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

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Overview

A rapid and robust method for 11 cannabinoids using a combination of LC with UV and MS/MS detectors 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 cananbinoids can be accurately detected and quantified in a single analysis with the same sample injection and dilution factor, thus increasing laboratory sample throughput.

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 cannibis potency values can vary based on the analytical technique and instrumentation used, which gives options for testing facilities to customize or streamline their workflows.

All analytical instruments exhibit a dynamic range of detection, and to accurately quantify the concentration of any component in a sample, that component must be diluted to a concentration within the dynamic range of the instrument. The dynamic range of an instrument is controlled by various factors, including detector performance, chromatographic efficiency, and ionization efficiency. At very high concentrations of a compound, a detector may not be able to distinguish small changes of concentration from one sample to the next and will not show a linear response of increasing detector response to analyte concentration.

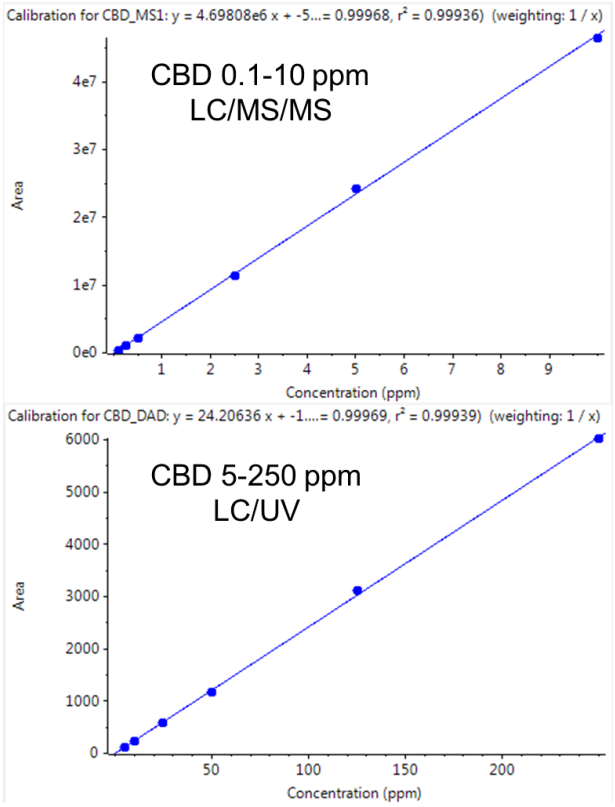


Figure 1: CBD Calibration Curves. (Top) CBD calibration curve using MS detector (0.1-10 ppm in vial; corresponds to 0.05-5% in samples) showing r2 of 0.999. (Bottom) CBD calibration curve using PDA (5-250 ppm in vial; corresponds to 2.5-125% in samples) showing r^2 of 0.999.

Key Advantages of HPLC-UV in Tandem with MS/MS Potency Analysis

- Assay panel covers 0.05-100% potency by weight allowing testing for both flower and pure distillate without any carryover or change in dilution factor
- SCIEX OS software provides custom flagging to determine whether the PDA or the MS is used as a detector automatically to generate accurate quantitative results

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. It is capable of a larger dynamic range and more specific detection because MS/MS detection measures the response of individual fragments of each compound. 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. Achieving an adequately low concentration for MS/MS analysis requires diluting the original extract multiple times to achieve final dilutions of 1:250,000 to 1:2,250,000. Due to the high hydrophobicity of cannabinoids, non-specific binding of cannabinoids on plastic or glass surfaces may occur, decreasing the apparent concentration of cannabinoids in the sample.

Table 1. 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	Hemp
Phenova Proficiency Test Hemp	Flower	Hemp
FLO Sativa	Flower	Cannabis
Gorilla Glue	Oil	Cannabis
M.H. Hemp D	Distillate	Hemp
Wedding Cake	Wax	Cannabis
Pachymama	Wax	Cannabis
Tropical Fruit	Oil	Cannabis
CBD Distillate	Distillate	Hemp

Therefore, performing multiple serial dilutions of cannabis extracts can lead to inaccurate results.

In this study, a workflow for analyzing 11 cannabinoids in cannabis and hemp products with varying levels of potency is presented using LC-UV in tandem with a triple quadrupole mass spectrometer. 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.

Experimental

Sample Preparation

Flower, Distillates and Concentrates

1. Homogenize flower samples, process concentrates without homogenization
2. Place 0.2 gram of sample in 10 mL of acetonitrile
3. Shake and sonicate for 30 minutes
4. Centrifuge for 5 min at 300xg
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 g 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.

Samples

Six cannabis and hemp flower strains were tested and six concentrates of different varieties were tested (Table 1).

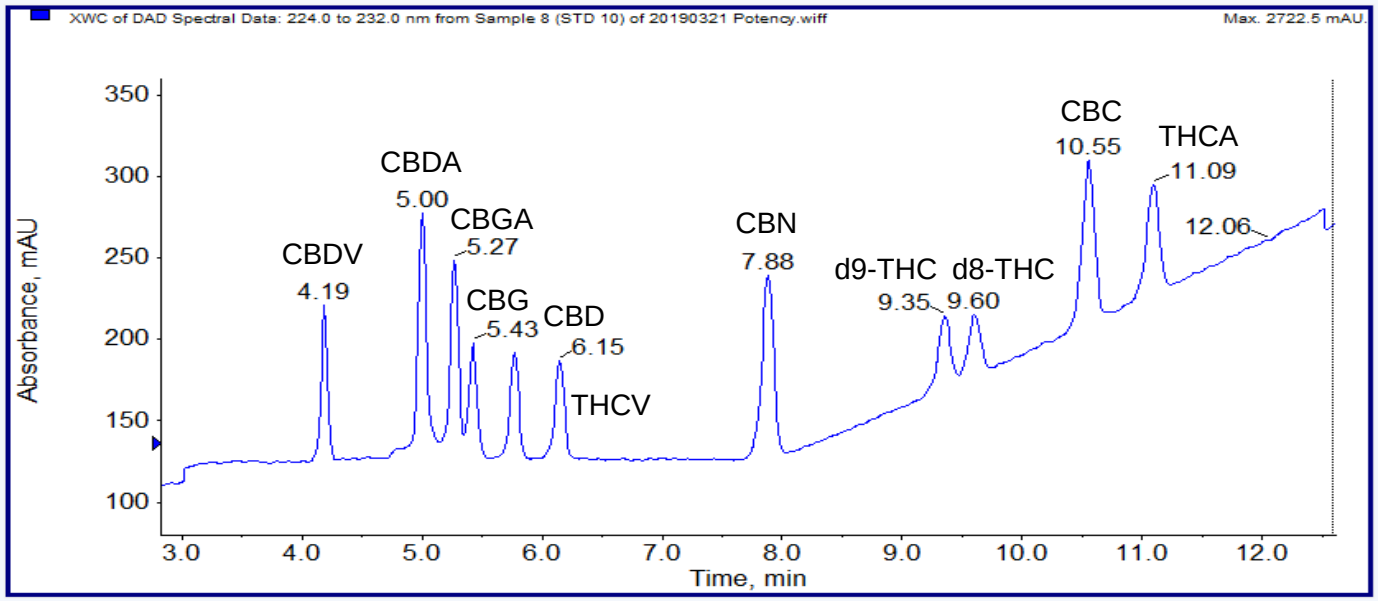


Figure 2: Cannabinoid Elution Profile of a 10 ppm Standard Showing UV trace Data.

LC Separation

A 2 µL volume of sample was injected using an ExionLC™ AD system with a PDA (photodiode array) detector coupled to a QTRAP® 6500+ system. Separation was performed using a Phenomenex Luna Omega Polar C18 (150×4.6 mm, 3 um) analytical column. The LC mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in 96% acetonitrile and 4% water (B) at a flow-rate of 1 mL/min and column temperature of 25°C.

Table 2: Gradient Conditions Used for the LC Separation. Flow rate of 1 mL/min was used.

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

Acquisition Method

Analysis was performed using the ExionLC system with integrated PDA UV detector and LC-MS/MS operated in both positive and negative polarity modes. The PDA detector was set to collect absorbance from a wavelength range of 210-230 nm. The following MS source conditions were used: CUR=40 psi, CAD=11, IS =5500/-4500 V, TEM=500°C, and GS1= 60 psi and GS2= 60 psi.

Data Processing

Data were processed using SCIEX OS-MQ Software 1.5. For the top 4 commonly detected cannabinoids (THC, THCA, CBD, CBDA), a high calibration range curve was generated using the PDA detector, and a low calibration range curve was generated using MS/MS on the MS detector. For the remaning cannabinoids, only an MS curve was analyzed because concentrations of these rarely exceed the maximum concentration of approximately 4% quantifiable by LC-MS/MS in this method. Once the curves were established, custom calculations were developed in SCIEX OS-MQ processing software to automatically convert the calculated concentrations to percent by weight of the plant using the mass extracted, volume extracted, and dilution factor, which were entered into Analyst® software when the samples were submitted for analysis.

Result and Discussion

An ExionLC system with integrated PDA detector and a SCIEX Triple Quad 6500+ mass spectrometer were used together in a single injection with a single dilution scheme to quantitate 11 cannabinoids in cannabis and hemp samples ranging from 0.05-100% total weight. 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, the software automatically determined whether the calculated value for the MS/MS or the PDA was to be reported. SCIEX OS-MQ 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. A customizable report template was then used to generate a report as shown in Figure 2.

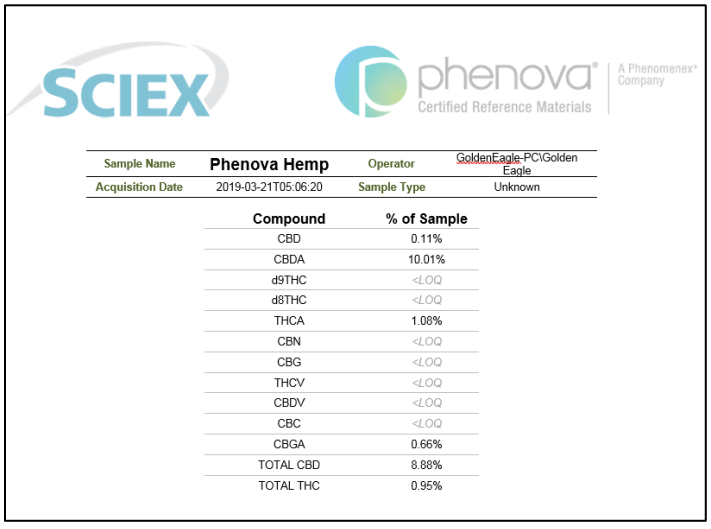


Figure 2: Custom Report Template Exported from Results Quantifiying Potency of Cannabinoids in Hemp Provided by Phenova.

In addition to an outstanding linear dynamic range, the method also exhibited good reproducibility, likely due to the single 1:5,000 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.

Table 3: Reproducibility 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%	

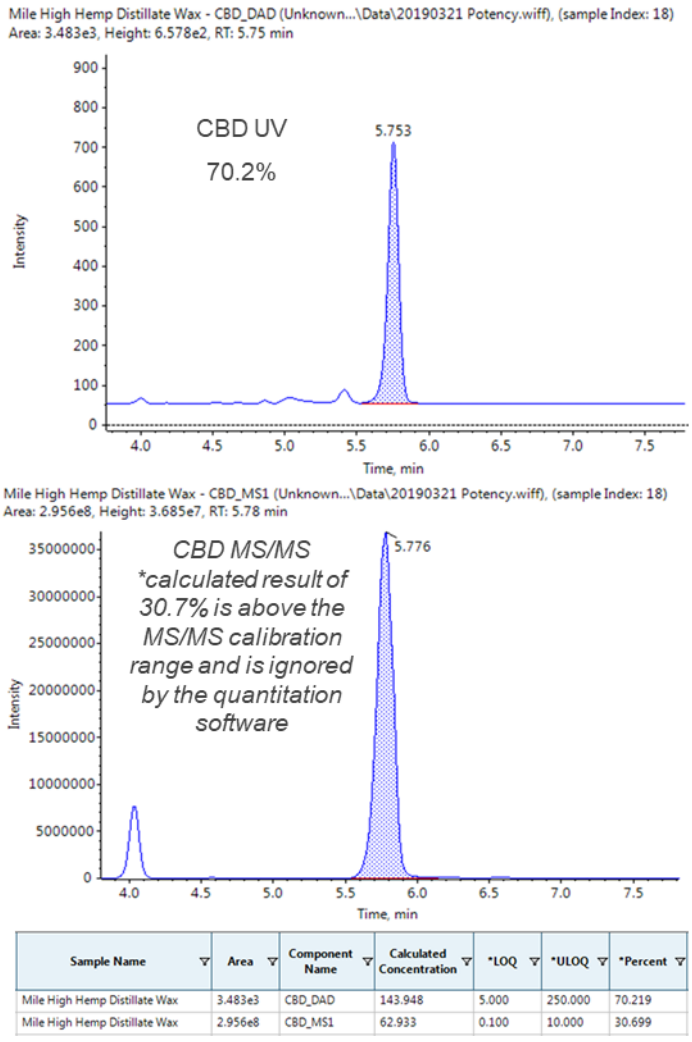
The results of 4 cannabis flower samples, 4 cannabis concentrates, 3 hemp flower samples, and 1 hemp concentrate are shown in Table 5. All 11 cannabinoids were detected in at least 1 sample. Because the moisture content was not analyzed for these samples, the values represent the percentage of each cannabinoid in the 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.

Conclusions

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.

Table 4. MRM Parameters.

Name	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_1	*357*	*191.2*	*-100*	*-42*



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

Figure 3: Quantitative Results for CBD in Hemp Wax. In this sample, SCIEX OS reported the UV value (top) because the MS/MS value was too high for the MS/MS calibration curve (bottom).

Table 5. 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%

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