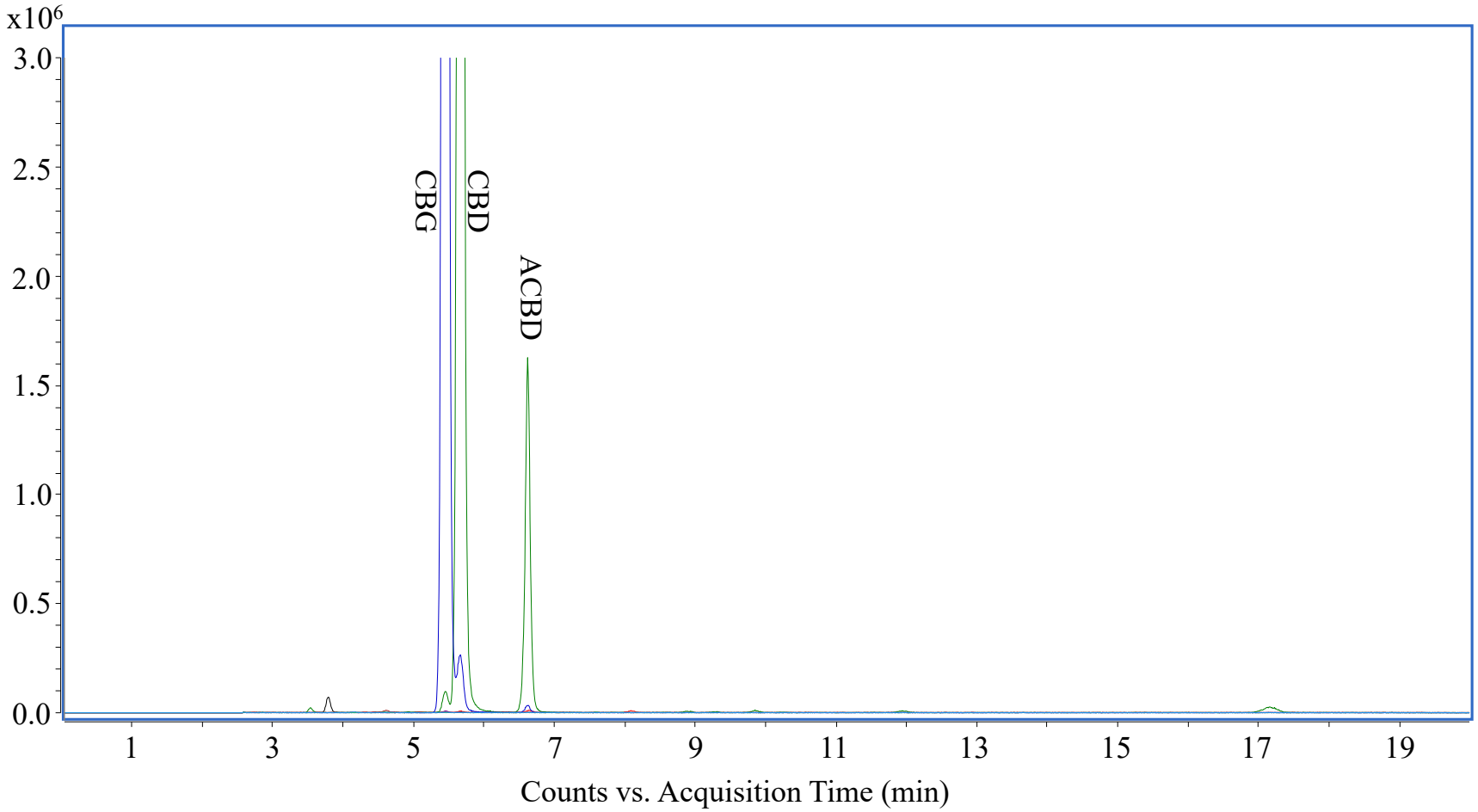
Supplementary Fig. S6. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL SCD probiotics + CBD spiked with 0.3% (w/w) ACBD.



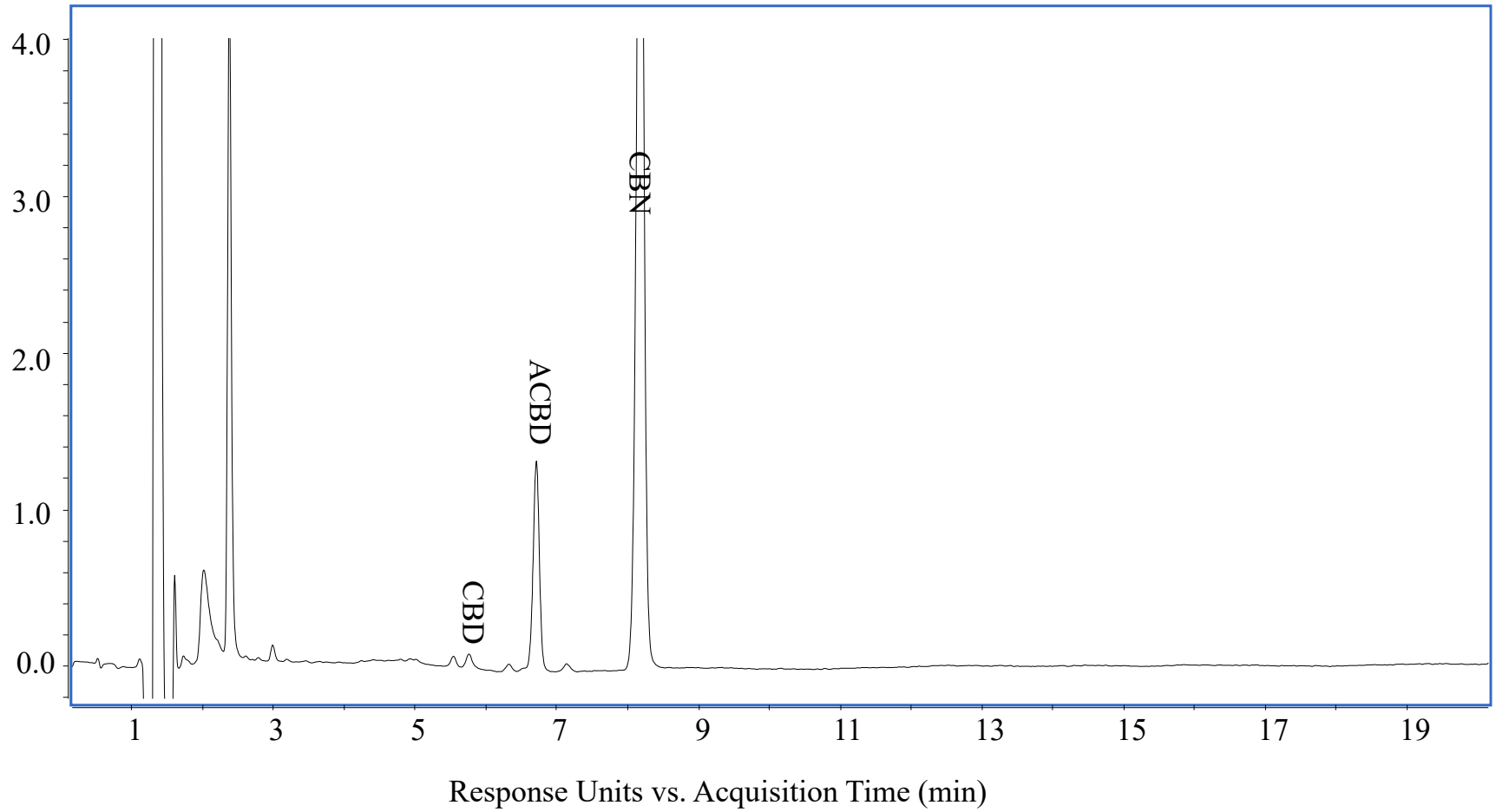

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

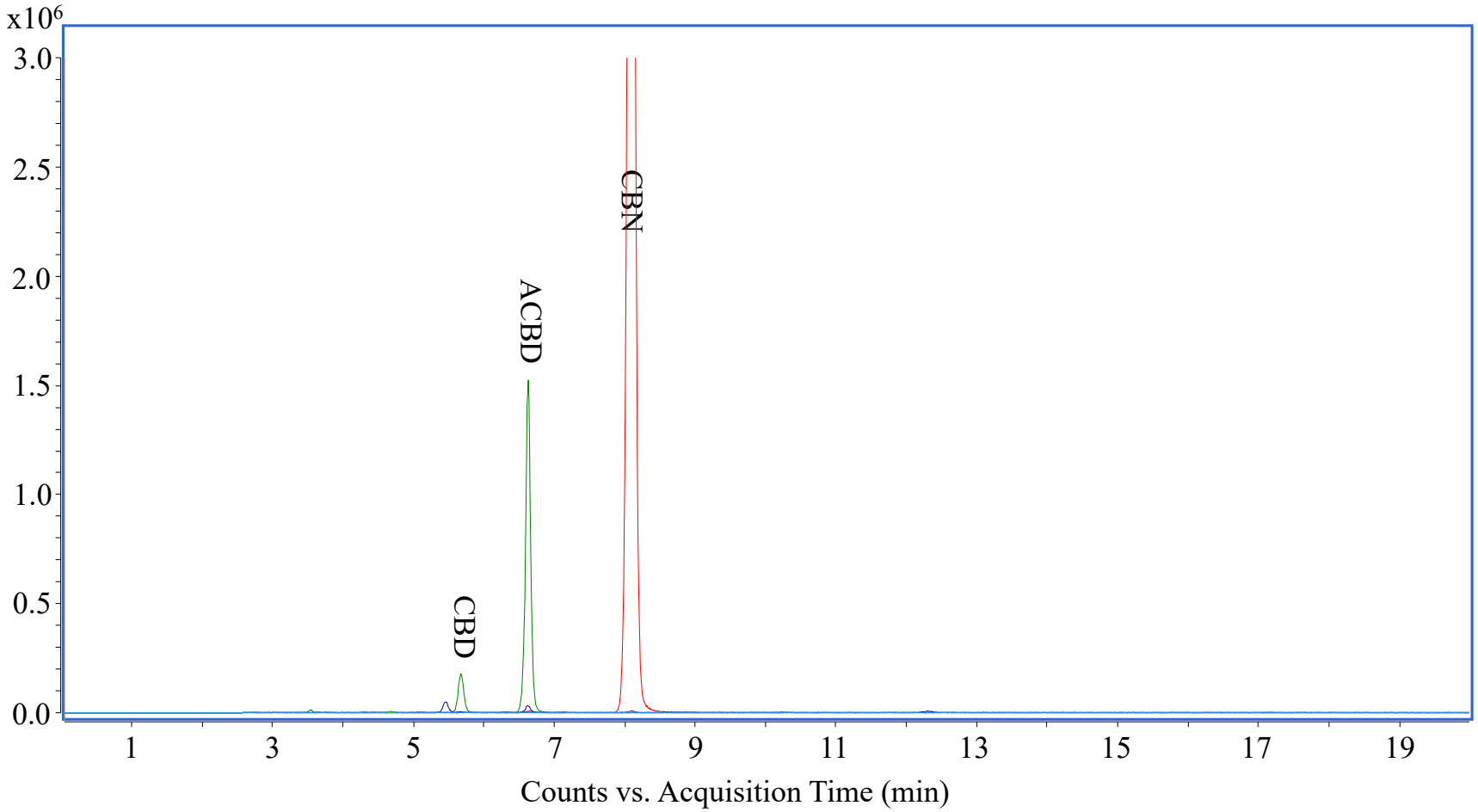
Supplementary Fig. S7. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL water soluble CBG oil spiked with 0.3% (w/w) ACBD.



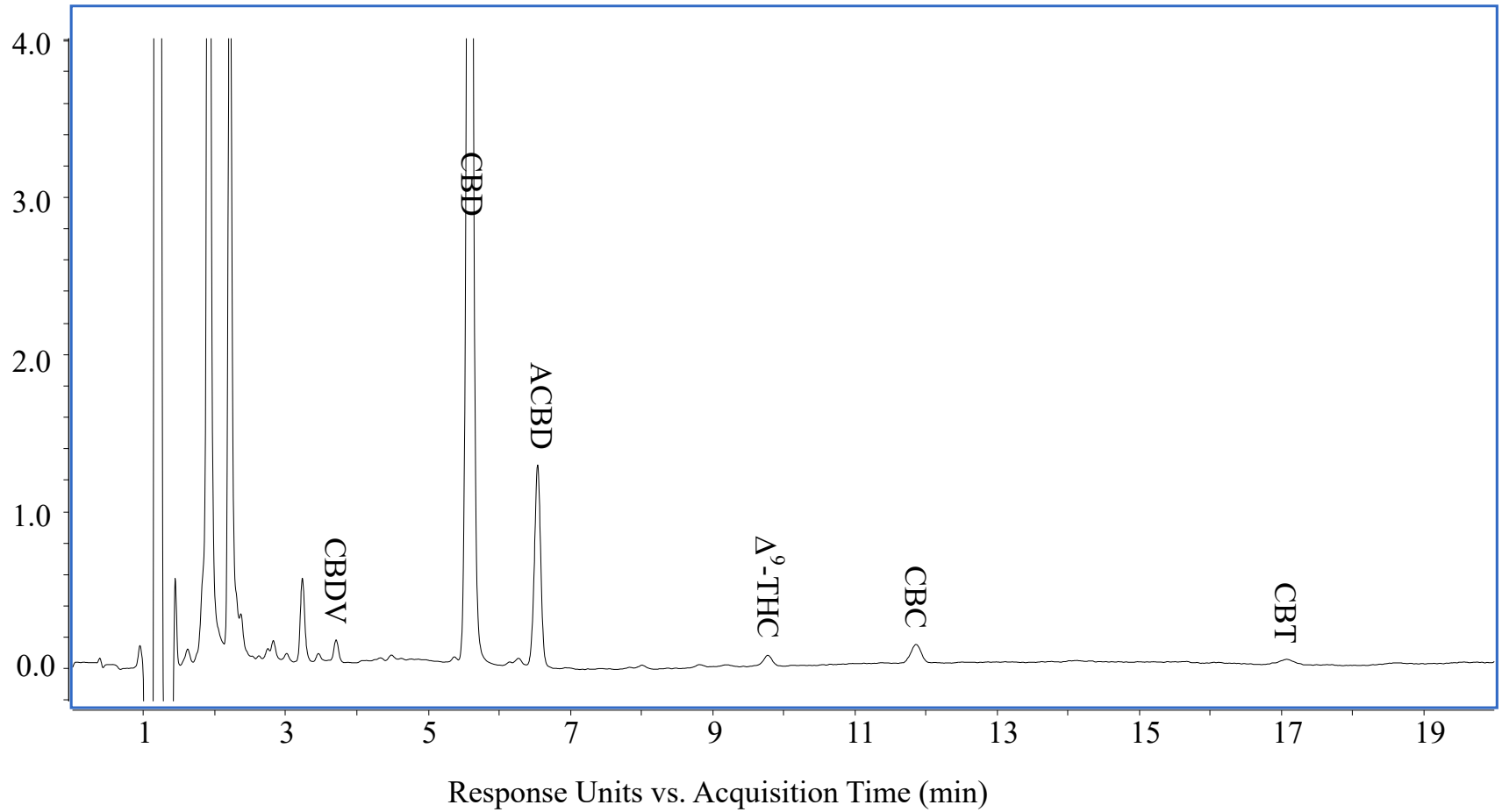

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBG
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

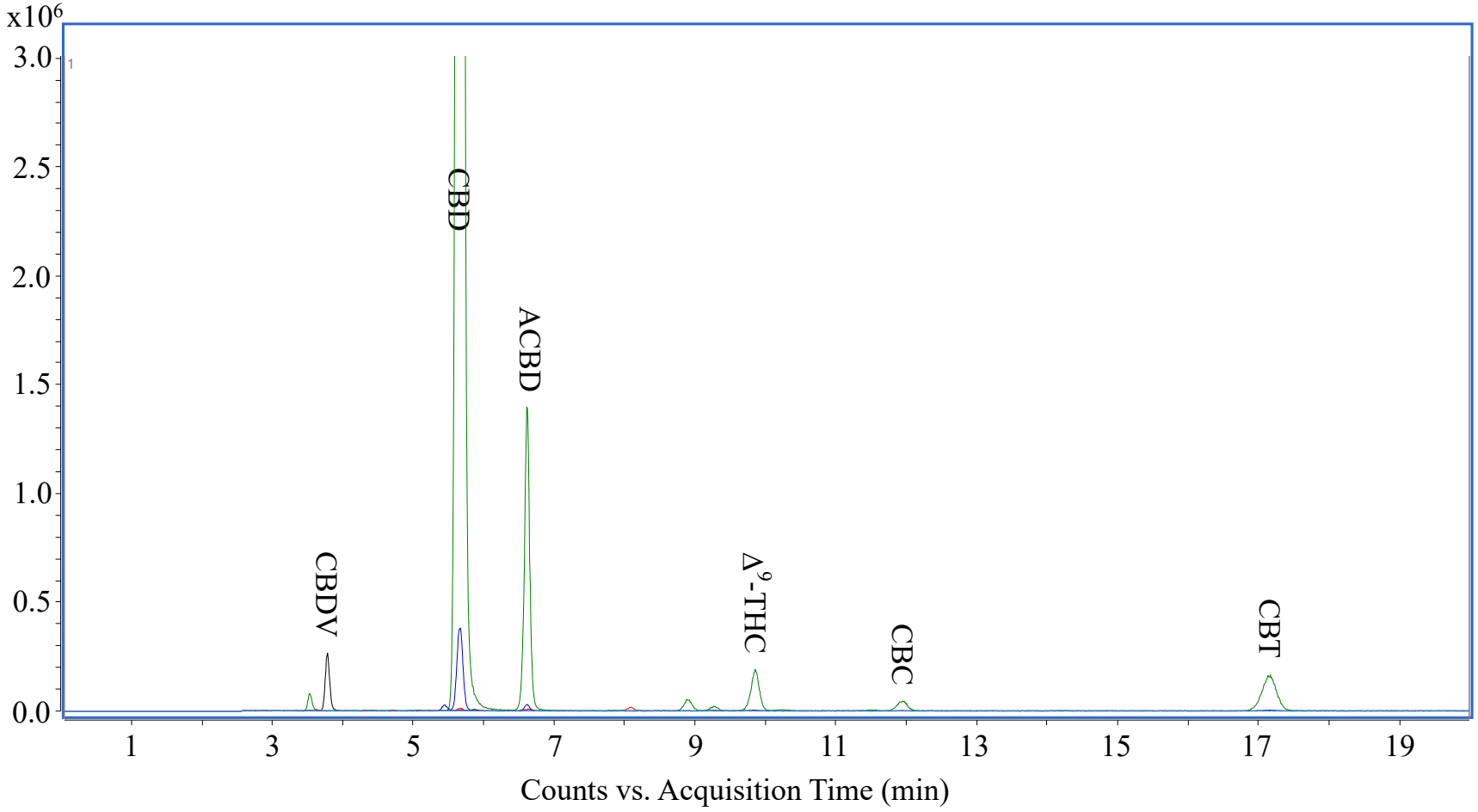
Supplementary Fig. S8. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBNight water soluble CBN oil spiked with 0.3% (w/w) ACBD.



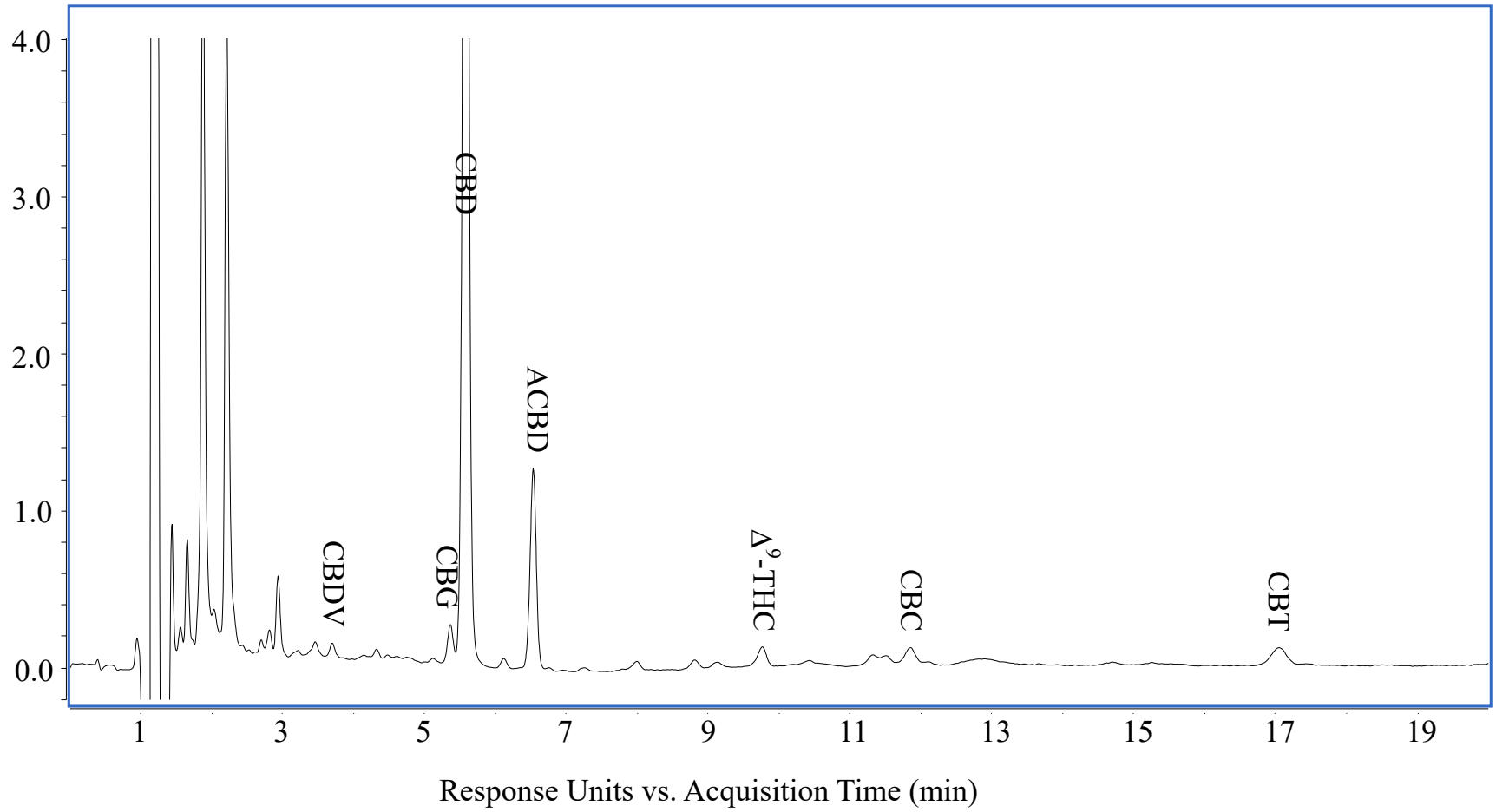

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
CBN
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

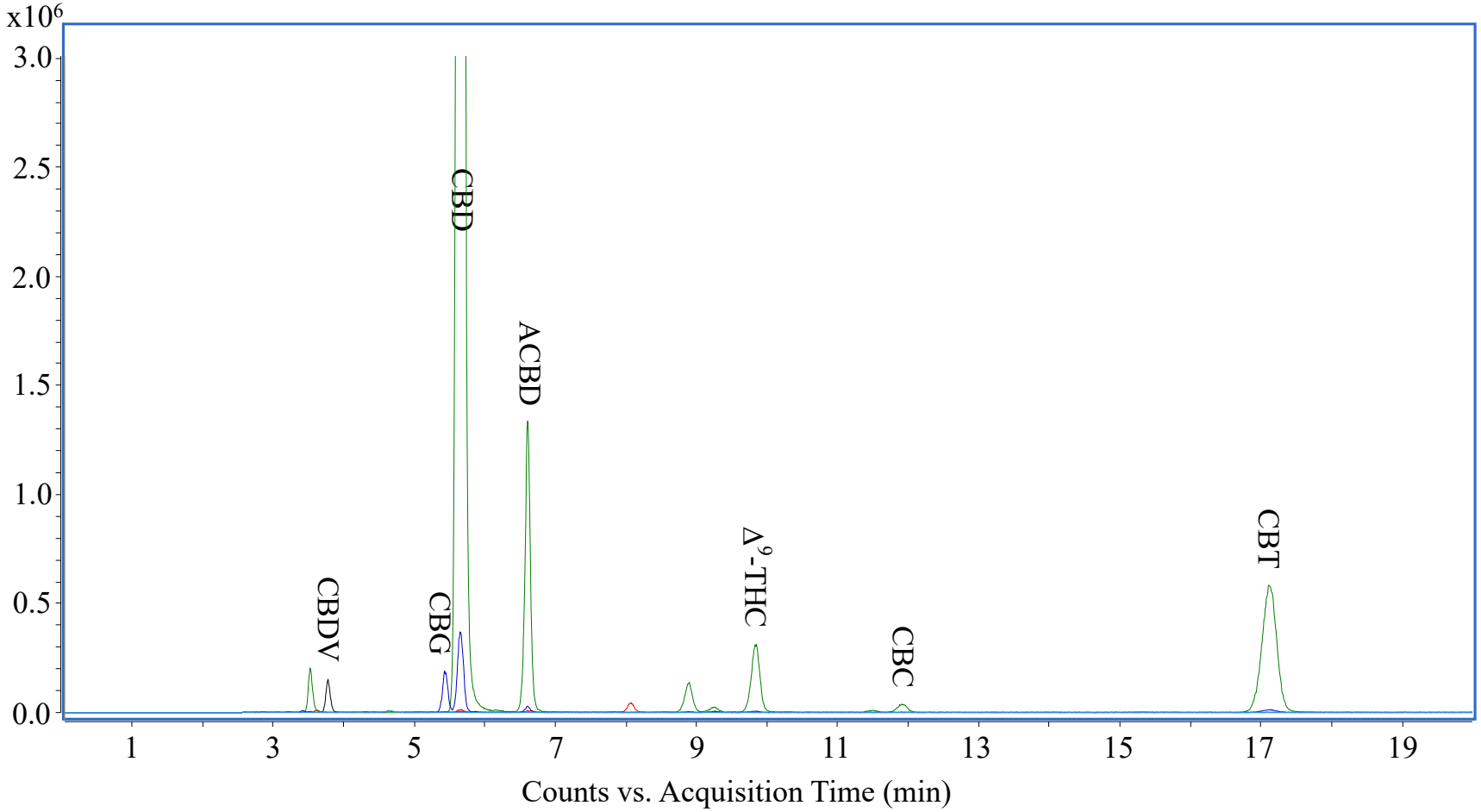
Supplementary Fig. S9. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL water soluble full spectrum hemp oil (grape flavor) spiked with 0.3% (w/w) ACBD.



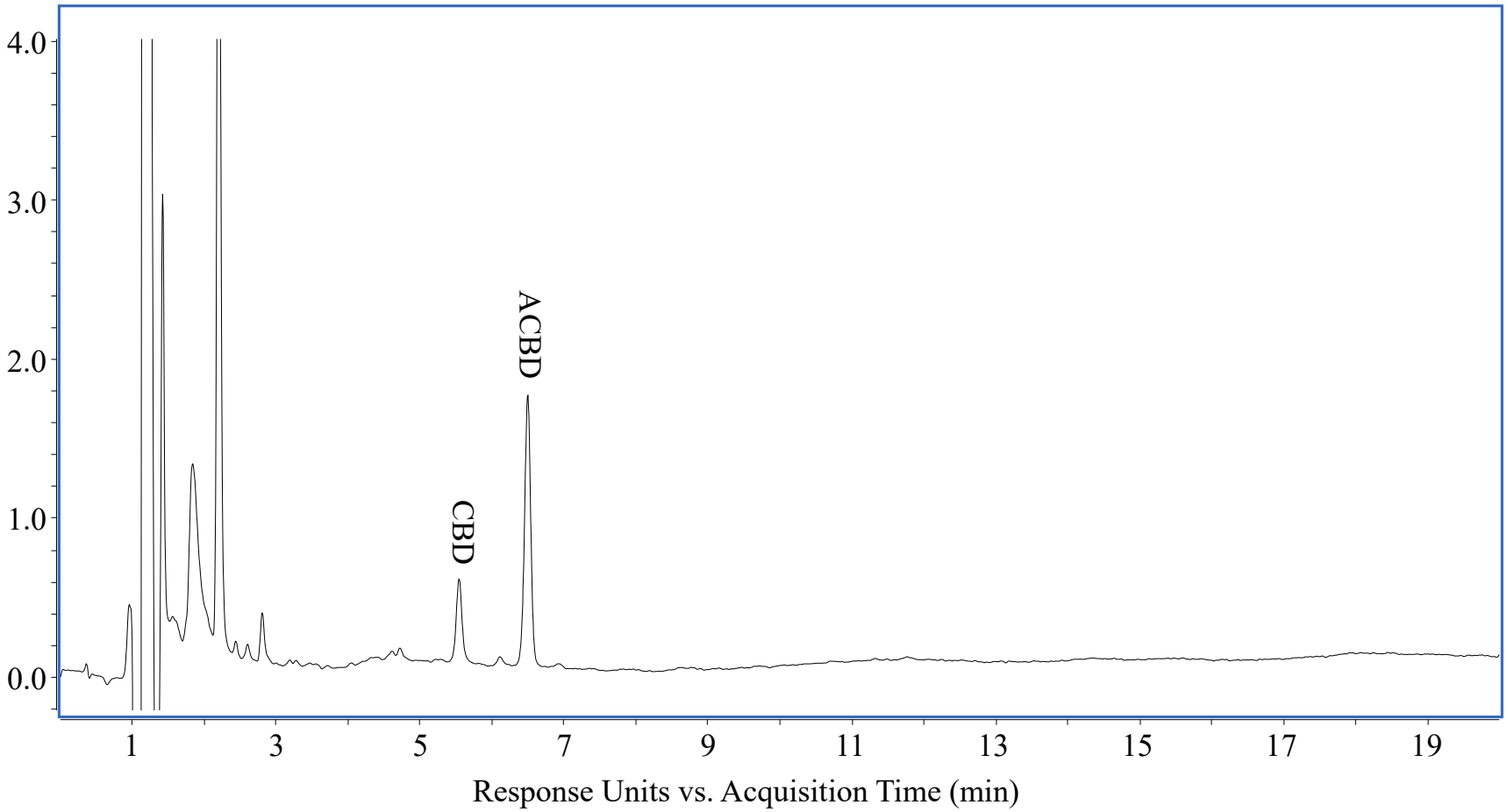

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBDV
CBD
ACBD
Δ9-THC
CBC
CBT
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

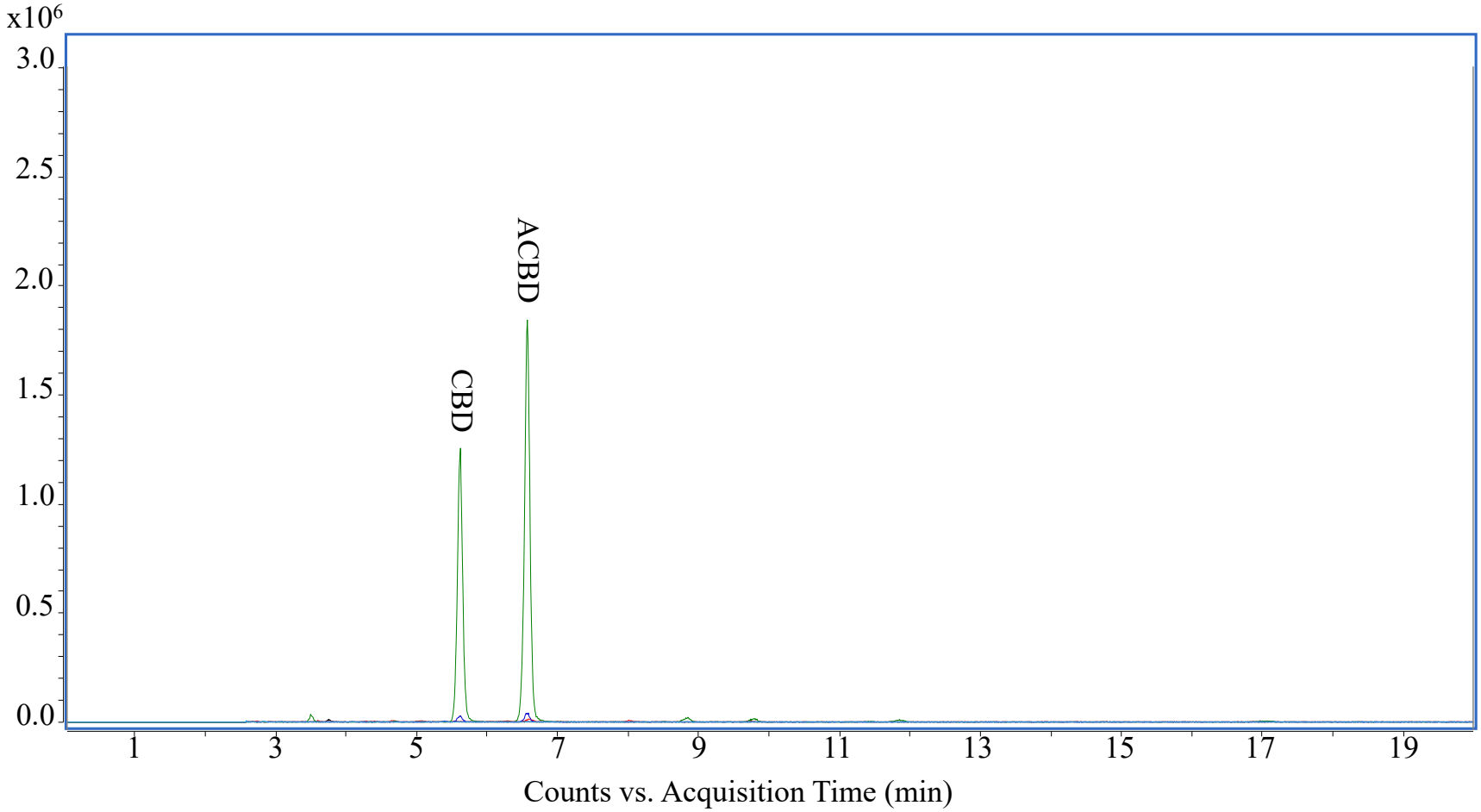
Supplementary Fig. S10. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL terpene rich hemp oil tincture (cinnamon flavor) spiked with 0.3% (w/w) ACBD.



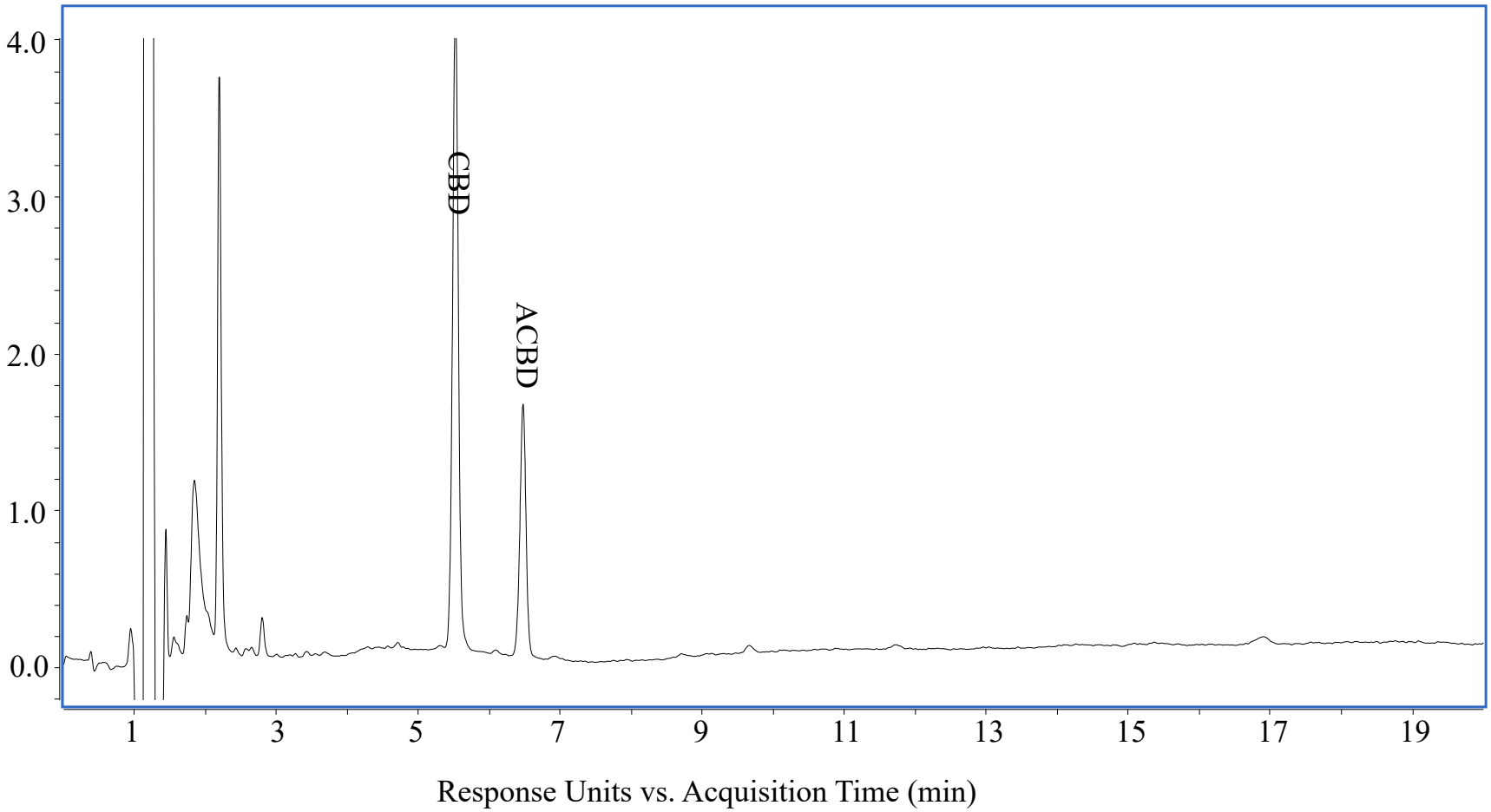

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBDV
CBG
CBD
ACBD
Δ9-THC
CBC
CBT
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

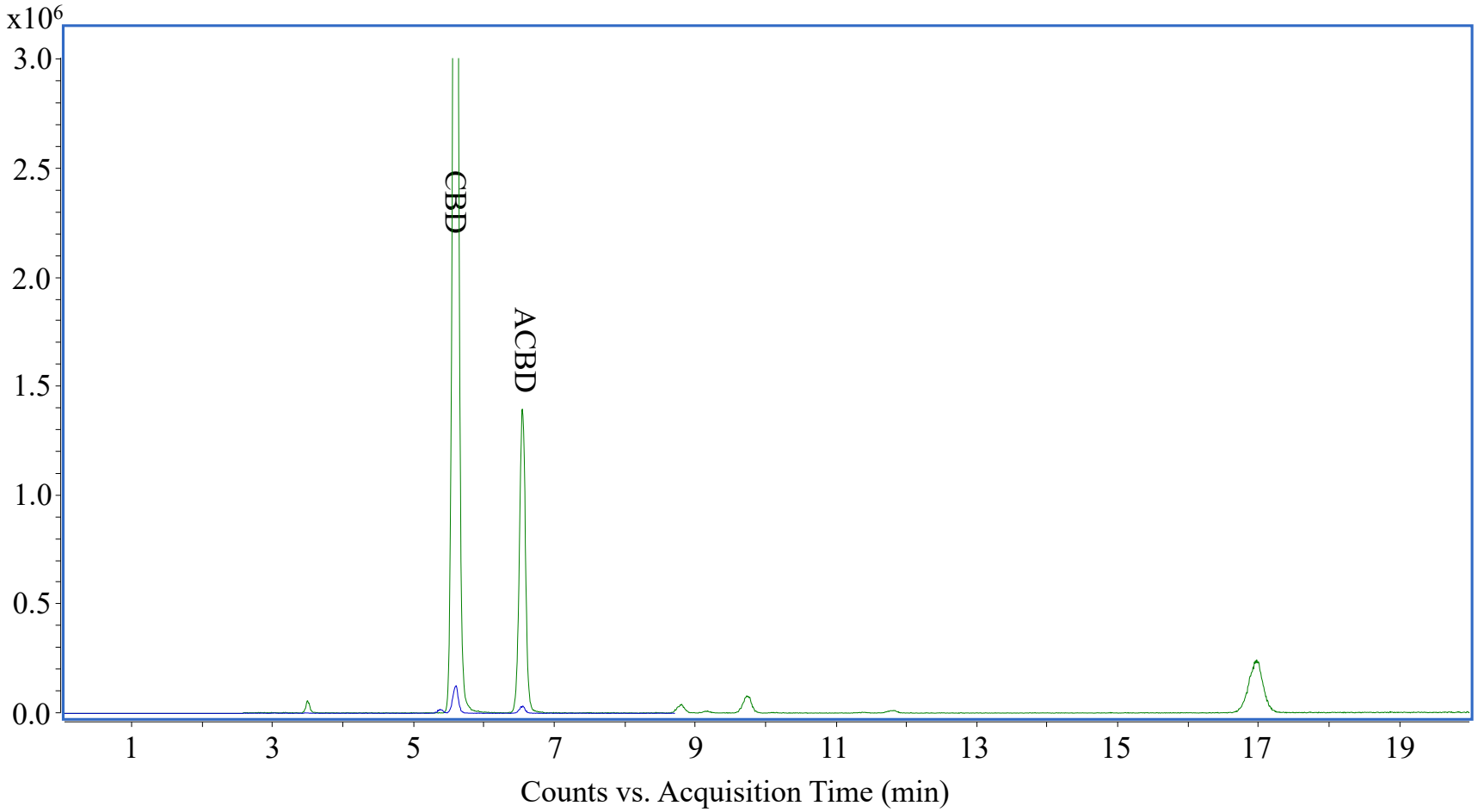
Supplementary Fig. S11. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD coffee spiked with 0.3% (w/w) ACBD.



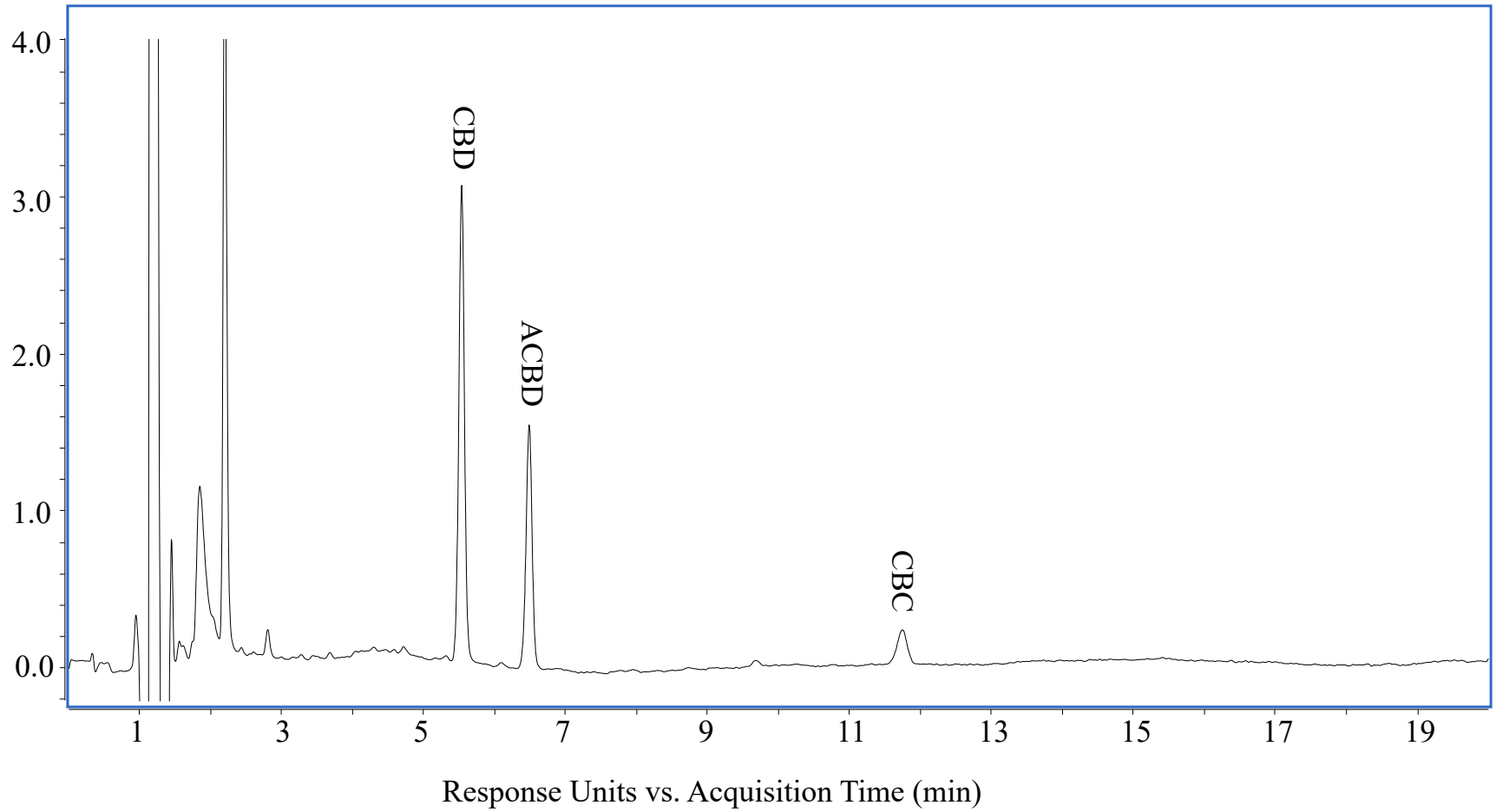

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

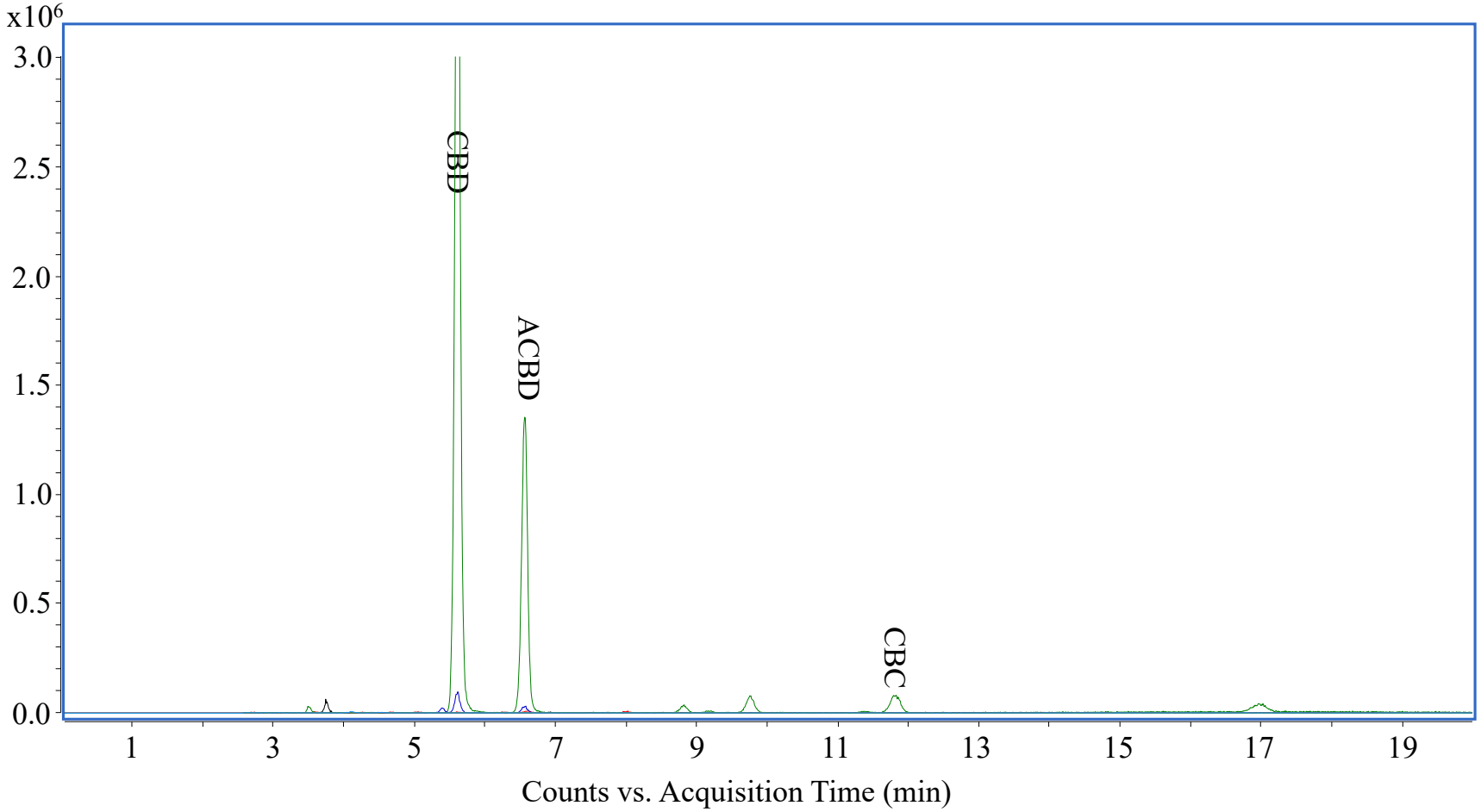
Supplementary Fig. S12. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD energy gummies (lemon flavor) with 0.3% (w/w) ACBD.



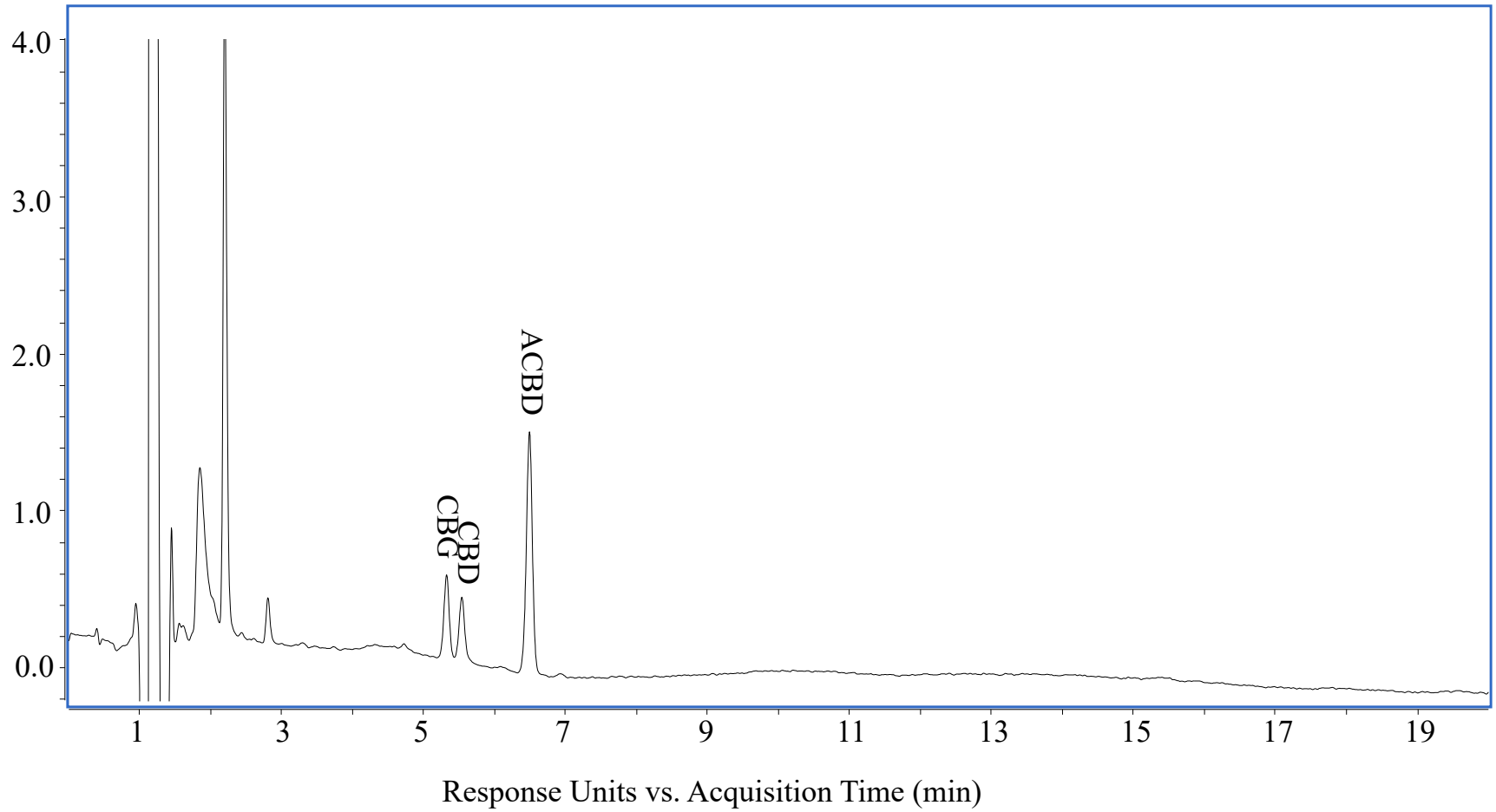

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

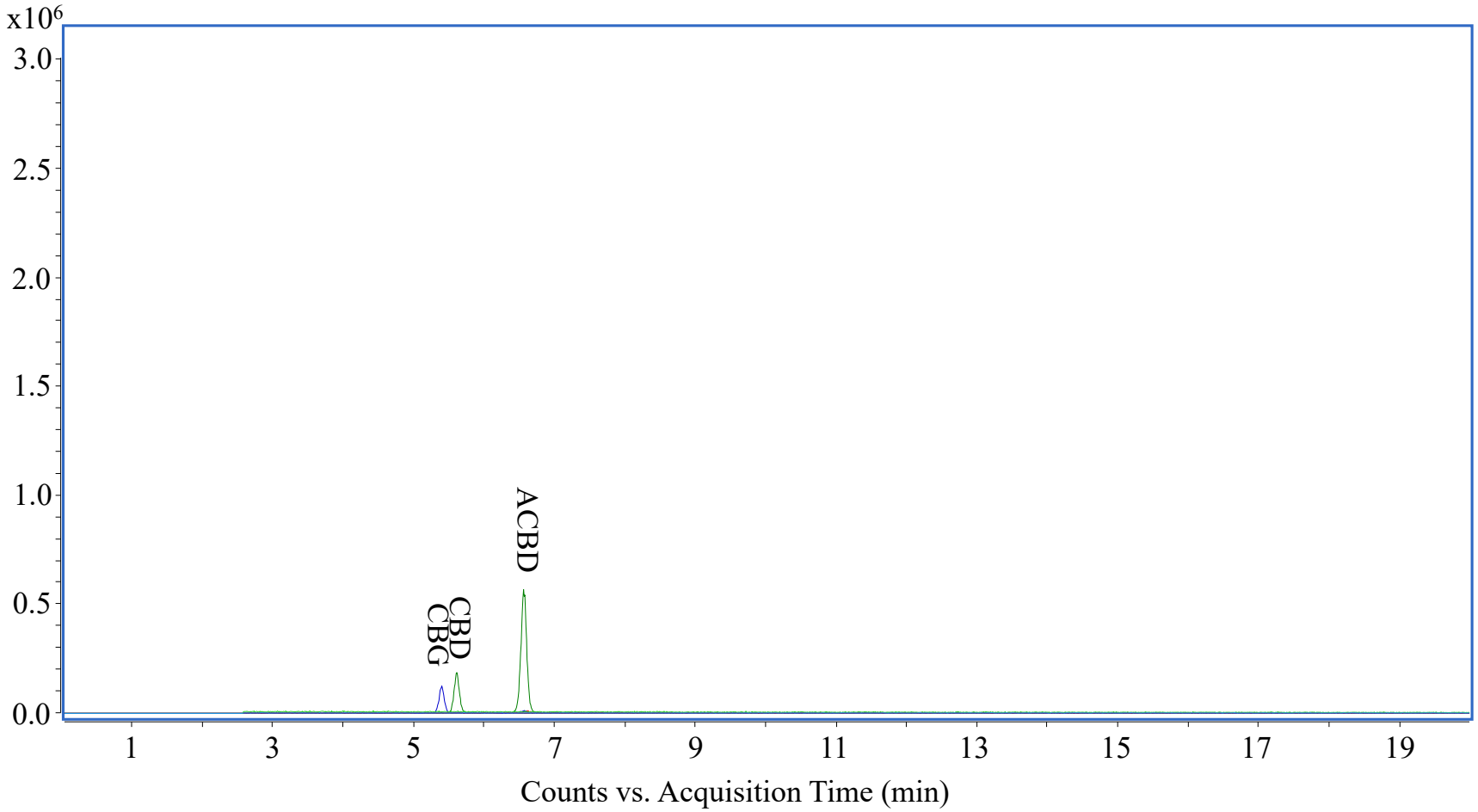
Supplementary Fig. S13. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD gourmet gummies (sweet watermelon flavor) spiked with 0.3% (w/w) ACBD.



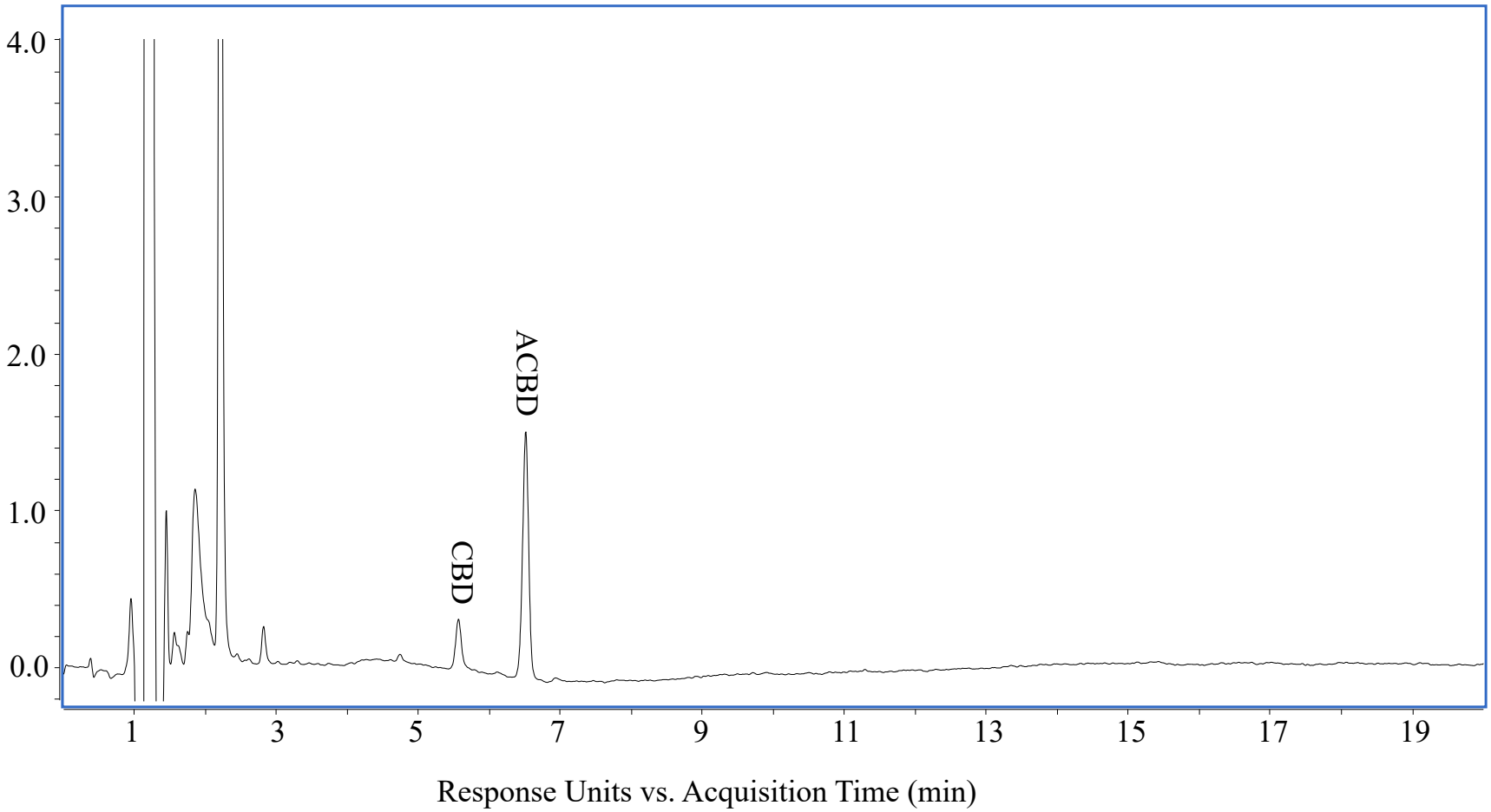

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

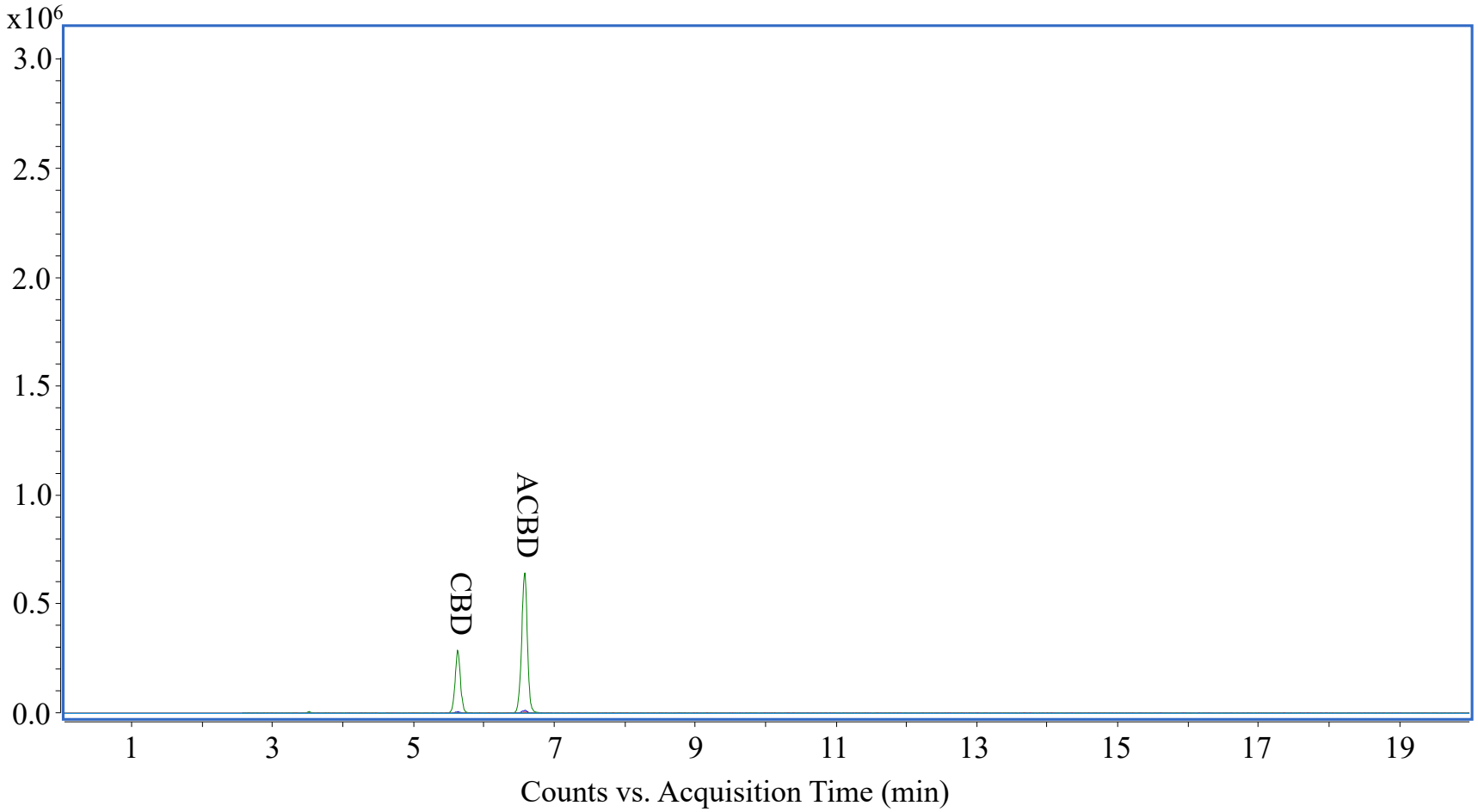
Supplementary Fig. S14. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBG gummies (cherry flavor) spiked with 0.3% (w/w) ACBD.



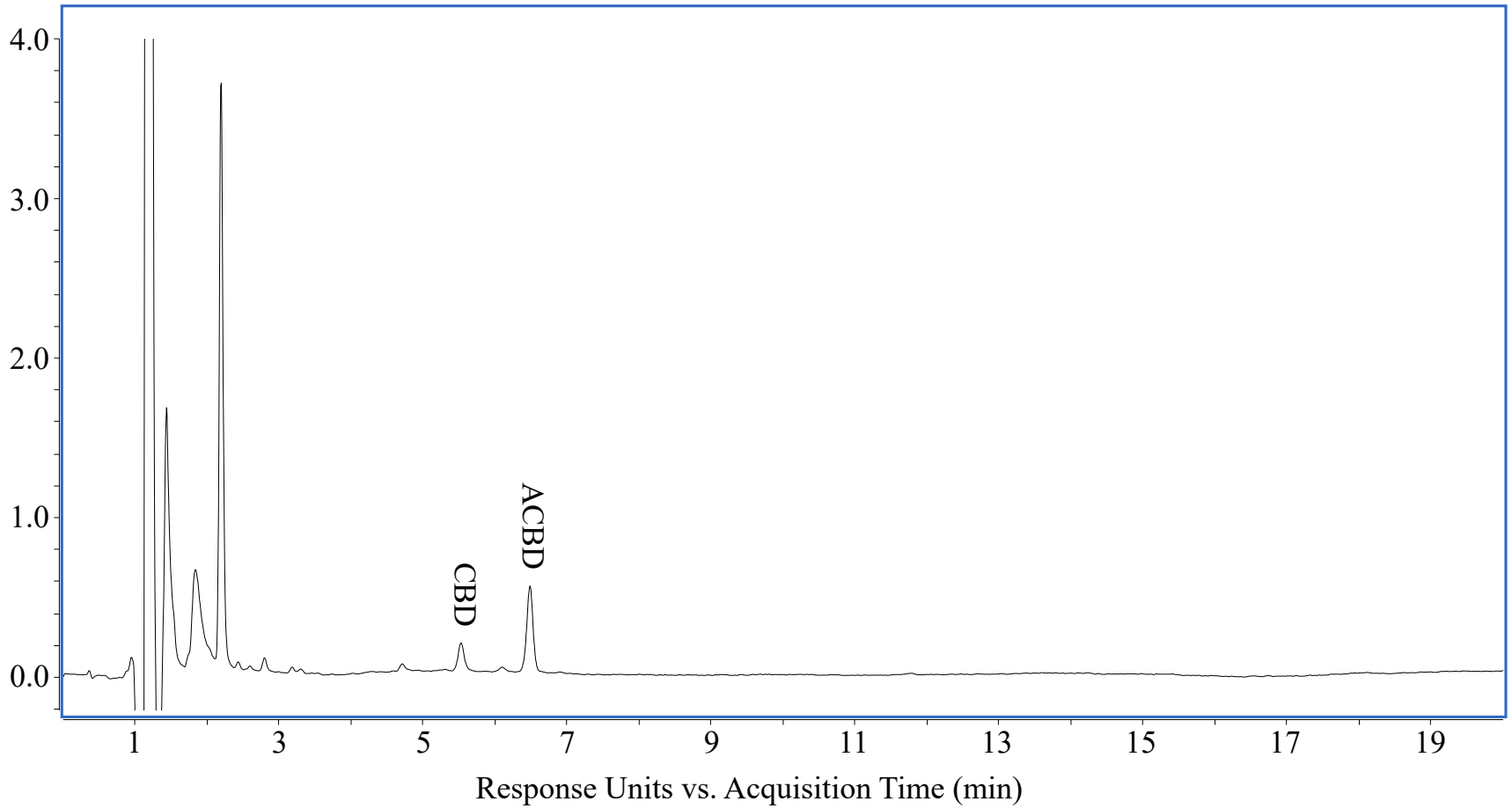

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBG
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

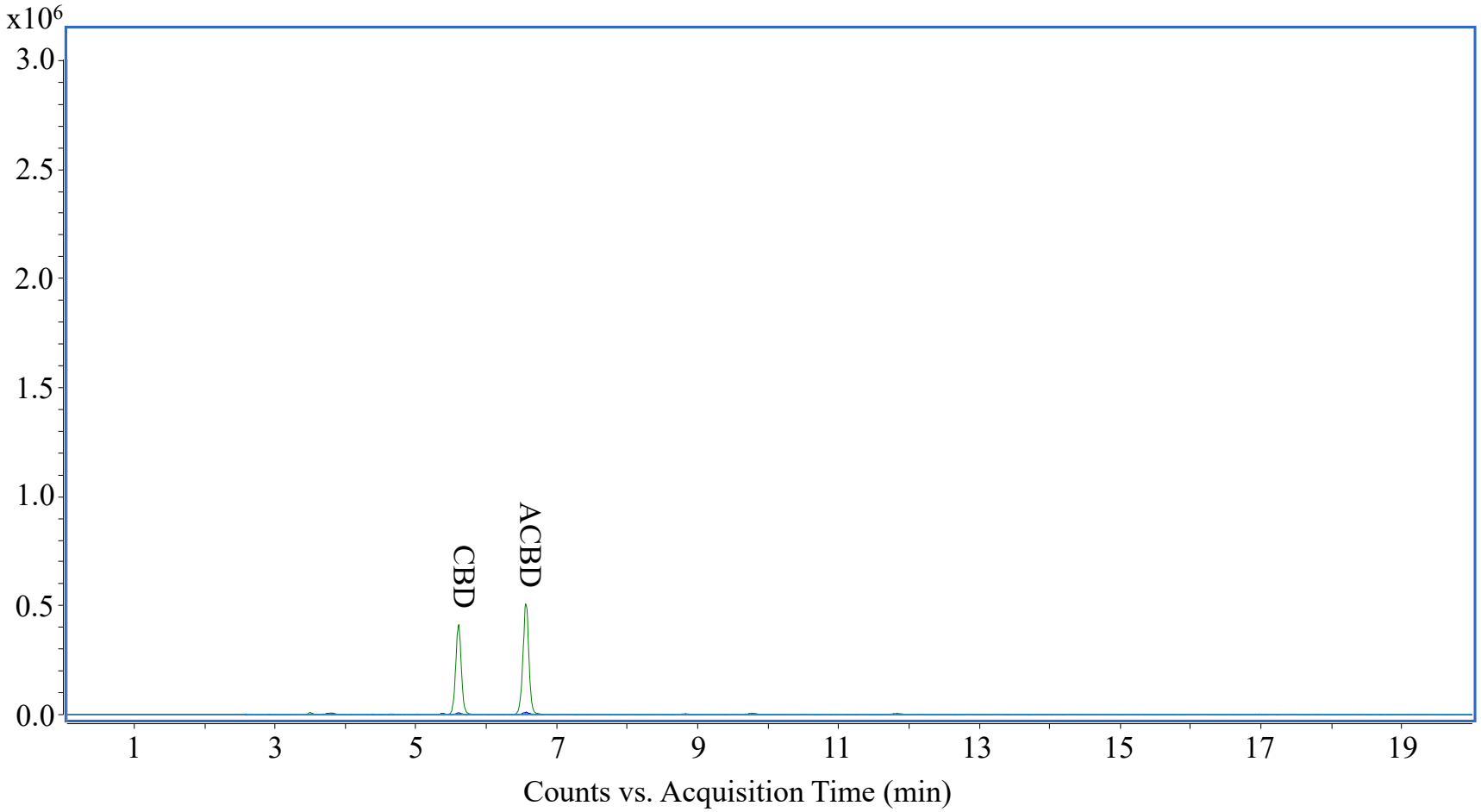
Supplementary Fig. S15. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD hard candy spiked with 0.3% (w/w) ACBD.



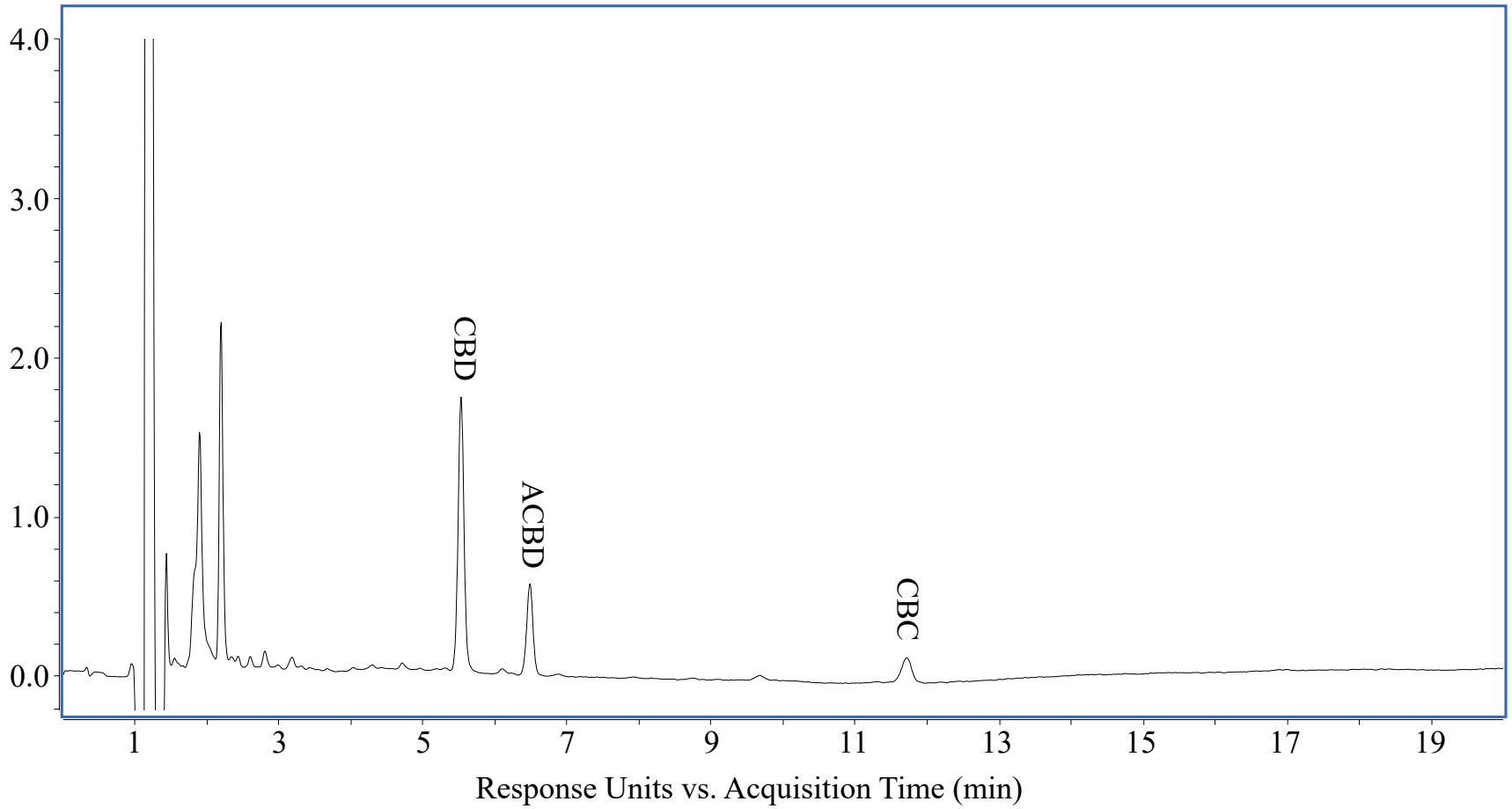

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

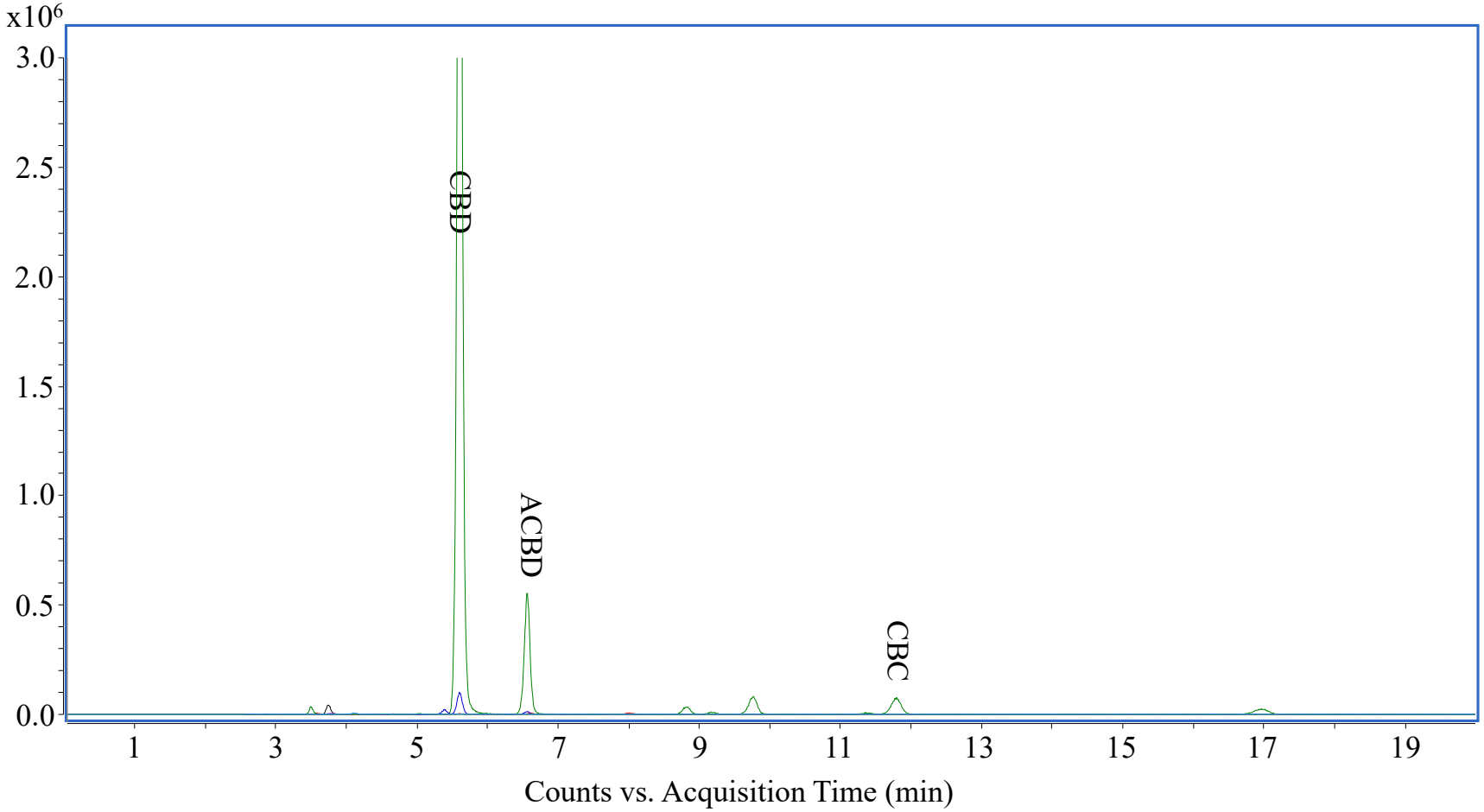
Supplementary Fig. S16. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD brownies spiked with 0.1% (w/w) ACBD.



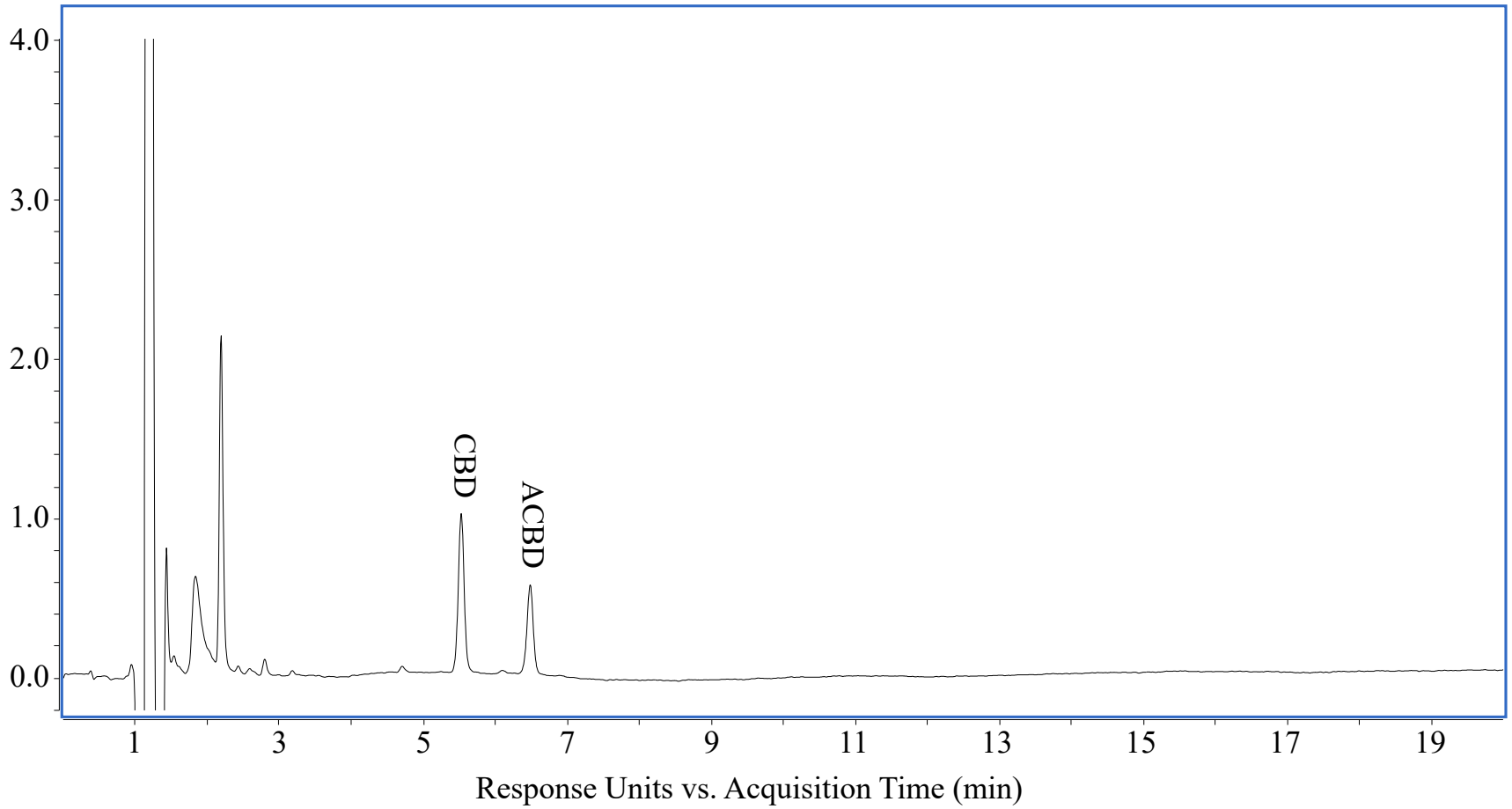

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

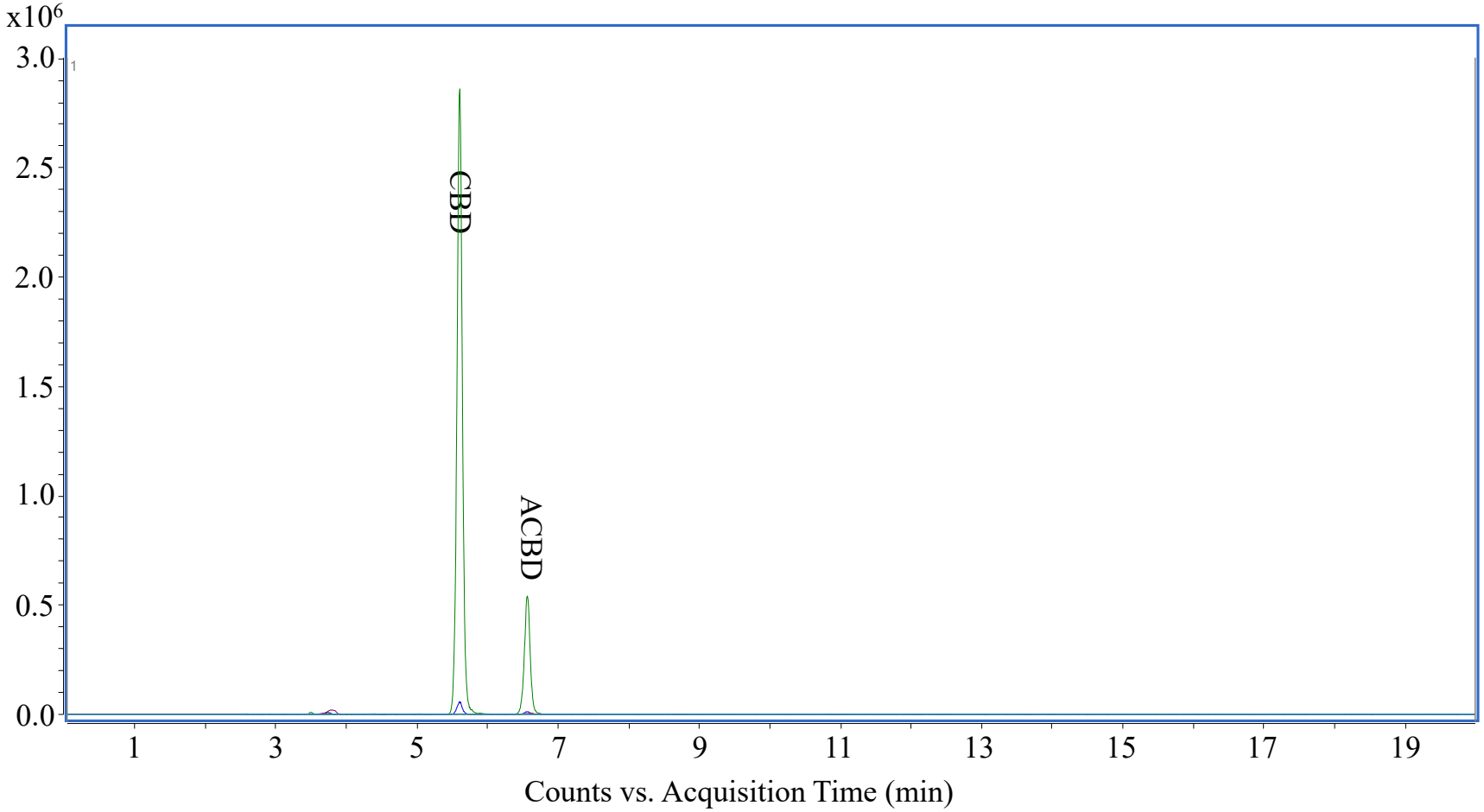
Supplementary Fig. S17. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD cookies (Hermit) spiked with 0.1% (w/w) ACBD.



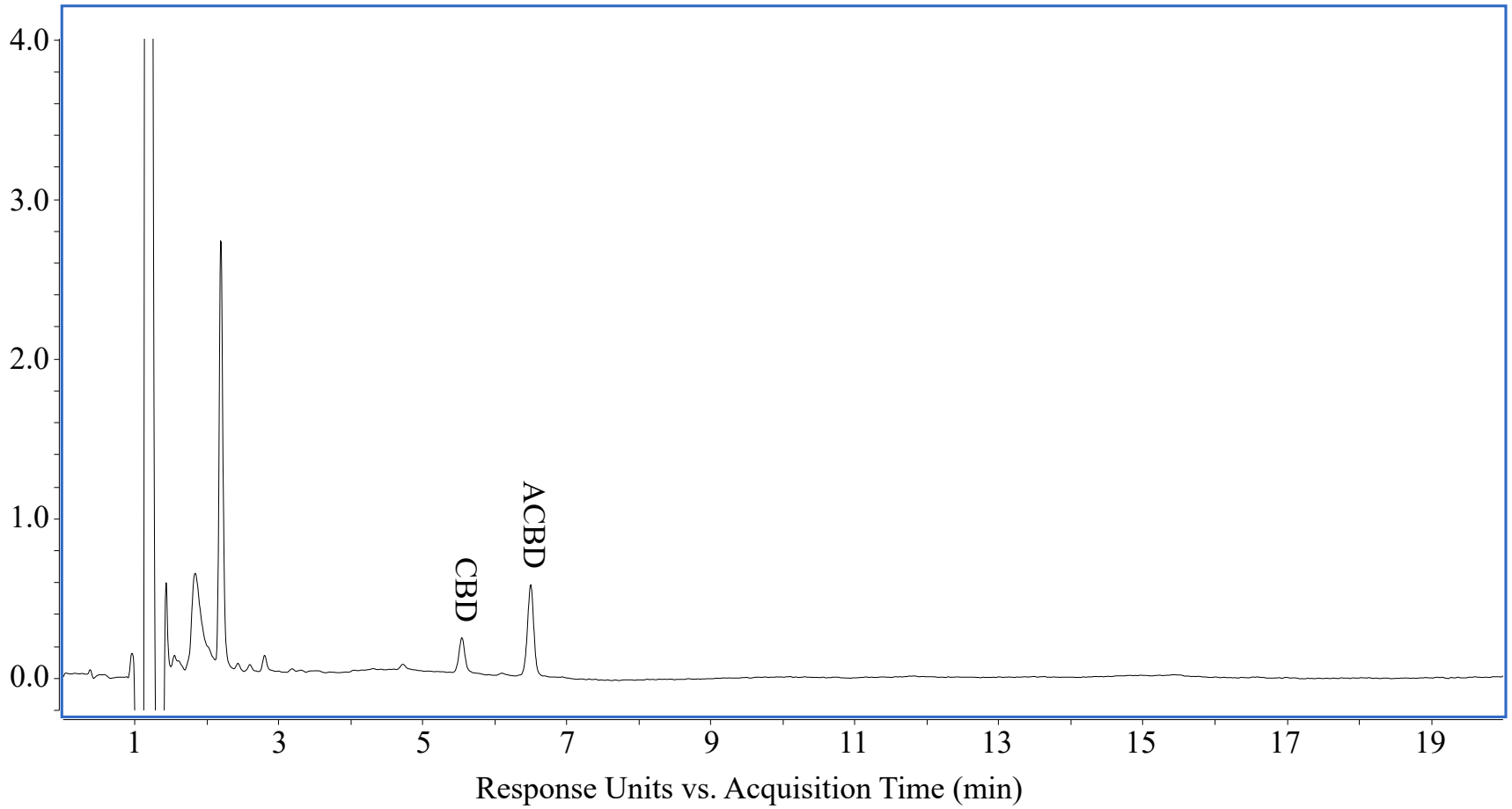

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

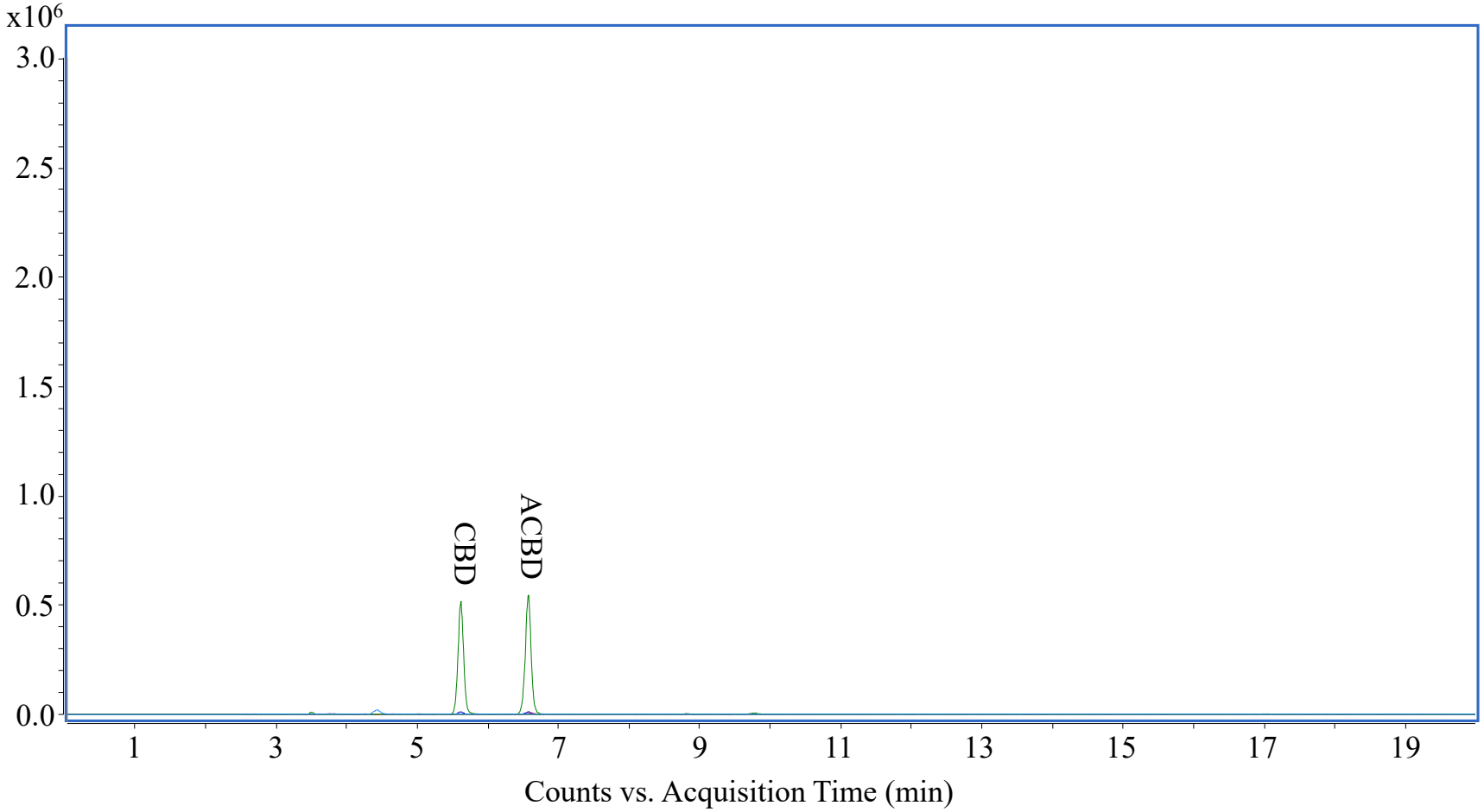
Supplementary Fig. S18. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD krispy cereal marshmallow treats spiked with 0.1% (w/w) ACBD.



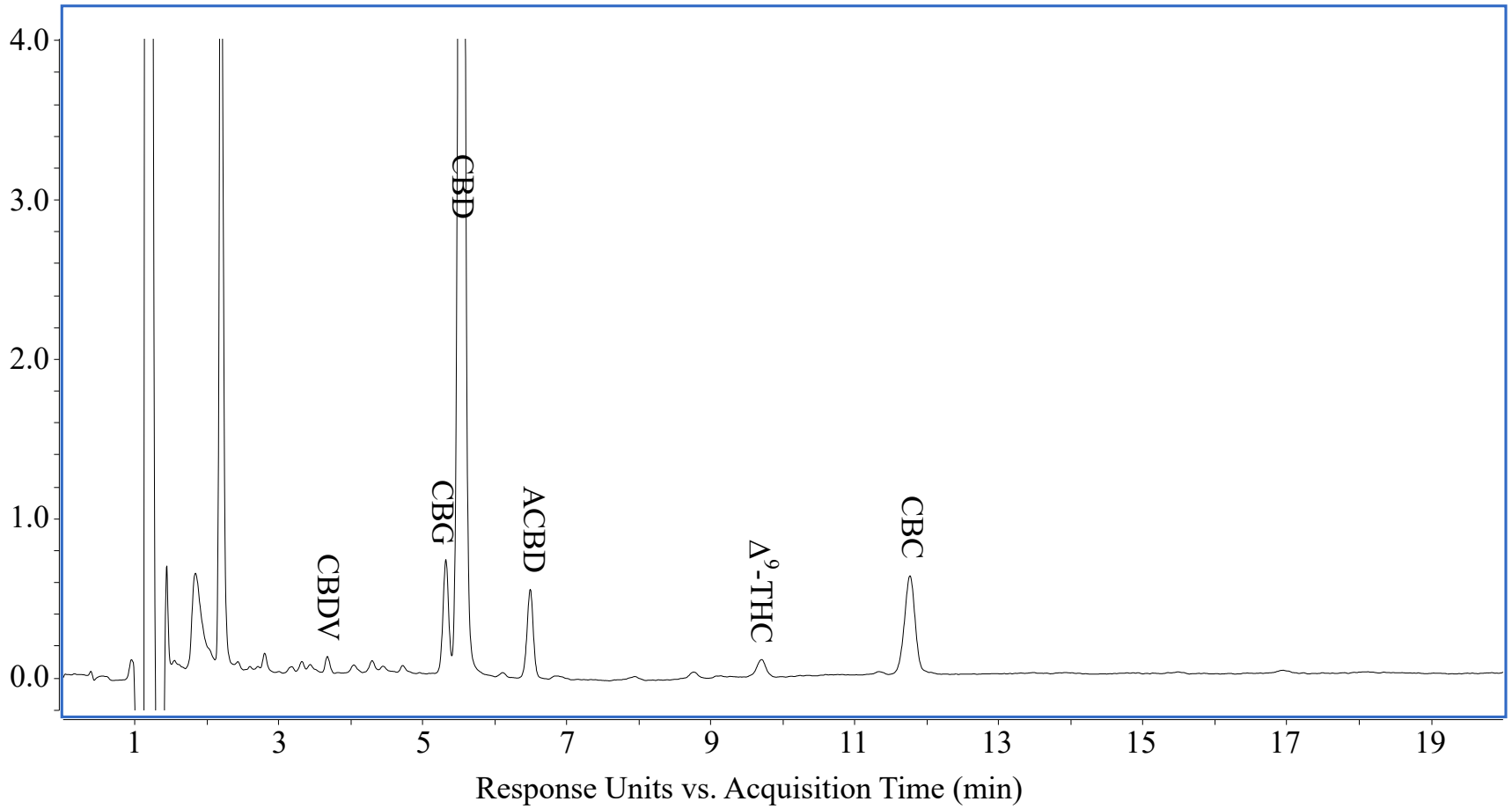

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

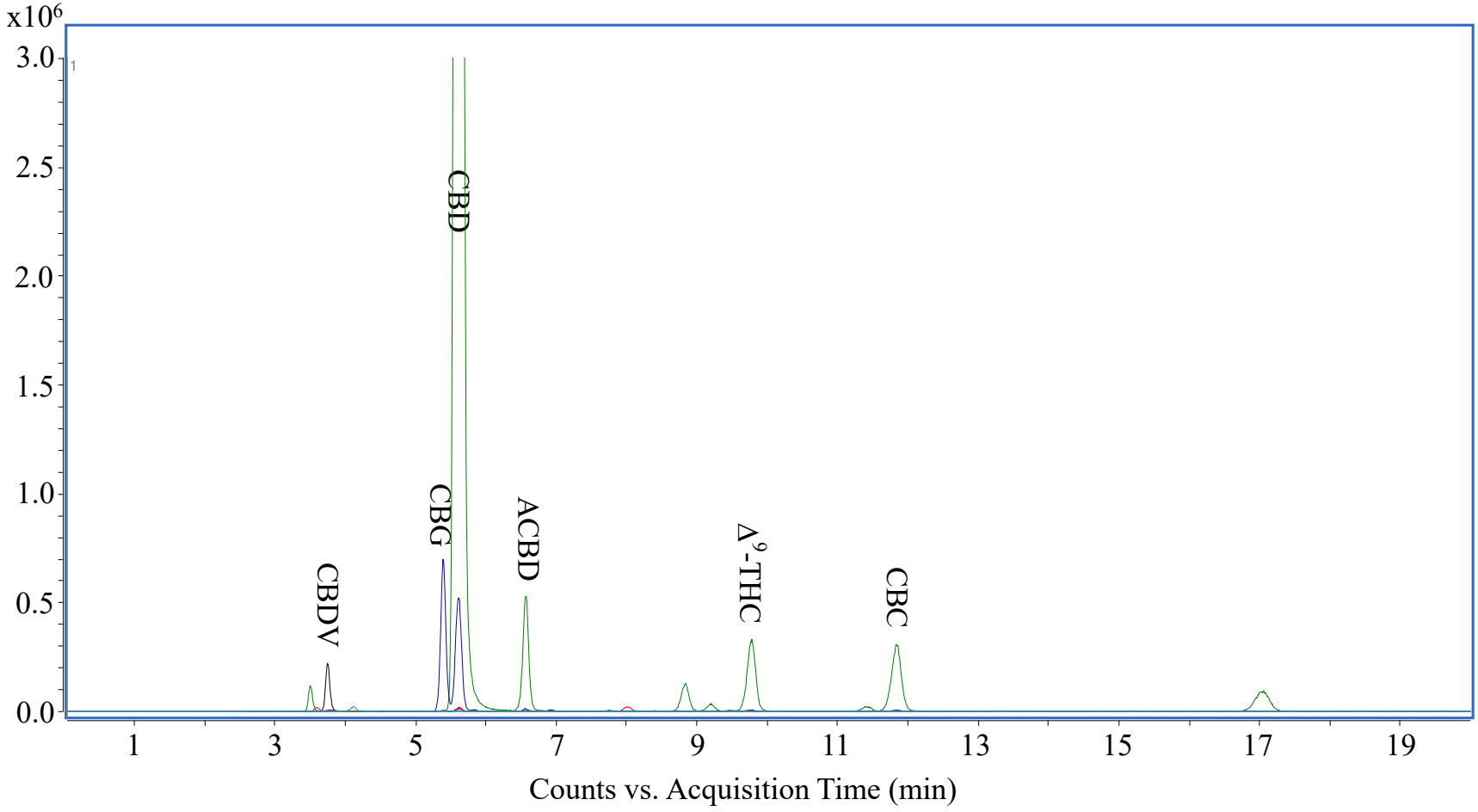
Supplementary Fig. S19. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD popcorn spiked with 0.1% (w/w) ACBD.



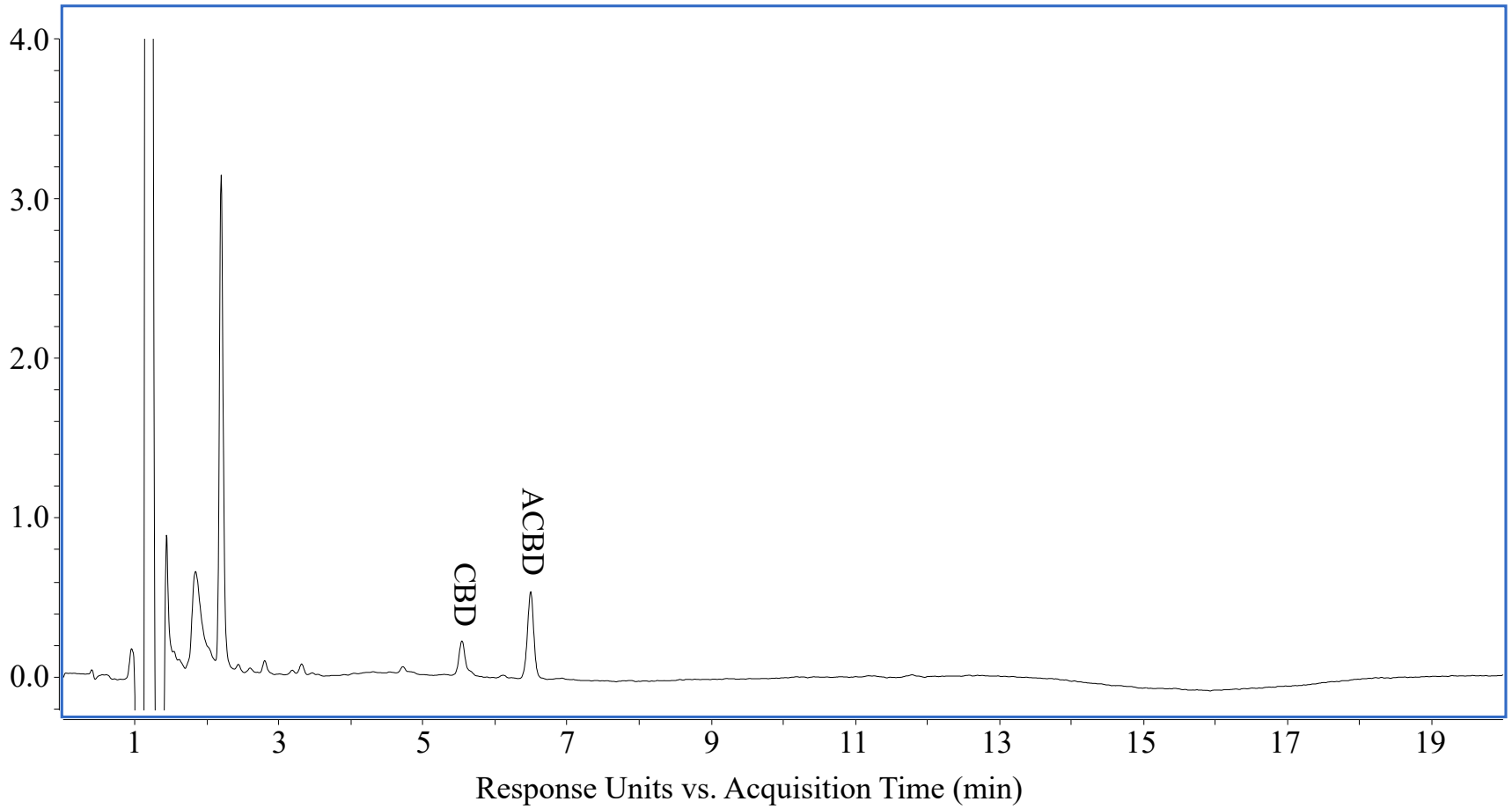

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

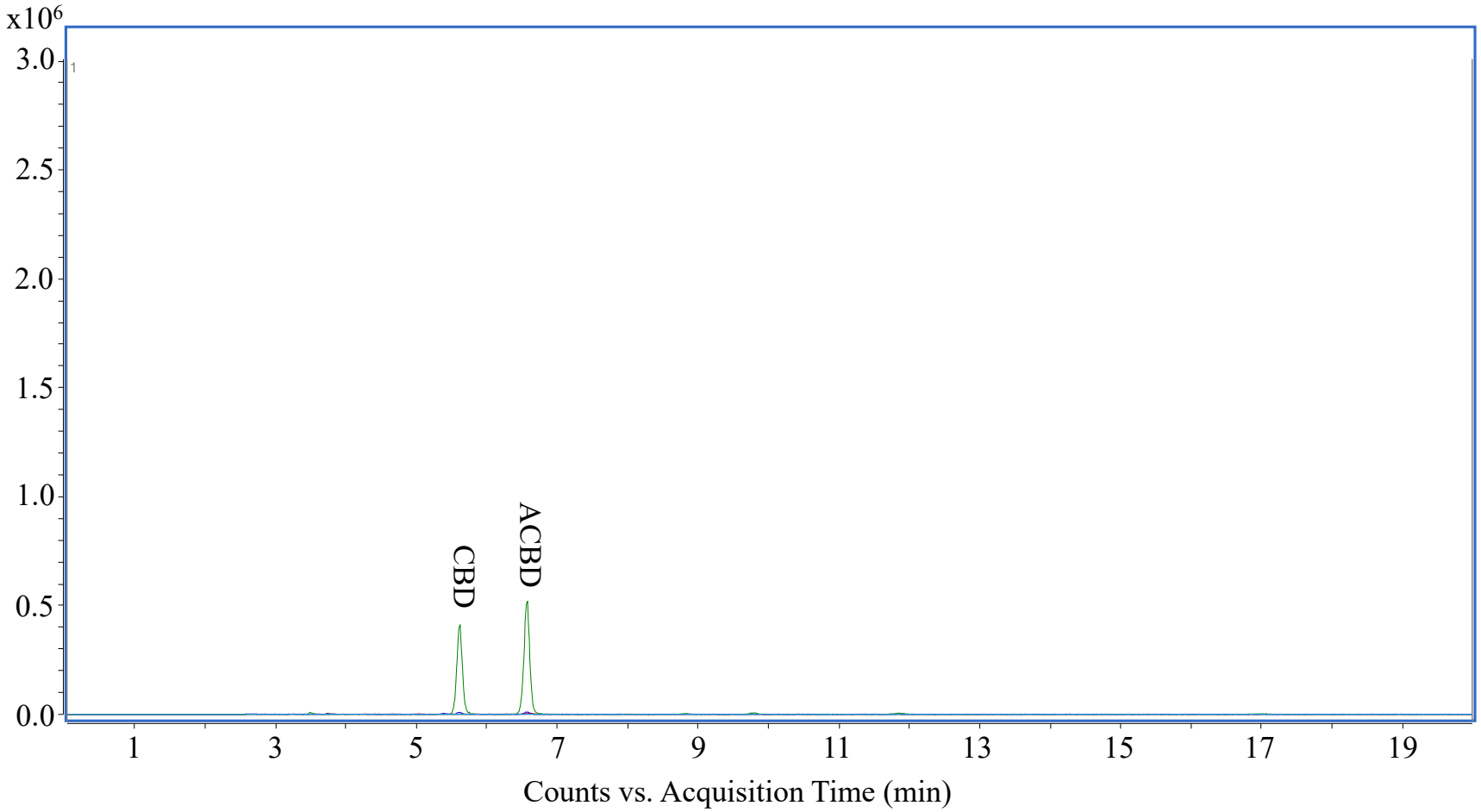
Supplementary Fig. S20. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD cat food kitty nugs spiked with 0.1% (w/w) ACBD.



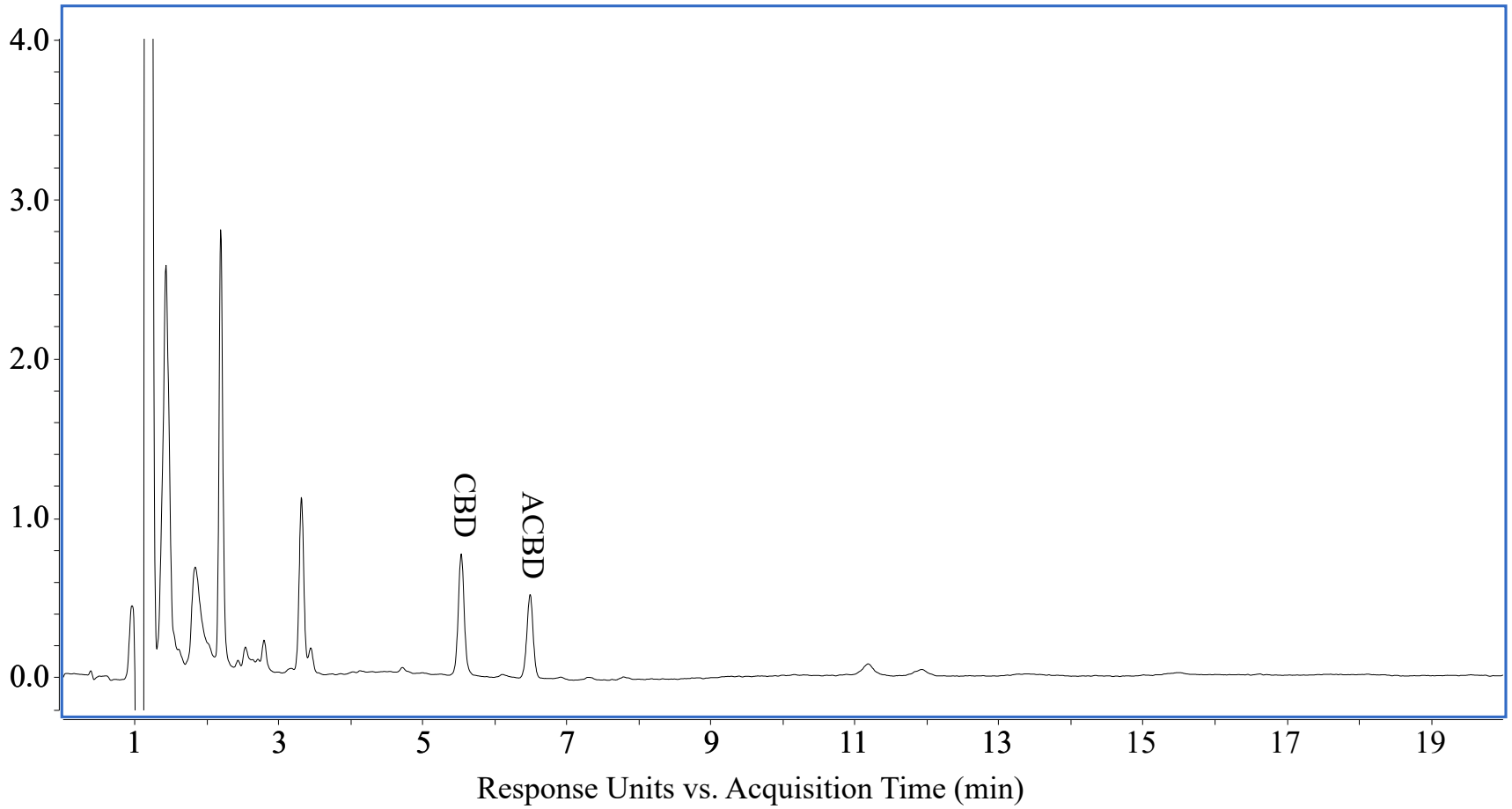

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBDV
CBG
CBD
ACBD
Δ9-THC
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

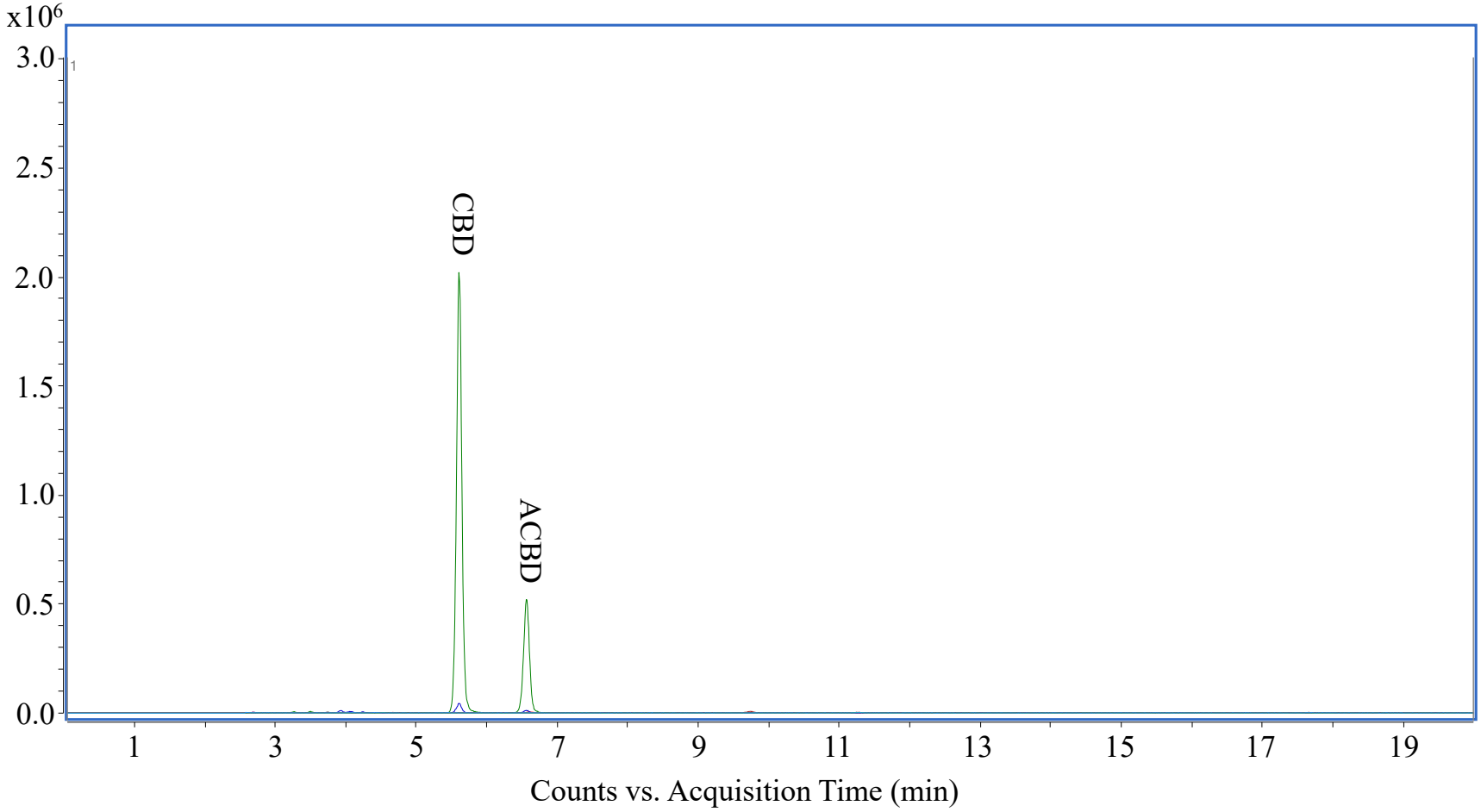
Supplementary Fig. S21. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD horse treats spiked with 0.1% (w/w) ACBD.



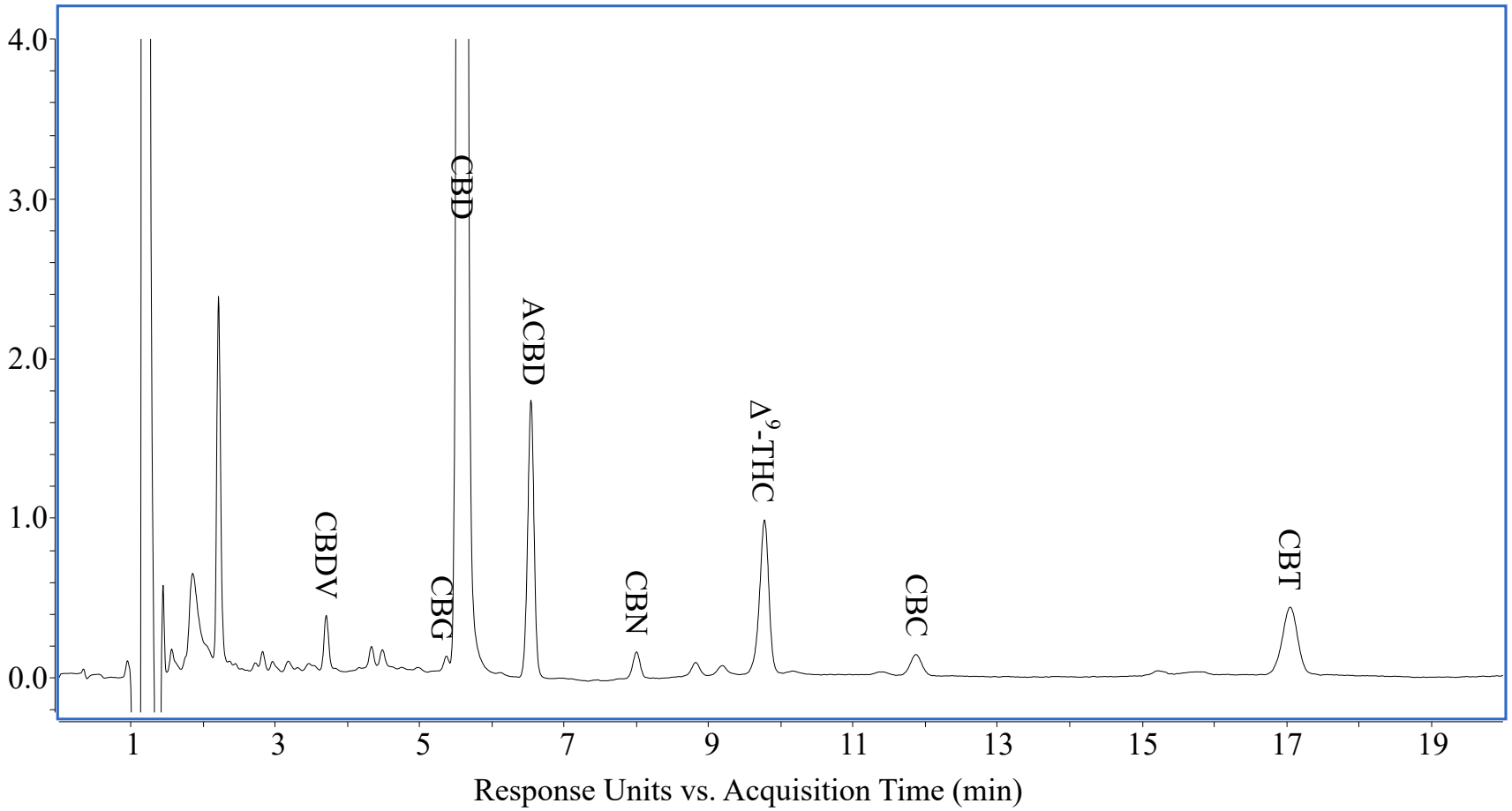

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

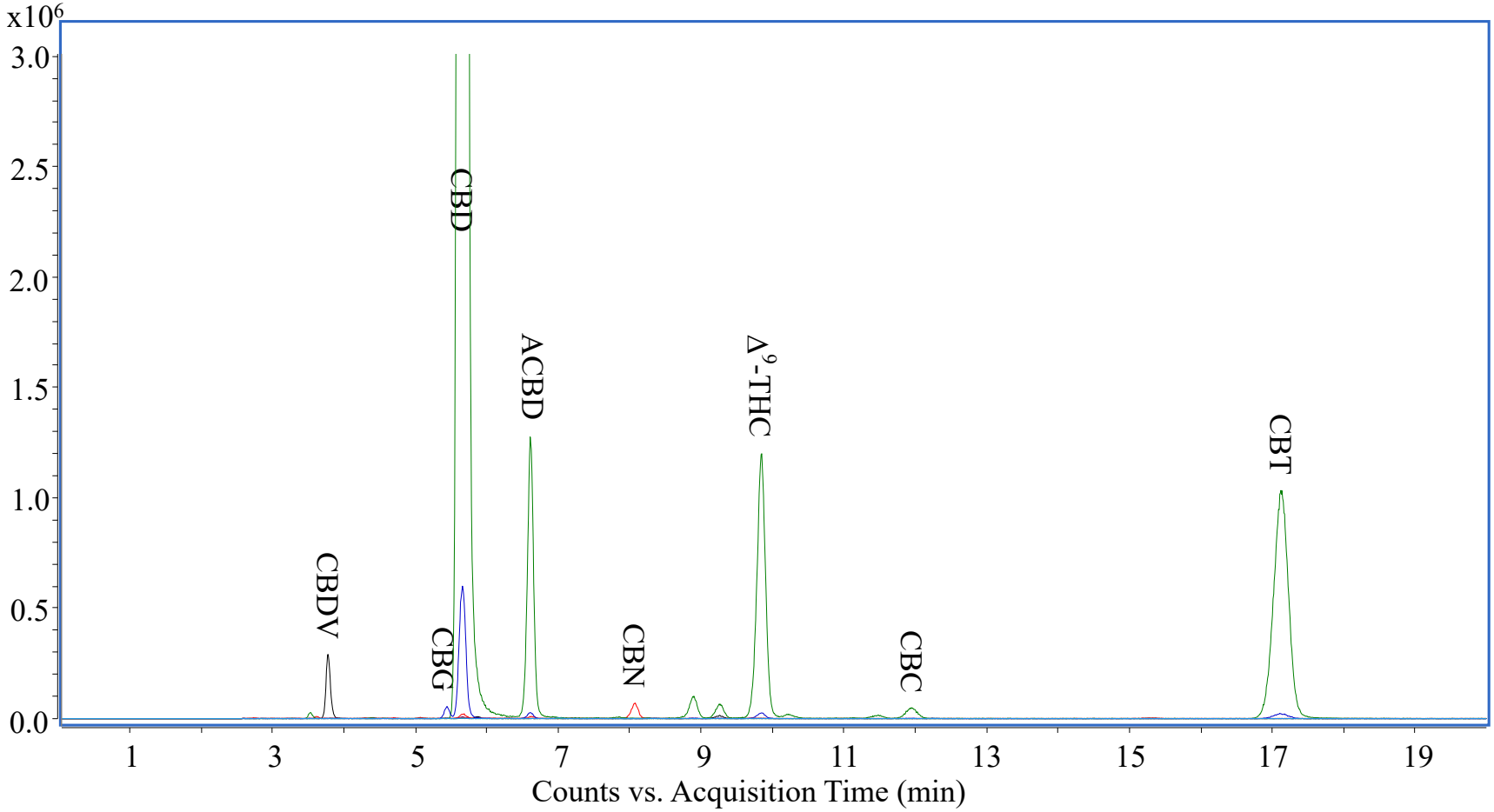
Supplementary Fig. S22. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL soft CBD dog treats spiked with 0.1% (w/w) ACBD.



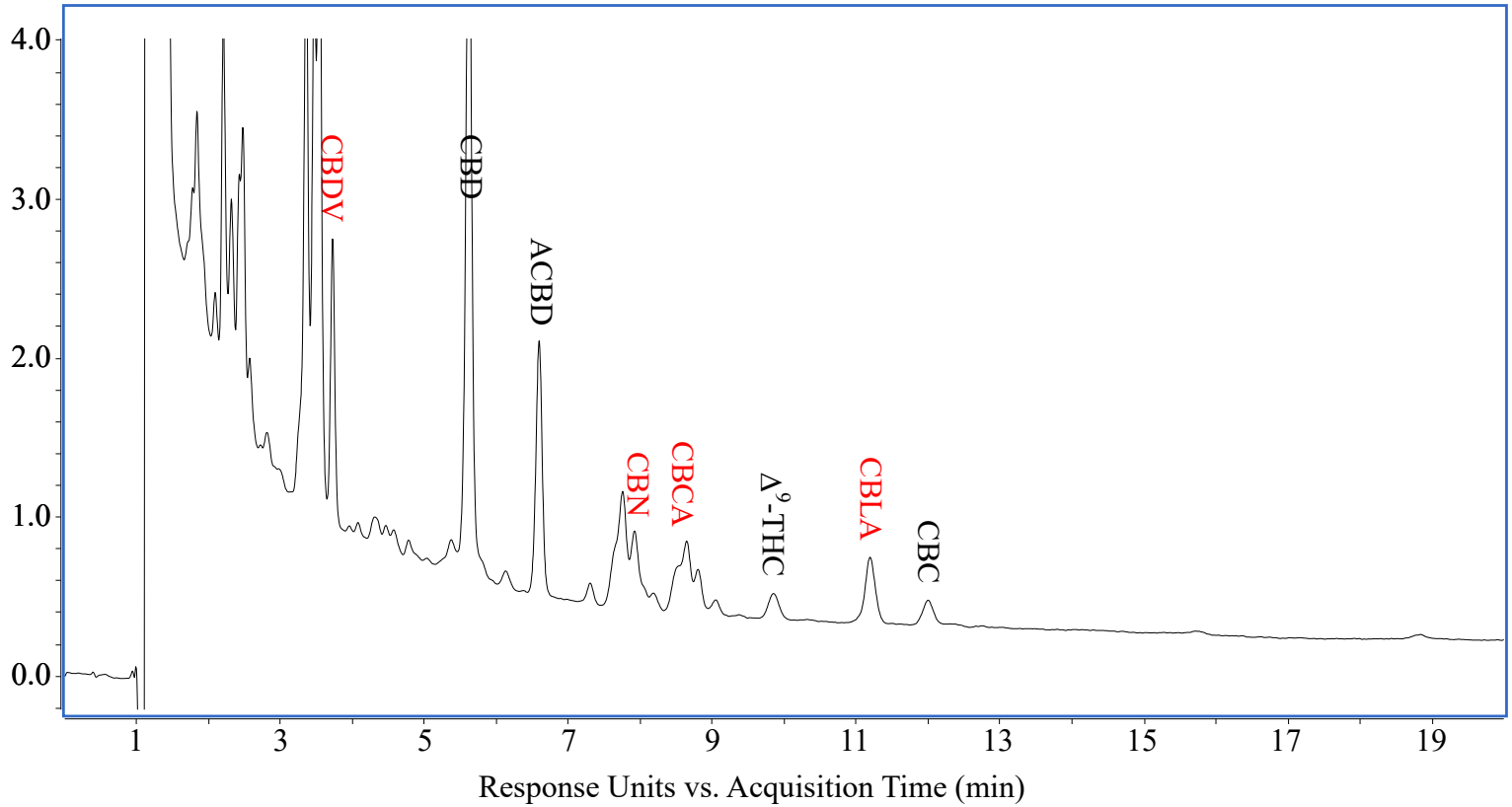

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

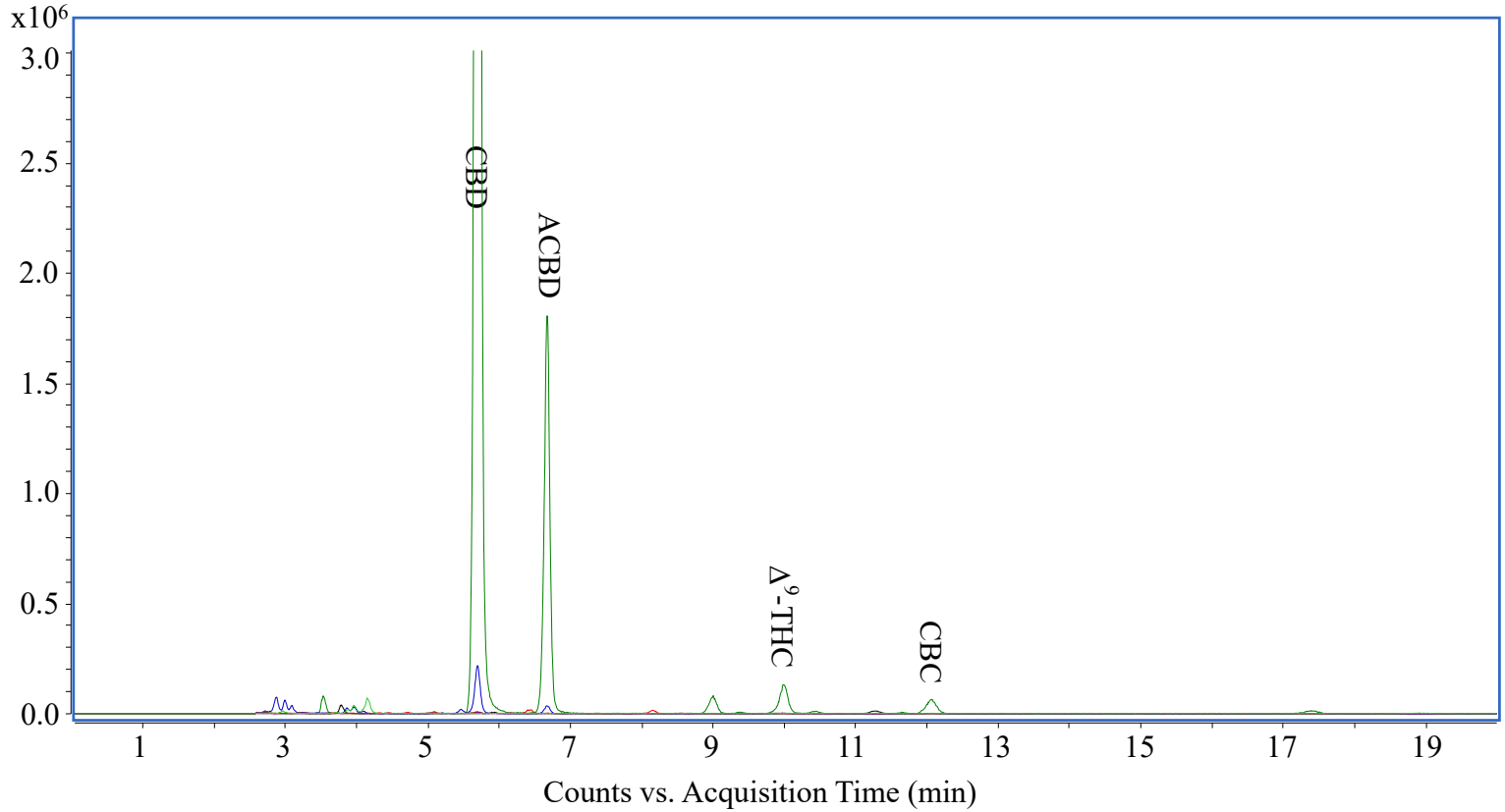
Supplementary Fig. S23. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 250 µg/mL CBD tablets spiked with 0.3% (w/w) ACBD.



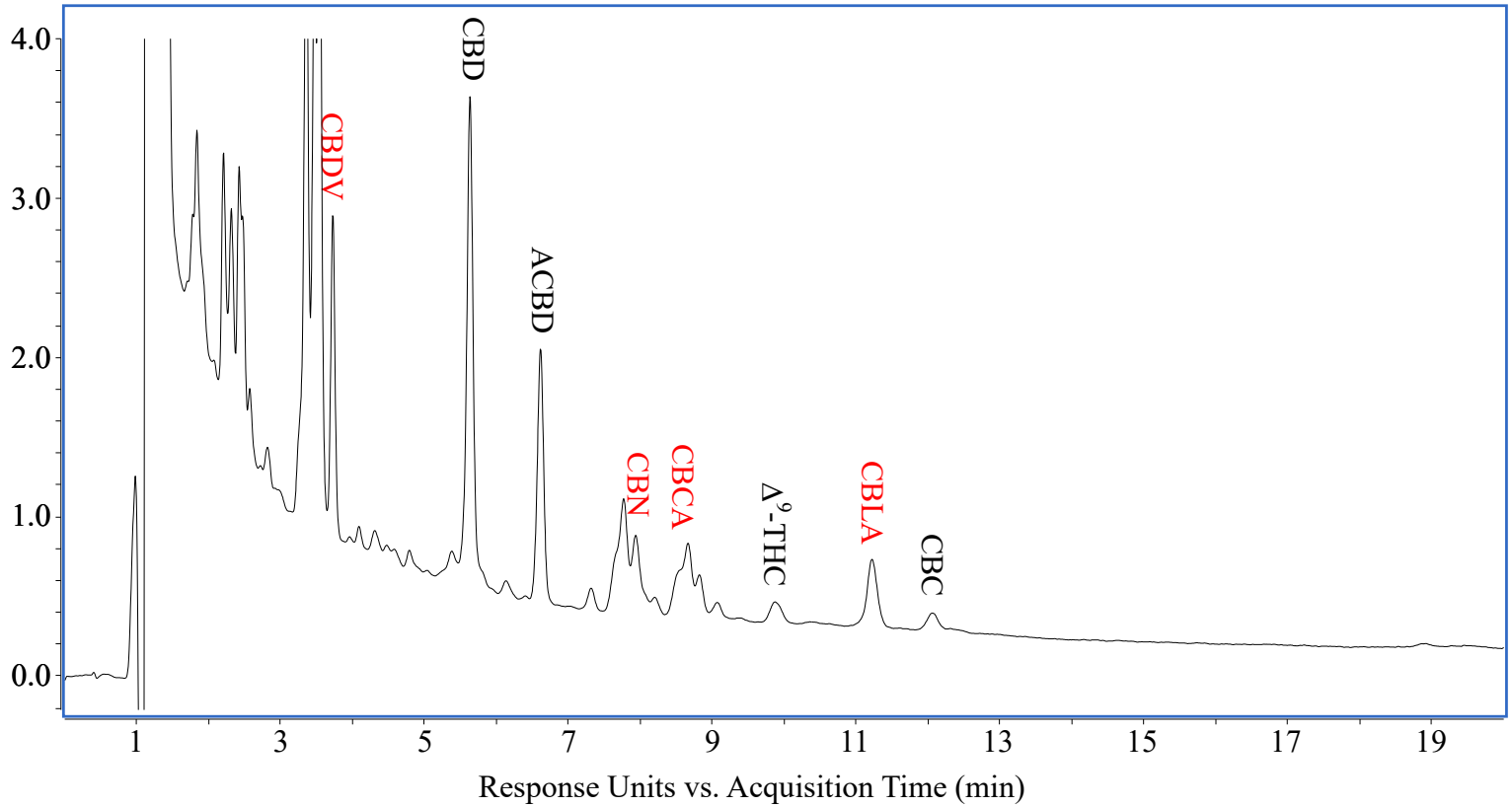

x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBDV
CBG
CBD
ACBD
CBN
Δ9-THC
CBC
CBT
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

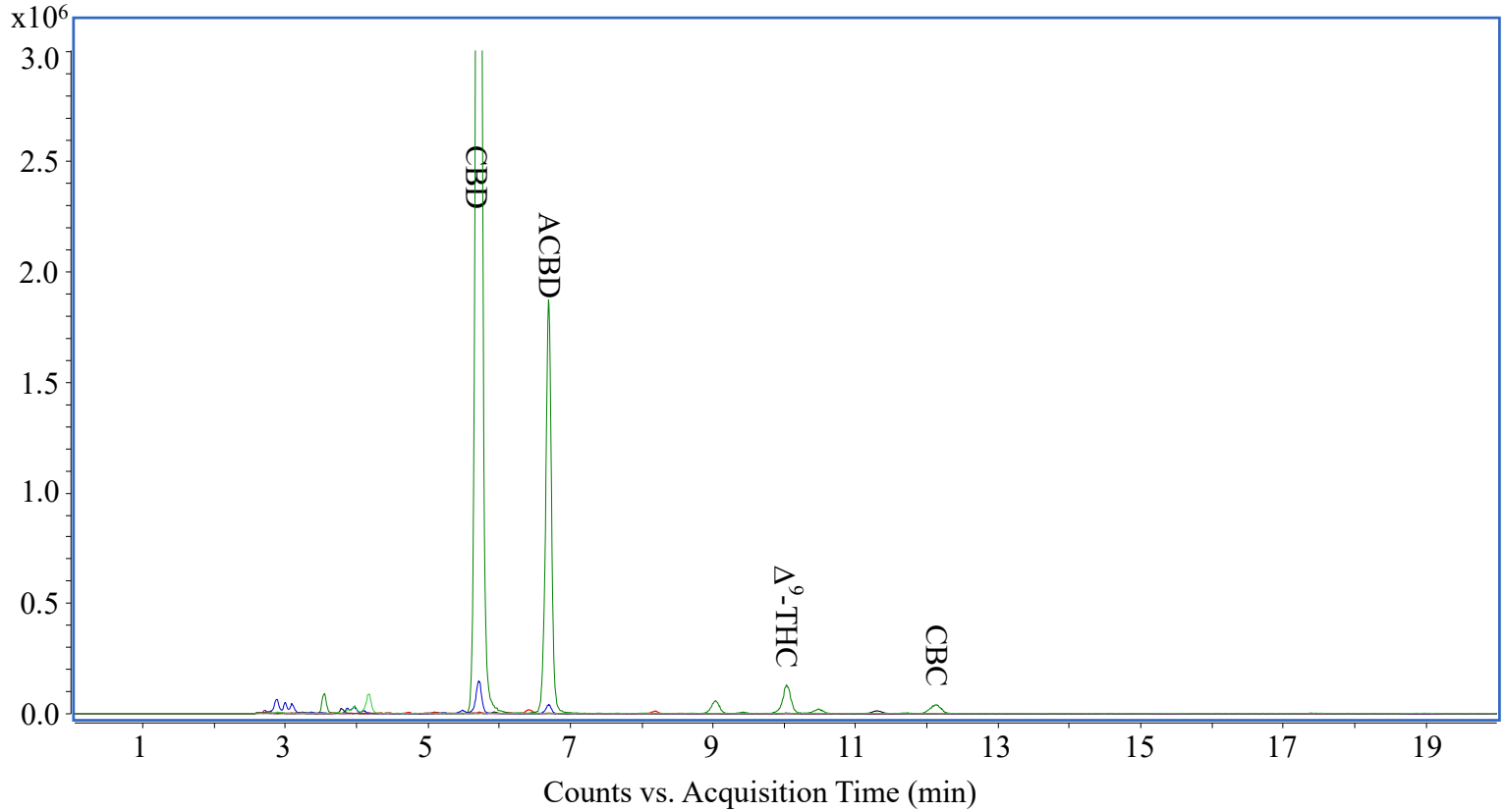
Supplementary Fig. S24. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 25 mg/mL grapefruit sparkling coconut water spiked with 0.003% (w/w) ACBD. False identification of CBN, CBCA and CBLA from intense matrix peaks by UV at 230 nm were discovered by UV absorption spectra and ESI/TOFMS, but false identification of CBDV from an intense matrix peak by UV at 230 nm were discovered only by ESI/TOFMS. The intended LOQ was 0.00008% (w/w), 10 times lower than the validated method.




x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
Δ9-THC
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

Supplementary Fig. S25. From top to bottom, LC-UV chromatogram (A) and LC-EICs (B) of 25 mg/mL lemon sparkling black tea spiked with 0.003% (w/w) ACBD. False identification of CBN, CBCA and CBLA from intense matrix peaks by UV at 230 nm were discovered by UV absorption spectra and ESI/TOFMS, but false identification of CBDV from an intense matrix peak by UV at 230 nm were discovered only by ESI/TOFMS. The intended LOQ was 0.00008% (w/w), 10 times lower than the validated method.




x10^6
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CBD
ACBD
Δ9-THC
CBC
1
3
5
7
9
11
13
15
17
19
Counts vs. Acquisition Time (min)

Supplementary Table S1

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	CBT
0.02	Day 1	96.8	104.1	103.1	91.6	102.6	103.3	112.4	103.5	104.4	99.6	98.6	110.7	109.9	99.3	108.6
	Day 2	103.2	101.9	106.0	105.9	95.2	92.9	101.2	97.4	98.2	94.9	92.0	99.5	112.3	97.8	91.8
	Day 3	101.5	101.8	96.6	100.0	92.5	103.9	102.2	99.9	104.5	101.2	97.1	107.2	102.6	103.0	109.7
	Interday	100.5	102.6	101.9	99.2	96.8	100.0	105.2	100.3	102.4	98.6	95.9	105.8	108.2	100.0	103.4
0.5	Day 1	99.7	105.1	97.6	98.4	95.1	103.1	100.6	100.4	100.4	96.6	98.7	97.9	101.5	100.4	99.9
	Day 2	99.2	98.4	99.4	97.5	97.9	97.9	101.7	100.6	100.3	94.8	98.1	100.3	101.2	99.6	100.3
	Day 3	99.0	102.6	99.4	96.6	100.5	103.5	101.6	102.0	102.7	96.7	99.6	101.2	104.6	102.1	100.6
	Interday	99.3	102.0	98.8	97.5	97.8	101.5	101.3	101.0	101.1	96.1	98.8	99.8	102.4	100.7	100.3
12.5	Day 1	94.6	98.3	94.2	95.1	101.3	97.8	92.8	95.1	95.2	102.8	95.3	95.3	95.3	95.5	94.6
	Day 2	94.5	94.6	97.0	94.5	107.6	94.4	89.9	94.3	94.1	110.4	95.5	93.9	93.7	94.0	95.9
	Day 3	95.1	95.4	96.7	94.0	109.1	96.8	90.1	94.9	95.2	108.9	95.0	94.8	94.8	94.7	93.7
	Interday	94.7	96.1	95.9	94.6	106.0	96.3	90.9	94.8	94.9	107.4	95.3	94.7	94.6	94.8	94.7

Supplementary Table S2

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

Concentration	Precision (%)	CBDVA	CBDV	CBDA	CBGA	THCVA	CBG	CBD	THCV	CBN	CBCA	Δ^9-THCA	Δ^9-THC	Δ^8-THC	CBC	CBT
0.02	Day 1	3.2	2.0	1.9	0.8	2.6	5.1	4.5	3.5	4.2	1.7	1.4	3.7	5.0	0.8	2.1
	Day 2	1.4	2.7	4.5	7.9	3.6	3.4	1.8	3.8	4.5	1.5	5.6	6.3	3.7	3.2	1.6
	Day 3	7.3	3.0	3.1	1.6	1.1	0.9	5.6	4.8	7.2	1.8	2.6	2.2	0.8	0.6	4.2
	Interday	3.3	1.3	4.7	7.3	5.4	6.2	5.9	3.1	3.5	3.3	3.6	5.4	4.7	2.6	9.7
0.5	Day 1	0.5	0.9	1.4	1.0	4.3	0.1	0.3	0.4	0.3	0.3	0.3	4.3	0.4	1.0	0.5
	Day 2	0.7	3.7	0.5	1.0	0.8	3.9	0.3	0.1	1.2	1.7	0.5	1.4	1.6	0.2	0.2
	Day 3	0.2	0.4	0.1	0.2	0.9	0.8	0.5	0.3	0.0	1.9	0.1	1.1	0.4	0.2	1.8
	Interday	0.4	3.3	1.0	0.9	2.8	3.1	0.6	0.9	1.3	1.1	0.8	1.7	1.8	1.3	0.4
12.5	Day 1	0.4	0.2	0.3	0.5	0.2	0.6	0.6	0.4	0.2	0.3	0.3	0.3	0.3	0.3	0.2
	Day 2	0.2	2.1	0.5	0.3	3.0	0.5	0.1	0.2	0.1	2.7	0.3	0.5	2.3	0.2	0.2
	Day 3	0.2	0.3	0.4	0.2	0.3	0.3	1.3	0.1	0.2	1.1	1.3	0.1	1.0	0.4	0.5
	Interday	0.3	2.0	1.6	0.6	3.9	1.8	1.8	0.4	0.7	3.8	0.3	0.8	0.9	0.8	1.2