A Fast, Sensitive, Comprehensive and Accurate Quantitative Assay of 16 Cannabinoids in Different Matrix by LC/MS/MS

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INTRODUCTION

In 2018, AOAC has published a method to quantify 10 cannabinoids (CBDs) with the range of 0.500-10.0 µg/mL in cannabis. However, there are more than 100 CBDs isolated from cannabis in addition to above 10 CBDs. Furthermore, CBDs have been legally and widely used for more and more products. Thus, AOAC method may not cover various needs from different CBD products. In order to cover various needs, Dyad Labs has developed UPLC-PDA and UPLC-MS/MS methods. In this poster, the fast, sensitive, accurate and comprehensive quantitative LC/MS/MS method for 16 major CBDs (**Table 1**) was presented in different matrix. The UPLC-PDA method is presented in P-T-019 poster.

Analyte and IS	Abbreviation	MRM Parameters			
		Q1	Q3	Typical CE (volts)	Typical DP (volts)
cannabidivarinic acid	CBDVA	331.5	191.0	38	75
Cannabidivarin	CBDV	287.4	231.0	26	100
cannabidiolic acid	CBDA	341.5	261.1	27	200
Tetrahydrocannabinolic Acid	THCA	359.5	285.0	35	75
cannabigerolic acid	CBGA	361.5	219.2	35	70
cannabigerol	CBG	317.2	193.1	17	60
cannabidiol	CBD	315.5	135.0	27	100
terahydrocannabivarin	THCV	287.0	165.2	-19	100
Tetrahydrocannabivarinic acid	THCVA	331.5	233.0	33	75
cannabinol	CBN	311.5	223.1	29	100
Cannabinolic Acid	CBNA	355.3	337.3	22	90
Delta-9-Tetrahydrocannabinol	delta9-THC	315.2	193.1	19	60
Delta-8-Tetrahydrocannabinol	delta8-THC	315.5	165.0	28.5	100
Cannabicyclol	CBL	315.5	235.0	22.7	100
cannabichromene	CBC	315.5	259.1	18	100
Cannabichromenic Acid	CBCA	359.3	277	20	85
Cannabidiol-D_3 (IS)	CBD-d3	318.2	135.0	30	100
Cannabinol-D_3 (IS)	CBN-d3	314.0	296.2	23	100
Delta-9-Tetrahydrocannabinol-D3 (IS)	delta9-THC-d3	318.2	196.1	-23	100

Table 1: The information and MRM parameters of 16 CBDs and IS

METHODOLOGY

Sample Preparation and Extraction:
Appropriate sample was hydrated with 5 mL of DI water, and then extracted with 35 mL of methanol. The sample is then diluted if needed, and then cleaned up with filter. Sample is diluted with equal volume of water to match with LC condition.

LC-MS/MS Conditions
LC system: Nexera UPLC system including SIL-30AC auto-sampler, controller, column heater and binary pump (Shimadzu) (see **Table 2**)

MS detector: Triple Quadrupole 5500 MS (AB Sciex) (See **Table 1** and **Table 3**)
MS Parameters: see Table 1

Column Temperature (°C)	35
Mobile Phase Gradient (%B)	70
Flow rate (mL/min)	1.50
Sample Manager Temperature (°C)	15
Run Time (min)	16

Table 2: LC Parameters

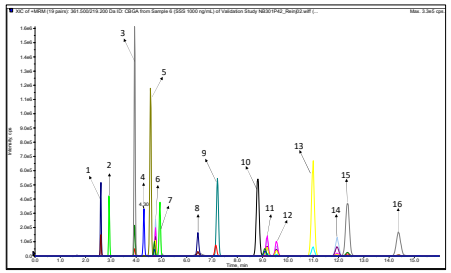
MS Parameters					
Scan Mode	Ion Mode	Source Temperature (°C)	Dwell Time (ms)	Ion Source Gas1	Ion Source Gas2
MRM	Positive	500	100	50	30

Table 3: MS Conditions

RESULTS and DISCUSSIONS

Chromatographic Separation

Some CBDs are isomers and have 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 1**).



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 1: The Representative Chromatogram of 16 CBDs

Specificity, Sensitivity and Linearity

The specificity results indicated that there is no interference between analyte, IS and in blank matrix (see **Figure 2**). The curve range of 20.0-2000 ng/mL was successfully validated. The regression is quadratic with 1/x as the weighing factor (***Figure 3***). The correlation coefficient R^2 is > 0.995 (***Figure 3***). The representative chromatograms for LLOQ and ULOQ samples were in ***Figure 3***.

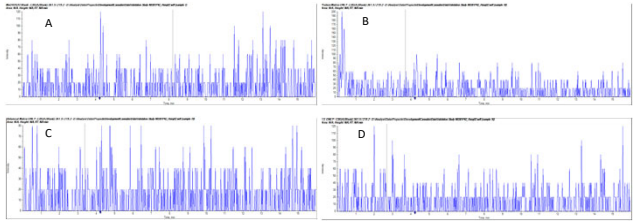


Figure 2: The CBGA specific chromatograms in A). diluent sample, B). Blank protein matrix sample, C). Blank botanical matrix sample and D). IS only sample.

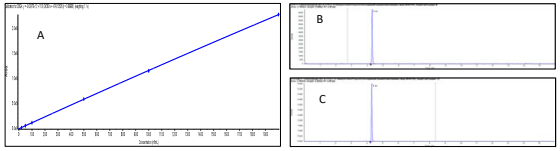


Figure 3: The CBGA calibration Curve (A), LLOQ (B) and ULOQ (C) chromatograms

Accuracy and Precision

The accuracy and precision were investigated in CBD oil sample and with post-spiking pesticides in blank botanical and non-botanical matrix at lower, medium and high regions of the established range of the calibration curve (***Tables 4***).

Analyte	Post-Spiking Low (75.0 ng/mL)		Post-Spiking Medium (300 ng/mL)				Post-Spiking High (1200 ng/mL)	
	Accuracy (%)	RSD%	Accuracy (%)	RSD%	Precision (%)	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 4: The Representative Accuracy and Precision (%) Data for post-spiking recovery in botanical matrix

CONCLUSIONS

This method is a fast, sensitive, comprehensive and accurate method, and the first validated method to simultaneously quantify 16 major CBDs in in CBD oil, botanical and protein matrix using UPLC-MS/MS.