Simultaneous Quantification of Cannabidiol, Δ-9-Tetrahydrocannabinol, and Their Major Metabolites in Rat Serum by LC-MS/MS

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Abstract

Cannabis products that contain no more than 0.3% Δ-9-tetrahydrocannabinol (THC) have become increasingly popular since the passage of the 2018 Farm Bill. However, along with gaps concerning the safety and use of cannabidiol (CBD) products, rigorous bioanalytical methods are needed to evaluate the metabolism of CBD and the possible contaminant THC in toxicological studies. A liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was developed and validated to measure CBD, THC, and their major metabolites (7-OH-CBD, 7-COOH-CBD, 11-OH-THC, and 11-COOH-THC) in rat serum according to FDA's Bioanalytical Method Validation Guidance. Rat serum was spiked with deuterium-labeled internal standards, proteins were precipitated with acetonitrile, and the analytes in the supernatant were separated with an ACQUITY UPLC HSS T3 column on a Waters ACQUITY UPLC System. The eluents were monitored on a Waters Quattro Premier mass spectrometer with an electrospray ion source in the positive ion mode (ESI^+) using multiple reaction monitoring. Matrix effects were observed for CBD, 11-OH-THC, and 11-COOH-THC, but these were minimized by using internal standards. Calibration curves were established in the range of 5 - 2000 ng/mL with r^2 >0.99. The limits of detection and lower limits of quantification were 1.1 - 4.1 ng/mL and 3.8 - 13.8 ng/mL, respectively, for all analytes. The intraday accuracy and precision for all analytes ranged from 85.0 to 113.0% and 0.2 to 12.2%, respectively; and the interday accuracy and precision were 92.0 - 110.6% and 0.3 - 8.25%, respectively. This sensitive and reliable LC-MS/MS method is useful to monitor the metabolism of CBD and possible THC contaminations in toxicological studies.

Introduction

The Agriculture Improvement Act of 2018 (the "Farm Bill") removed hemp and its derivative products from the definition of marijuana in the Controlled Substance Act in the US. This "Farm Bill" has contributed to an increased popularity and availability of CBD and other cannabis consumer products. However, along with gaps concerning the safety and use of CBD products, rigorous bioanalytical methods are needed to evaluate the metabolism of CBD and the possible contaminant THC in toxicological studies. A sensitive and reliable LC-MS/MS method was developed and validated to measure CBD, THC, and their major metabolites (Figure 1) in rat serum according to FDA's Bioanalytical Method Validation Guidance.

Methods

- CBD, 7-OH-CBD, 7-COOH-CBD, THC, 11-OH-THC, 11-COOH-THC, and their deuterated internal standards were purchased from Cerilliant (Round Rock, TX). Formic acid and acetonitrile were LC-MS grade.
- After addition of the internal standards, rat serum was subjected to protein precipitation with acetonitrile. The supernatants were analyzed with a Waters ACQUITY UPLC coupled with a Premier mass spectrometer (Tables 1 and 2).
- Male Sprague-Dawley rats were treated by intravenous injection with 0.1% or 1% CBD in Intralipid 20% at a dose volume of 1 ml/kg body weight. Blood was collected from the tail vein at 10 min and by cardiac puncture at 30 min post dosing and processed to serum.

Methods (continued)

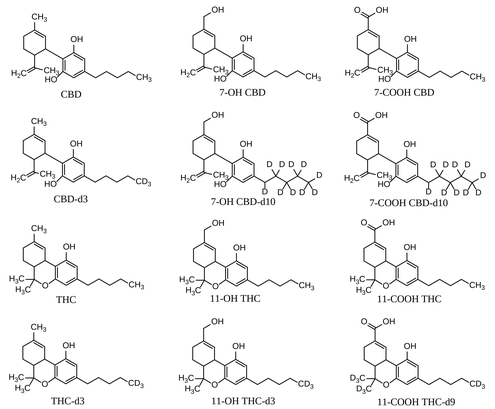

Figure 1. Chemical structures of CBD, THC, their major metabolites (7-OH-CBD, 7-COOH-CBD, 11-OH-THC, and 11-COOH-THC), and respective internal standards

Table 1. LC-MS/MS chromatographic conditions

UPLC conditions	
Column	ACQUITY UPLC HSS T3 column (2.1 mm × 50 mm, 1.8 µm)
Guard column	ACQUITY UPLC HSS T3 VanGuard Pre-column (2.1 mm × 5 mm, 1.7 µm)
Column Temp.	40 ˚C
Mobile phases	A: LC-MS grade H_2O, 0.1% Formic acid B: LC-MS grade Acetonitrile, 0.1% Formic acid
Gradient elution	Time (min) / Flow rate (ml/min) / A% / B% 0 / 0.4 / 95 / 5 5.0 / 0.4 / 0 / 100 5.1 / 0.4 / 95 / 5 7.0 / 0.4 / 95 / 5
Weak needle wash	H_2O, 0.1% Formic acid
Strong needle wash	Acetonitrile, 0.1% Formic acid
Seal wash	50% Methanol
Autosampler Temp.	15 ˚C
Injection volume	15 µL

MS conditions	
MS system	Waters Quattro Premier Mass Spectrometer
Ion source	ESI+
Operation mode	Multiple reaction monitoring (MRM)

Table 2. Optimized MS parameters for MRM transitions of analytes

Analyte	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision (V)	Retention time (min)
CBD	315.2	193.0	0.100	33	23	4.73
CBD-D3	318.2	196.0	0.050	33	23	4.72
7-OH-CBD	313.1	193.0	0.075	35	23	3.71
7-OH-CBD-D10	323.2	203.0	0.075	35	23	3.69
7-COOH-CBD	345.2	299.2	0.075	35	20	3.61
7-COOH-CBD-D10	355.2	309.1	0.075	35	20	3.60
THC	315.2	193.0	0.100	33	23	5.18
THC-D3	318.2	196.0	0.050	33	23	5.18
11-OH-THC	313.2	193.0	0.075	45	28	4.27
11-OH-THC-D3	316.2	196.2	0.075	45	28	4.26
11-COOH-THC	345.2	299.2	0.075	35	22	4.28
11-COOH-THC-D9	354.2	308.2	0.075	35	22	4.26

Results

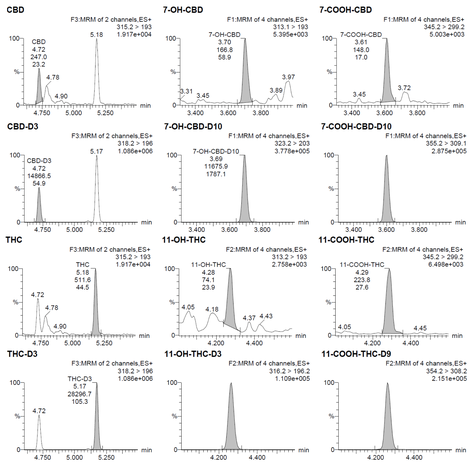

Figure 2. Chromatograms of CBD, THC, and their major metabolites (10 ng/mL in rat serum, 15 pg on column).

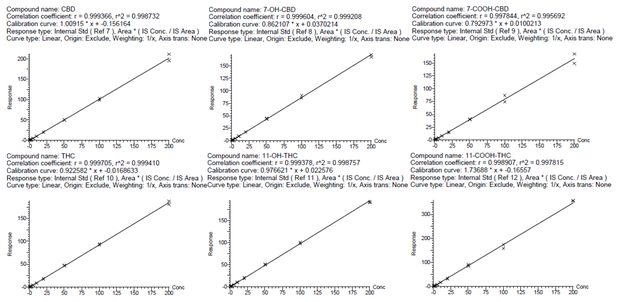

Figure 3. Calibration curves of CBD, THC, and their major metabolites (5 - 2000 ng/mL in rat serum).

Table 3. Matrix effects and recovery for all analytes in rat serum

Analyte	Absolute matrix effect	Relative recovery	Absolute recovery
CBD	57.9	103.5	59.9
CBD-D3	56.1	104.9	58.9
7-OH-CBD	81.8	111.0	90.7
7-OH-CBD-D10	89.6	102.3	91.6
7-COOH-CBD	94.8	96.7	91.7
7-COOH-CBD-D10	94.5	103.2	97.5
THC	75.8	151.0	114.5
THC-D3	79.1	148.2	117.2
11-OH-THC	35.4	108.9	38.6
11-OH-THC-D3	34.0	107.3	36.4
11-COOH-THC	45.5	104.8	47.6
11-COOH-THC-D9	33.6	104.5	35.1

Absolute matrix effect=Spiked after extraction/Neat solution standard*100
Relative recovery=Spiked before extraction/Spiked after extraction*100
Absolute recovery=Spiked before extraction/Neat solution standard*100

Results (continued)

Table 4. Accuracy and precision of the LC-MS/MS method for quantification of CBD, THC, and their major metabolites in rat serum

Analyte	Conc. (ng/mL)	Intraday (n=5)			Interday (n=3)		
		Mean ± SD	CV (%)	Accuracy (%)	Mean ± SD	CV (%)	Accuracy (%)
CBD	10	9.8 ± 1.1	11.1	98.0	10.6 ± 0.4	3.9	105.6
	50	47.4 ± 1.1	2.4	94.8	46.2 ± 2.7	5.8	92.4
	200	192.5 ± 9.9	5.1	96.3	198.3 ± 11.9	6.0	99.1
	1000	990.0 ± 32.5	3.3	99.0	980.0 ± 14.0	1.4	98.0
7-OH-CBD	10	9.6 ± 1.1	11.4	96.0	9.7 ± 0.5	5.6	96.7
	50	49.9 ± 1.3	2.7	99.8	48.6 ± 0.7	1.5	97.2
	200	199.0 ± 3.8	1.9	99.5	201.6 ± 7.0	3.4	100.8
	1000	1019.0 ± 5.5	0.5	101.9	980.3 ± 32.8	3.3	98.0
7-COOH-CBD	10	10.2 ± 0.5	5.0	102.0	9.6 ± 0.8	8.5	96.1
	50	47.7 ± 1.8	3.7	95.4	46.7 ± 2.1	4.4	93.3
	200	194.0 ± 9.4	4.8	97.0	200.9 ± 6.3	3.1	100.4
	1000	1023.4 ± 15	1.5	102.3	958.3 ± 50.7	5.3	95.8
THC	10	9.8 ± 0.5	4.8	98.0	9.7 ± 0.7	7.2	97.2
	50	48.5 ± 0.6	1.3	97.0	46.8 ± 1.6	3.4	93.6
	200	191.0 ± 5.1	2.7	95.5	198.0 ± 5.4	2.7	99.0
	1000	993.8 ± 1.6	0.2	99.4	988.8 ± 3.4	0.3	98.9
11-OH-THC	10	8.5 ± 1.0	12.2	85.0	9.6 ± 0.8	8.2	95.6
	50	46.9 ± 0.9	2.0	93.8	46.0 ± 1.0	2.1	92.0
	200	197.5 ± 5.9	3.0	98.8	199.4 ± 12.6	6.3	99.7
	1000	1023.1 ± 16.6	1.6	102.3	976.0 ± 23.1	2.4	97.6
11-COOH-THC	10	11.3 ± 0.7	5.8	113.0	11.1 ± 0.5	4.7	110.6
	50	50.0 ± 2.2	4.3	100.0	49.5 ± 3.0	6.0	99.0
	200	208.2 ± 7.4	3.5	104.1	196.4 ± 11.0	5.6	98.2
	1000	1033.2 ± 50.4	4.9	103.3	965.7 ± 8.5	0.9	96.6

Table 5. Limit of detection (LOD) and lower limit of quantification (LLOQ)

Analyte	S/N*	LOD (ng/mL)	LLOQ (ng/mL)
CBD	38	1.6	5.3
7-OH-CBD	42	1.4	4.8
7-COOH-CBD	14.5	4.1	13.8
THC	52.5	1.1	3.8
11-OH-THC	16.5	3.6	12.1
11-COOH-THC	29.5	2.0	6.8

* Based on the rat serum standard of 10 ng/mL. The LOD and LLOQ were estimated based on a signal to noise ratio of 3 and 10, respectively.

Table 6. Levels (ng/mL) of CBD and its metabolites in the serum of male SD rats treated by IV injection with 0.1% or 1% CBD

Treatment	Analyte	10 min	30 min
0.1% CBD	CBD	149.0 ± 16.4	48.9 ± 12.9
	7-OH-CBD	37.7 ± 0.1	10.9 ± 3.2
	7-COOH-CBD	108.6 ± 1.4	85.6 ± 18.4
1% CBD	CBD	2330.5 ± 99.5	922.5 ± 174.3
	7-OH-CBD	466.4 ± 69.7	182.2 ± 11.8
	7-COOH-CBD	1284.3 ± 98.9	1530.1 ± 225.9

Mean ± SD. n=2 or 3. No THC, 11-OH-THC, or 11-COOH-THC was detected in the serum samples.

Conclusions

- Matrix effects were observed for CBD, 11-OH-THC, and 11-COOH-THC, but these were minimized by using internal standards.
- Calibration curves were established for CBD, THC, and their major metabolites in the range of 5 – 2000 ng/mL with r^2 >0.99.
- The method accuracy and precision met the requirements of the FDA's Bioanalytical Method Validation Guidance.
- The limits of detection and lower limits of quantification were 1.1 - 4.1 ng/mL and 3.8 - 13.8 ng/mL, respectively, for all analytes.
- A sensitive and reliable LC-MS/MS method was developed and validated to measure simultaneously CBD, THC, and their major metabolites in rat serum.

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