A Fast, Sensitive and Comprehensive Assay to Quantify 16 Cannabinoids in Hemp Flower and Leaf using LC/MS/MS

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INTRODUCTION

Marijuana is defined as any cannabis sativa plant that has greater than 0.3 percent tetrahydrocannabinol (THC), while hemp is a phenotype of the Cannabis sativa plant species that has 0.3 percent or less THC. In 2018, AOAC published a method to quantify 10 cannabinoids (CBDs) with the range of 0.500-10.0 µg/mL in finished goods. However, there are more than 100 CBDs isolated from cannabis in addition to these 10 CBDs in the AOAC method. In 2019, Dyad Labs validated a LC/MS/MS method for 16 CBDs (see Table 1) in distillate, extract, and other finished products. In this paper, Dyad Labs has successfully developed and validated another fast, sensitive, and comprehensive LC/MS/MS assay to quantify 16 cannabinoids in the hemp leaf and flower samples

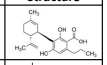
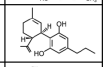
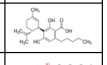
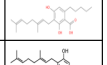
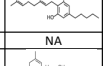
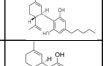
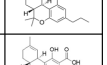
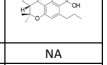
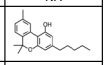
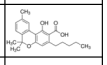
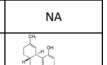
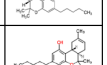
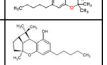
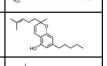
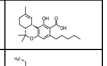
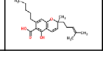
#	Common name	Abbreviation	IUPAC name	CAS No.	Molecular Structure
1	Cannabivarinic Acid	CBDVA	2,4-Dihydroxy-3-[(1R,6R)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1-yl]-6-propylbenzoic acid	31932-13-5	
2	Cannabidivarin	CBDV	2-((1S,6S)-3-methyl-6-(prop-1-en-2-yl) cyclohex-2-enyl)-5-propylbenzene-1,3-diol	24274-48-4	
3	Cannabidiolic Acid	CBDA	2,4-Dihydroxy-3-[(1R,6R)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1-yl]-6-pentylbenzoic acid	1244-58-2	
4	Cannabigerolic Acid	CBGA	3-[(2E)-3,7-dimethylocta-2,6-dienyl]-2,4-dihydroxy-6-pentylbenzoic acid	25555-57-1	
5	Cannabigerol	CBG	2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-pentyl-benzene-1,3-diol	25654-31-3	
6	Cannabidiol-d3	CBD-d3	NA	NA	NA
7	Cannabidiol	CBD	2-[(1R,6R)-6-isopropenyl-3-methylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol	13956-29-1	
8	Tetrahydrocannabivarin	THCV	6,6,9-Trimethyl-3-propyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol	28172-17-0	
9	Tetrahydrocannabivarinic acid	THCVA	(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-3-propyl-6a,7,8,10a-tetrahydrobenzo[c]chromene-2-carboxylic acid	39986-26-0	
10	Cannabinol-d3	CBN-d3	NA	NA	NA
11	Cannabinol	CBN	6,6,9-Trimethyl-3-pentyl-benzo[c]chromen-1-ol	521-35-7	
12	Cannabinolic Acid	CBNA	1-hydroxy-6,6,9-trimethyl-3-pentylbenzo[c]chromene-2-carboxylic acid	2808-39-1	
13	Delta9-Tetrahydrocannabinol -d3	delta9-THC-d3	NA	NA	NA
14	(-)-Delta9-Tetrahydrocannabinol	delta9-THC	(−)-(6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol	8/3/1972	
15	(-)-Delta8-Tetrahydrocannabinol	delta8-THC	6,6,9-Trimethyl-3-pentyl-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol	5957-75-5	
16	Cannabicyclol	CBL	9,13,13-trimethyl-5-pentyl-8-oxatetracyclo-7,4,1,1-tetradeca-2,4,6-trien-3-ol	21366-63-2	
17	Cannabichromene	CBC	2-Methyl-2-(4-methylpent-3-enyl)-7-pentyl-5-chromenol	20675-51-8	
18	Tetrahydrocannabinolic Acid	THCA	(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6h-benzo[c]chromene-2-carboxylic acid	23978-85-0	
19	Cannabichromenic Acid	CBCA	5-Hydroxy-2-methyl-2-(4-methyl-3-penten-1-yl)-7-pentyl-2H-chromene-6-carboxylic acid	20408-52-0	

Table 1: **The Information of 16 CBDs and its Internal Standard**

METHODOLOGY

Sample Preparation and Extraction

The hemp sample was homogenized to powder by Resch Mixer Mill MM400. 0.500 gram of hemp powder was weighed and extracted with ethanol: water (90:10, v/v) in an amber container, followed by filtration with 0.45 µm membrane filter. The internal standards, including cannabidiol-d3, cannabinol-d3, delta9-THC-d3, were used in this method. Extracts were injected for analysis on Shimadzu Nexera UPLC system with an ARC-18 column at 35 °C.

LC-MS/MS Conditions

LC system: Nexera UPLC system including SIL-30AC auto-sampler, controller, column heater and binary pump (Shimadzu)
Column: C_{18} Column
Mobile Phase A: Ammonium Formate and Formic Acid in water
Mobile Phase B: Formic Acid in acetonitrile
Pump Gradient Cycle time: 16 minutes

MS detector: Triple Quadrupole 5500 MS (Sciex)
MS Parameters: see below Table 2.

MS CONDITIONS											
Scan Mode		Ion Mode		Source Temperature (°C)				Dwell Time (ms)			
MRM		Positive		500				100			
Compounds Parameters											
Analyte	Q1	Q3	Analyte	Q1	Q3	Analyte	Q1	Q3	Analyte	Q1	Q3
CBDVA	331.5	191	CBD-d3	318	135	CBN	312	223.1	CBL	315.5	165
CBDV	287.4	231	CBD	316	135	CBNA	355	337.3	CBC	315.5	235
CBDA	341.5	261.1	THCV	287	165	delta9-THC-d3	318	196.1	THCA	315	295.1
CBGA	361.5	219	THCVA	331	233	delta9-THC	315	193.1	CBCA	359	341
CBG	317.2	193	CBN-d3	314	296	delta8-THC	316	165			

Table 2: **The MS Parameters**

RESULTS and DISCUSSIONS

Stability

In the AOAC method, the standard solutions are expensive and only have 3 days of stability. In order to better understand the stability of CBD, we comprehensively investigated on the stability of all CBDs with different parameters including light, heat and pH (see Figure 1). The study indicated that CBDs are sensitive to light, heat and pH. Neutral condition with light protection and low temperature was adopted during sample preparation. Dyad Labs has successfully established stability up to 149 days.

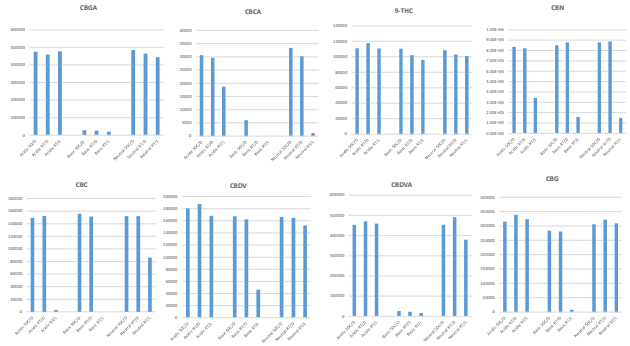


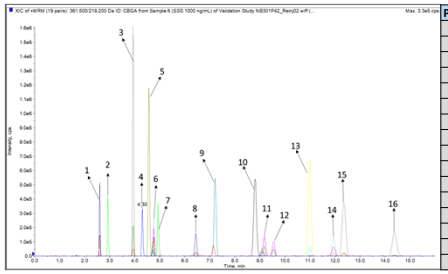
Figure 1: **Representative Data of Stability Study**

Note: "D", "50C", "RT" and "L" represent "dark", "50°C", "Room Temperature" and "Light", respectively.

RESULTS and DISCUSSIONS

Chromatographic Separation

Some CBDs are isomers and have the same MRM transitions, thus chromatographic separation is very critical in this method. We have investigated on different columns, mobile phases and buffers to achieve enough separation, sharp and symmetric peak shape. A special C_{18} column provided sufficient separation (see Figure 2).



Peak #	Analyte	RT (min)
1	CBDVA	2.65
2	CBDV	2.98
3	CBDA	3.96
4	CBGA	4.21
5	CBG	4.57
6	CBD	4.76
7	THCV	4.94
8	THCVA	6.39
9	CBN	7.15
10	CBNA	8.78
11	delta9-THC	9.07
12	delta8-THC	9.43
13	CBL	11.03
14	CBC	11.90
15	THCA	12.15
16	CBCA	14.38

Figure 2: **The Representative Chromatogram of 16 CBDs**

Accuracy and Precision

The accuracy and precision were investigated with post-spiking all sixteen CBDs in hemp leaf and flower at lower, medium, and high regions of the established range of the calibration curve (20.0-2000 mg/mL) (*Table 3*).

Analyte	Post-Spiking Low (75.0 ng/mL)		Post-Spiking Medium (300 ng/mL)				Post-Spiking High (1200 ng/mL)	
	Accuracy (%)	RSD%	Accuracy (%)	RSD%	Accuracy (%)	RSD%	Accuracy (%)	RSD%
CBDVA	91.3	2.64	88.7	1.72	92.1	4.34	96.2	1.72
CBDV	96.5	0.74	100.7	1.53	102	2.35	103	4.18
CBDA	96.2	4.83	90.3	1.94	93.5	4.02	98.2	2.55
CBGA	98.6	0.86	97.8	2.39	98.9	3.04	96.7	2.72
CBG	109	2.00	107	2.39	102	5.45	109	1.59
CBD	101	3.57	102	2.88	101	2.76	101	0.15
THCV	93.2	5.95	93.7	3.11	95.3	2.98	95.8	0.94
THCVA	98.6	3.77	97.5	3.96	98.4	3.31	100	1.45
CBN	93.1	1.92	94.2	2.23	96.2	3.40	100	1.82
CBNA	95.6	0.86	95.8	2.84	96.7	2.96	98.8	0.61
delta 9 THC	93.1	5.92	96.5	1.89	98.2	3.04	98.7	1.49
delta 8 THC	85.7	4.52	93.1	4.11	94.8	4.00	100	0.89
CBL	101	4.35	105	3.48	101	4.53	108	2.71
CBC	97.7	3.15	96.2	1.88	98.6	3.50	98.2	0.73
THCA	89.6	3.78	90.2	3.74	91.8	4.34	99.1	1.78
CBCA	97.9	3.29	99.1	2.29	99.0	2.49	101.0	1.11

Table 3: **The Accuracy and Precision (%) Data for post-spiking recovery in the hemp leaf samples**

CONCLUSIONS

This method is a fast, sensitive, comprehensive and accurate method and is the first validated method to simultaneously quantify 16 major CBDs in hemp flower and leaf samples using UPLC-MS/MS.