A Fast, Comprehensive and Accurate Quantitative Assay of 16 Cannabinoids in Different Matrix by UPLC-PDA

Aihua Liu, Paul Kelly, Daniel Taylor, Spencer Carter
Dyad Labs, 1945 S Fremont Dr| Salt Lake City, UT 84104

Poster Number: P-T-019

AOAC Educate Network Collaborate
ANNUAL MEETING & EXPOSITION
133rd Annual Meeting | September 6-12, 2019 | Denver, Colorado

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 CBDs products. In order to cover various needs, Dyad Labs has developed UPLC-PDA and UPLC-MS/MS methods for 16 major CBDs. In this poster, the fast, comprehensive and accurate quantitative UPLC-PDA assay for 16 CBDs (see **Table 1** and **Figure 1**) is presented in different matrix. The UPLC-MS/MS method is presented in P-T-018 poster.

Analyte and IS	Abbreviation
Cannabidivarinic acid	CBDVA
Cannabidivarin	CBDV
Cannabidiolic acid	CBDA
Tetrahydrocannabinolic Acid	THCA
Cannabigerolic acid	CBGA
Cannabigerol	CBG
Cannabidiol	CBD
Terahydrocannabivarin	THCV
Tetrahydrocannabivarinic acid	THCVA
Cannabinol	CBN
Cannabinolic Acid	CBNA
Delta-9-Tetrahydrocannabinol	delta9-THC
Delta-8-Tetrahydrocannabinol	delta8-THC
Cannabicyclol	CBL
Cannabichromene	CBC
Cannabichromenic Acid	CBCA
Cannabidiol-D_3 (IS)	CBD-d3
Cannabinol-D_3 (IS)	CBN-d3
Delta-9-Tetrahydrocannabinol-D3 (IS)	delta9-THC-d3

Table 1: The information of 16 CBDs and IS

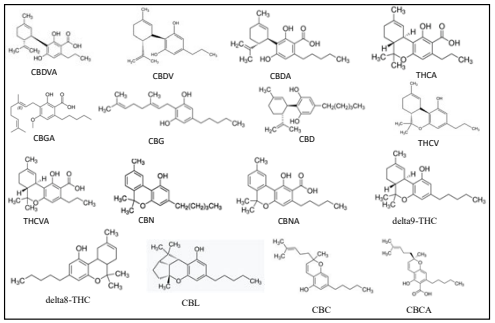

Figure 1: The Chemical Structures of 16 CBDs

METHODOLOGY

Sample Preparation and Extraction:
Appropriate sample was weighed in 50 mL centrifuge tube. 5 mL of DI water was added to hydrate sample. 35 mL of methanol was then added to sample to precipitate protein and other types of interferences. The supernatant is diluted if needed, and then cleaned up with filter. Filtered sample is mixed with equal volume of DI water to match with LC initial solvent before analysis on instrument.

UPLC-PDA Conditions
UPLC system: Waters Acquity UPLC System including quaternary solvent manager, sample manager FTN, column manager and PDA detector. (see *Table 2* for parameters)

PDA detector: Scanning range of 200 nm to 400 nm. Quantitation uses 220 nm as channel.

Column Temperature (°C)	35
Mobile Phase Gradient (%B)	70
Flow rate (mL/min)	1.50
PDA range (nm)	200-400
Sample Manager Temperature (°C)	15
Run Time (min)	16

Table 2: UPLC-PDA Parameters

RESULTS and DISCUSSIONS

Stability

In AOAC method, the standard solutions are expensive, but only have 3 days stability. In order to understand the stability of CBD better, we comprehensively investigated on the stability of all CBDs with different parameters including light, heat and pH (see **Figure 2**). 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.

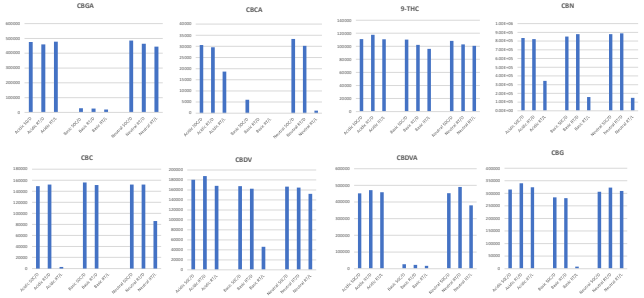

Figure 2: Representative Data of Stability Study

In order to establish longer stability, we also compared different solvents including water, methanol, acetonitrile and methanol:water (70:30 v/v). The data indicated that CBDs are most stable in methanol:water (70:30 v/v) (see **Figure 3**). During validation, we established up to 7 days stability for the standard solutions at 4.00-40.0 ug/mL using methanol:water (70:30 v/v) as solvent in fridge. The extracted sample is stabile for 7 days with storing in fridge (see **Table 3**)

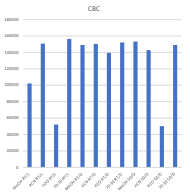

Figure 3: The CBC Solvent Stability Data

Sample	Storing Time (days)	Storing Condition	Accuracy
Std 1 (4 ug/mL)	7	Amber/Fridge	95-105%
Std 6 (40 ug/mL)	7	Amber/Fridge	95-105%
Botanical Low (10 ug/mL)	7	Amber/Fridge	90-110%
Botanical High (30 ug/mL)	7	Amber/Fridge	90-110%
Protein Low (10 ug/mL)	7	Amber/Fridge	90-110%
Protein High (30 ug/mL)	7	Amber/Fridge	90-110%

Table 3: The data of standard solution and extracted sample

Sensitivity and Linearity

The curve range of 4.00-40.0 ug/mL was successfully validated. The regression is quadratic with 1/x as the weighing factor. The correlation coefficient R^2 is > 0.995. The representative chromatograms of LLOQ and ULOQ were in *Figure 5*.

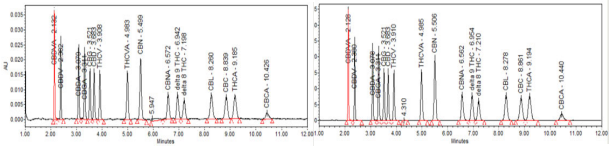

Figure 5: Representative Chromatograms of LLOQ (left) and ULOQ (right)

Accuracy and Precision

The accuracy and precision were investigated with CBD oil sample and post-spiking CBDs in blank botanical and protein matrix at lower, medium and high regions of the established range of the calibration curve (*Tables 7*).

Analyte	Post-Spiking Low (10 ug/mL)		Post-Spiking Medium (20 ug/mL)				Post-Spiking High (30 ug/mL)	
	Accuracy (%)	RSD%	Accuracy (%)	RSD%	Precision (%)	RSD%	Accuracy (%)	RSD%
CBDVA	105	0.95	101	0.68	100	0.59	101	0.57
CBDV	104	0.96	100	0.23	100	0.76	100	0.58
CBDA	104	0.55	99.0	0.62	101	1.53	102	0.56
CBGA	105	1.45	100	0.21	100	0.69	101	0.57
CBG	104	2.00	100	0.65	101	0.85	101	0.57
CBD	106	1.44	101	0.96	100	1.33	101	0.57
THCV	104	1.11	100	0.68	100	0.89	101	0.57
THCVA	105	0.55	100	0.59	99.0	1.36	101	0.57
CBN	105	0.95	100	0.63	101	0.91	101	0.57
CBNA	105	1.46	98.0	1.89	101	3.27	101	0.57
delta 9 THC	101	1.51	98.0	0.89	100	2.13	100	0.97
delta 8 THC	103	0.56	100	0.88	100	1.31	101	0.00
CBL	106	0.55	99.0	0.59	100	0.89	101	0.00
CBC	107	1.08	100	1.36	101	1.39	100	1.48
THCA	104	2.00	99	1.49	98.0	1.76	100	1.10
CBCA	102	6.57	95.0	4.47	102	7.94	97.0	3.34

Table 7: The Representative Accuracy and Precision (%) Data for post-spiking recovery in protein matrix

CONCLUSIONS

This is the first validated method for a fast, comprehensive and accurate quantification of the 16 major CBDs in CBD oil, botanical and protein matrix using UPLC-PDA .