TN-1330

Potency Analysis in Hemp and Cannabis Products Using a Single-dilution Combined LC-UV-MS/MS Approach

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Introduction

Based on individual state regulatory requirements in the US, the potency of commercial cannabis products must be reported as the percentage of THC and printed on cannabis product labels after being certified by a licensed cannabis testing facility. The methodology for obtaining cannabis potency values can vary based on the analytical technique and instrumentation used, which gives options for testing facilities to customize or streamline their workflows.

The simplest approach to cannabinoid analysis is LC separation with UV detection in the 200-230 nm wavelength range. Due to limitations in the linear dynamic range of UV and photodiode array (PDA) detectors, it may be difficult to accurately quantitate a wide range of cannabinoids in a single injection using a single dilution scheme for all samples. The concentrations of highly abundant cannabinoids, such as delta-9-THC and Tetrahydrocannabinolic Acid A (THCA) in cannabis or Cannabidiol (CBD) and Cannabidiolic Acid (CBDA) in hemp, may exceed 90 %. However, other cannabinoids may only be present at concentrations less than 0.5 %. Therefore, with UV analysis alone, a multiple dilution protocol may be necessary to analyze a wide panel of cannabinoids to ensure that the calculated concentrations fall within the linear dynamic range of the UV detector.

LC separation with MS/MS detection is another commonly used technique for cannabis potency analysis. Modern mass spectrometers are designed to be sensitive enough to measure compounds in the fg/mL and pg/mL range, however some cannabinoids may be present in concentrations exceeding 90 % of the weight of the product.

In this technical note, the unique selectivity and increased retention for both polar and non-polar analytes of the Luna Omega Polar C18 column and the robustness of the SCIEX® QTRAP® 6500+ system delivered a robust analysis and determination of 11 cannabinoids in cannabis and hemp products with varying levels of potency. The mass spectrometer provides sensitivity for low abundance cannabinoids and the HPLC-UV detector provides quantitation up to 100 % THC or CBD potency by weight.

Sample Preparation

Step	Description
1.	Homogenize flower samples, process concentrates without homogenization
2.	Place 0.2 g of sample in 10 mL of Acetonitrile
3.	Shake and sonicate for 30 minutes
4.	Centrifuge for 5 min at 300 x g
5.	Filter extract with a 0.2 µm nylon syringe filter
6.	Dilute filtered extract 1:100 (v/v) with Acetonitrile
7.	Inject 2 µL for analysis

The mass of sample extracted can be modified if necessary. For example, 0.5 grams of sample may be extracted into 25 mL of Acetonitrile. Water content was not determined in this study. Therefore, the percent results represent the weight as received of each sample. Moisture content analysis must be performed separately to normalize results to the water content of each sample. Six cannabis and hemp flower strains were tested, and six concentrates of different varieties were tested (**Table 2**).

LC Conditions

Column: Luna™ Omega 3 µm Polar C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4760-E0
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile / Water (96:4, v/v)

Gradient:

Time (min)	%B
0	75
0.5	82
6	82
12	90
12.5	100
14	100
14.1	75
16	75

Flow Rate: 1 mL/min
Injection Volume: 2 µL
Temperature: 25 °C
LC System: SCIEX ExionLC™ with a PDA Detector
Detection: UV @ 210-230 nm and MRM
Detector: SCIEX QTRAP 6500+

MS/MS Conditions

Polarity: Positive and Negative
Gas Temperature: 500 °C
GS1: 60 psi
GS2: 60 psi
CUR: 40 psi
CAD: 11
IS: 5500/-4500 V

Table 1. MRM Parameters.

Analyte	Q1 (m/z)	Q3 (m/z)	DP	CE
CBG_1	317	193	200	10
CBG_2	317	123	100	30
THCV_1	287.1	165	125	30
THCV_2	287.1	231.3	125	24
CBDV_1	287.1	165.3	150	32
CBDV_2	287.1	123.1	150	41
CBC_1	315	193	94	27
CBC_2	315	81.2	94	17
THC_1	315	193.1	150	25
THC_2	315	135	150	25
CBN_1	311.2	223	50	15
CBN_2	311.2	241	50	15
CBD_1	315	259	200	27
CBD_2	315	193	150	27
CBGA_1	359	191.1	-200	-45
CBGA_2	359	315.3	-200	-30
CBDA_1	357	245.3	-200	-39
CBDA_2	357	179.1	-200	-32
THCA_1	357	313.4	-100	-34
THCA_2	357	191.2	-100	-42

Table 2. List of Cannabis and Hemp Samples Tested.

Name	Product Type	Plant
Blue Dream	Flower	Cannabis
Lemon Kush	Flower	Cannabis
Mile High Hemp	Flower	Hemp
Phenova Hemp	Flower	Hemo
Phenova Proficiency Test Hemp	Flower	Hemp
FLO Sativa	Flower	Cannabis
Gorilla Glue	Oil	Cannabis
M.H. Hemp D	Distillate	Hemp
Wedding Cake	Wax	Cannabis
Pachamama	Wax	Cannabis
Tropical Fruit	Oil	Cannabis
CBD Distillate	Distillate	Hemp

Results and Discussion

11 cannabinoids in cannabis and hemp samples ranging from 0.05-100 % total weight were quantitated in a single injection with a single dilution scheme. At the low end of this range, sufficient signal was present using the MS/MS system to calibrate even lower than the limit used in this study (approximately 0.005 %). This extra sensitivity could be important when analyzing low abundance cannabinoids or small sample masses for research purposes. The PDA detected the high end of the potency range for the abundant cannabinoids at 2.5-100 % by weight without detector saturation at the highest point in the calibration curve. An example of the two overlapping calibrations curves from two different detectors is shown in **Figure 1**.

Using the custom flagging features in SCIEX® OS-MQ Software, the software automatically determined whether the calculated value for the MS/MS or the PDA was to be reported. SCIEX OS-MQ Software also automatically converted the results to a percentage using the extracted sample mass entered into the batch and the total dilution factors. Finally, the software calculated the total percentage of CBD and THC by adding the acid and neutral forms of each (CBD + CBDA and THC + THCA) after applying a 0.877x molar correction factor to the acids, due to the extra molecular weight of the acid before decarboxylation.

In addition to an outstanding linear dynamic range, the method also exhibited good reproducibility, likely due to the single 1:5000 dilution used during sample preparation coupled with a 2 µL injection. Continuing calibration verifications (CCVs) were analyzed every 10 samples, and their responses were consistent over the course of the batch. **Table 3** shows good reproducibility of THCA in a 0.5 ppm MS/MS CCV and a 25 ppm PDA CCV with RSDs of 1.6 % and 2.0 %, respectively. The calculated concentrations of the CCVs were within the desired 25 % of the expected concentration throughout the course of the run, which included approximately 60 injections of cannabis flower, hemp flower, and concentrate samples.

Concentrates were also quantified using the same workflow, including the same dilution factor, injection volume, and calibration standards. In **Figure 3**, CBD results are shown using the PDA curve or the MS curve. Because the concentration was higher than the linear dynamic range of the MS, the calculated result of 30 % by weight CBD is inaccurate. However, the PDA detector, which can accurately quantify up to 100 % by weight, showed that the CBD concentration in the wax was 70.2 %. The automatic flagging rules used in SCIEX OS-MQ Software reported the 70.2 % CBD value to the report and ignored the inaccurate 30.7 % MS/MS calculated value.

The results of 4 cannabis flower samples, 4 cannabis concentrates, 3 hemp flower samples, and 1 hemp concentrate are shown in **Table 4**. All 11 cannabinoids were detected in at least 1 sample. Because the moisture content was not analyzed for these samples, the values represent the percentage of each cannabinoid in the entire sample and were therefore not directly comparable to reported label values. All 12 samples were prepared using the protocol described in the sample preparation section without modification based on sample type. The advantage of this workflow is this ability to accurately analyze this diverse set of samples without changing the mass of sample extracted, dilution factor, injection volume, or any other parameter.

Figure 1. CBD Calibration Curves. (Top) CBD Calibration Curve Using MS Detector (0.1-10 ppm in Vial; Corresponds to 0.05-5 % in Samples) Showing R^2 of 0.999. (Bottom) CBD Calibration Curve Using PDA (5-250 ppm in Vial; Corresponds to 2.5-125 % in Samples) Showing R^2 of 0.999.

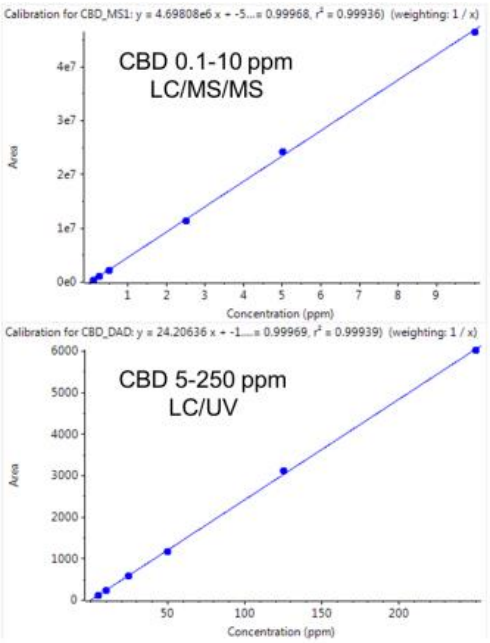


Table 3. Reproducibilty of CCV Standards Analyzed Throughout the 60 Sample Batch

Sample	Expected Concentration THCA (ppm)	Calculated Concentration THCA (ppm)	Accuracy (%)
MS QC1	0.5	0.538	108
MS QC2	0.5	0.533	107
MS QC3	0.5	0.555	111
MS QC4	0.5	0.547	109
MS QC5	0.5	0.532	106
MS QC6	0.5	0.540	108
MS QC Summary		**RSD = 1.6 %**	
UV QC1	25	24.9	100
UV QC2	25	23.8	95
UV QC3	25	24.9	100
UV QC4	25	24.7	99
UV QC5	25	24.8	99
UV QC6	25	25.3	101
UV QC Summary		**RSD = 2.0 %**	

Figure 2. Cannabinoid Elution Profile of a 10 ppm Standard Showing UV Trace Data.

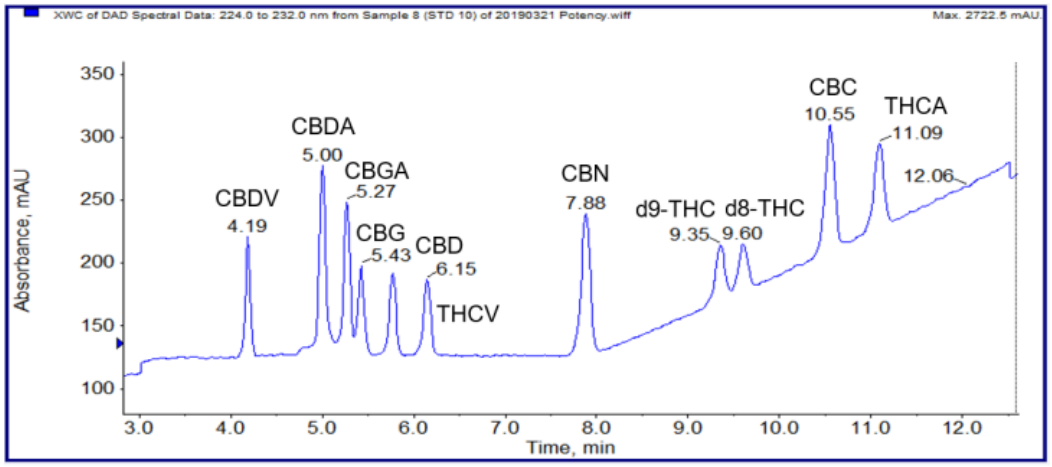
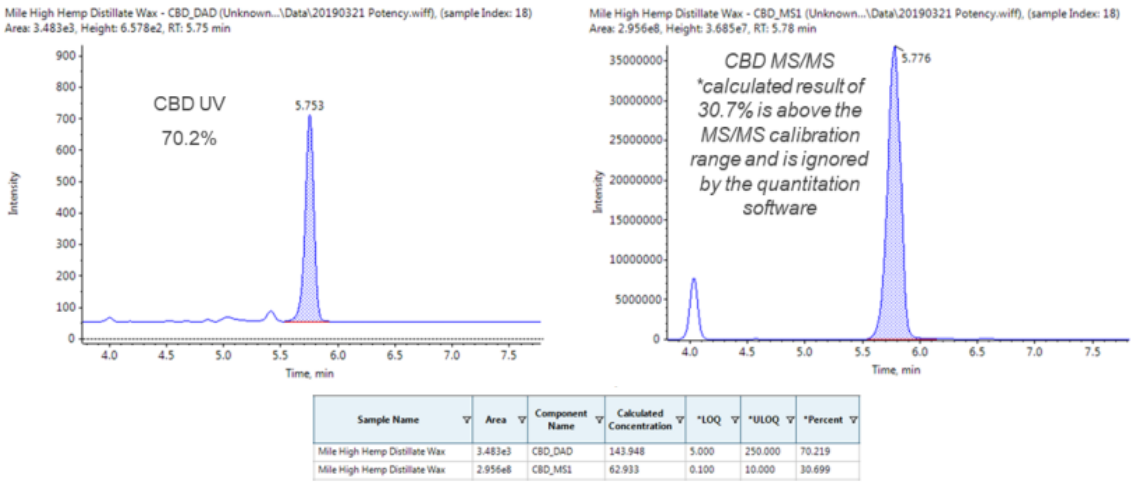


Figure 3. Quantitative Results for CBD in Hemp Wax. In This Sample, SCIEX® OS Software Reported the UV Value (Top) Because the MS/MS Value was Too High for the MS/MS Calibration Curve (Bottom).



Sample Name	Area	Component Name	Calculated Concentration	*LOQ	*ULOQ	*Percent
Mile High Hemp Distillate Wax	3.483e3	CBD_DAD	143.948	5.000	250.000	70.219
Mile High Hemp Distillate Wax	2.956e8	CBD_MS1	62.933	0.100	10.000	30.699

Table 4. Summary Table of Cannabinoid Concentrations for All Samples Analyzed in This Study. *Total CBD and THC Concentrations Assume 100 % Decarboxylation of CBDA and THCA to CBD and THC, Respectively, on a Molar Basis.

Sample Name	CBD	CBDA	d9THC	d8THC	THCA	CBN	CBG	THCV	CBDV	CBC	CBGA	Total CBD*	Total THC*
Blue Dream Cannabis Flower	<LOQ	0.06 %	0.14 %	<LOQ	18.38 %	<LOQ	0.07 %	<LOQ	<LOQ	<LOQ	0.19 %	0.05 %	16.26 %
FLO Cannabis Flower	<LOQ	<LOQ	<LOQ	<LOQ	12.67 %	<LOQ	<LOQ	<LOQ	<LOQ	0.05 %	0.18 %	0 %	11.11 %
Lemon Kush Cannabis Flower	<LOQ	0.06 %	0.83 %	<LOQ	17.48 %	<LOQ	0.12 %	<LOQ	<LOQ	<LOQ	0.91 %	0.05 %	16.16 %
Phenova Cannabis Flower	3.37 %	3.90 %	<LOQ	<LOQ	2.31 %	0.14 %	0.15 %	0.19 %	<LOQ	0.27 %	0.15 %	6.79 %	2.02 %
Pachamama Sugar Wax	0.38 %	3.25 %	9.25 %	<LOQ	59.88 %	<LOQ	0.39 %	0.45 %	<LOQ	0.21 %	1.05 %	3.23 %	61.76 %
Wedding Cake Sugar Wax	<LOQ	0.22 %	4.90 %	<LOQ	69.83 %	<LOQ	0.27 %	<LOQ	<LOQ	0.11 %	2.13 %	0.19 %	66.14 %
Evolabs Tropical CO2 Oil	3.77 %	<LOQ	72.45 %	<LOQ	<LOQ	0.76 %	1.78 %	0.66 %	<LOQ	1.18 %	<LOQ	3.77 %	72.45 %
Gorilla Glue CO2 Oil	0.16 %	0.25 %	41.08 %	<LOQ	13.02 %	1.02 %	1.58 %	0.37 %	0.12 %	1.14 %	1.17 %	0.38 %	52.50 %
Phenova Hemp Flower 1	0.12 %	12.27 %	<LOQ	<LOQ	1.15 %	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.68 %	10.9 %	1.01 %
Phenova Hemp Flower 2	4.13 %	5.70 %	<LOQ	<LOQ	0.58 %	<LOQ	0.22 %	<LOQ	<LOQ	0.25 %	0.36 %	9.12 %	0.50 %
Mile High Hemp Flower	1.62 %	4.92 %	0.07 %	<LOQ	0.10 %	<LOQ	<LOQ	<LOQ	<LOQ	0.11 %	0.06 %	5.93 %	0.15 %
Mile High Hemp Distillate	69.97 %	<LOQ	3.76 %	<LOQ	<LOQ	0.39 %	3.53 %	<LOQ	0.14 %	4.45 %	<LOQ	69.97 %	3.76 %

Conclusion

A rapid and robust method for 11 cannabinoids using a combination of LC with UV and MS/MS detectors, coupled with a Luna™ Omega 3 µm Polar C18 column, in a single analytical run is presented for cannabis and hemp potency testing. The method separates the psychoactive delta-9-Tetrahydrocannabinol (delta-9-THC) and its isomer delta-8-Tetrahydrocannabinol (delta-8-THC) in a 16-minute gradient providing accurate levels of total THC for potency labeling of cannabis products. This two-detector approach covers a wide quantitation range of individual cannabinoid content from 0.05-100 % by product weight. By simultaneously utilizing both UV and MS detectors, higher and lower abundant cannabinoids can be accurately detected and quantified in a single analysis with the same sample injection and dilution factor, thus increasing laboratory sample throughput. The feasibility of using a dual detector approach to analyze 11 cannabinoids for potency reproducibly with a 1:5000-fold sample dilution is shown to be possible with very small replicate deviation. The method was tested on both hemp and cannabis matrices for flowers and concentrates that cover the entire potency range. Sample preparation no longer requires a multiple injection or multiple dilution sample method to monitor both the low- and high-abundant cannabinoids.

Luna Omega Ordering Information

3 µm Analytical Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0*/10pk**
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJ0-7606
C18	00B-4784-E0	00D-4784-E0	00F-4784-E0	00G-4784-E0	AJ0-7612
SUGAR	—	00D-4775-E0	00F-4775-E0	00G-4775-E0	AJ0-4495

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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