



Extraction and UPLC Analysis of THC, CBN, and CBD from Hemp Flower

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SUMMARY

With the passing of the 2018 Farm Bill legalizing hemp, new hemp producers are entering the market with new and large hemp crops. This increase in raw material has led to the need for efficient extraction methods to assess the potency of the material. For cannabis to be defined as hemp, the total THC content must be at, or lower than a specific level, therefore accurate determination of cannabinoid content is essential to the industry. Cannabis is a difficult and complex matrix to analyze. Extraction and cleanup generally go hand in hand utilizing sorbents to remove undesirable constituents from the total extract. Typically, the QuEChERS method is used to extract the cannabinoid content from cannabis. This method has several disadvantages, including multiple sample transfers, substantial waste generation, and its manual nature. In this study, hemp flower was milled to a coarse powder in the MiniG® and the CEM EDGE® was used to extract cannabinoids from the sample, this was shown to be more efficient than traditional methods at both low and high temperatures.

INTRODUCTION

In light of the legalization of industrial hemp, methods to examine the potency of cannabinoids from cannabis plants and products are necessary. Potency testing is crucial for assessing the legality of the material. The major compounds of interest in cannabis are Δ^9 -tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), cannabinol (CBN), cannabidiol (CBD), and cannabidiolic acid (CBDA). Currently, regulations require industrial hemp to contain less than 0.3% of THC and THCA combined on a dry weight basis. Furthermore, the cannabinoid content of cannabis is important for its use medicinally or recreationally. Cannabis that contains high total THC content and low total CBD (sum of both CBD and CBDA) is typically used recreationally and is sold at a higher price, while cannabis that has lower total THC content and higher total CBD is usually for medicinal purposes.

Using the EDGE, extractions are performed in a single vessel, the Q-Cup™, in one automated step without the use of cleanup materials. The EDGE also filters the extract, yielding a cooled sample, ready for direct liquid chromatography analysis.

- :: APPLICATION NOTE SP030
Plant tissue
homogenization
- :: APPARATUS
1600 MiniG®
- :: APPLICATION
Cannabinoid extraction
and analysis by UPLC



MATERIALS AND METHODS

REAGENTS

A bag containing 3.5 g of "Pine Berry" variety hemp flower was obtained from a retail CBD dispensary in Matthews, NC. ACS grade methanol and isopropanol were purchased from Sigma Aldrich. Methanol was used as the extraction solvent, while isopropanol was used as an additional wash solvent.

EXTRACTION

All samples were extracted and filtered on an EDGE system from CEM Corporation (Matthews, NC). A Q-Cup was assembled using a C9+G1+C9 Q-Disc® sandwich. This combination provides filtration of particulate sizes greater than 0.3 µm. The hemp flower was milled to a coarse powder in a SPEX SamplePrep 1600 MiniG with 2 x 11 mm balls per 50 mL tube for a 1 min grind at 1500 strokes per min. 0.25 g of the ground hemp flower was weighed directly into the Q-Cup. No additional cleanup matrix was added to the sample. Six extraction conditions were developed for comparison where temperature and hold times were varied to optimize extraction efficiency. All samples and blanks were prepared in triplicate.

EDGE METHOD

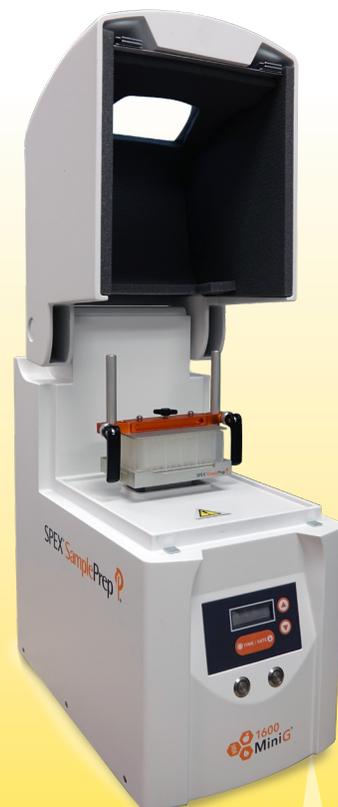
- Q-Disc: C9+G1+C9
- Extraction Solvent: Methanol
- Top Add: 10 mL
- Bottom Add: 5 mL
- Rinse: 5 mL
- Temperature: 35 °C or 90 °C
- Hold Time: 3 min, 6 min, or 9 min
- Wash 1: 30 mL Isopropanol, 15 s hold at 50 °C
- Wash 2: 30 mL Methanol, no hold or heat applied

ANALYSIS

Analysis was performed by Avazyme (Durham, NC). The resulting concentrations for total THC (the sum of THC and THCA) and total CBD (the sum of CBD and CBDA) were compared to the certificate of analysis provided at the time of purchase of the hemp flower to determine EDGE extraction recovery. The measurements for total THC and total CBD were utilized instead of the individual cannabinoids themselves because the temperature of the extracted method affects the ratio of these compounds.

RESULTS AND DISCUSSION

For all methods tested, the EDGE efficiently extracted the total THC and total CBD from the samples. Table 1 displays the levels of THC, THCA, CBD, CBN, and CBDA obtained for each extraction condition tested on the EDGE. Table 2 shows the recovery data of the total THC and CBD for each method tested. Each method yielded recoveries greater than 92%.



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TABLE 1: EXTRACTION RESULTS OF EACH OF THE SIX METHODS

*Compound concentrations are measured in mg of cannabinoid per g of dry weight material.

COMPOUND	S1 (MG/G)	S2 (MG/G)	S3 (MG/G)	S4 (MG/G)	S5 (MG/G)	S6 (MG/G)	ORIGINAL COA
CBN	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
CBD	12.93	13.62	13.94	15.49	16.48	20.27	1.25
CBDA	106.89	110.84	111.88	100.76	98.2	108.13	119.01
THC	1.74	1.82	1.85	1.83	1.86	2.17	0.17
THCA	2.65	2.65	2.67	2.43	2.4	2.61	4.76
Total CBD	106.67	110.83	112.06	103.86	102.6	141.99	105.62
Total THC	4.06	4.14	4.19	3.96	3.96	4.46	4.34

TABLE 2: EXTRACTION RECOVERIES

SAMPLE	TEMPERATURE (°C)	HOLD TIME (MIN)	TOTAL THC RECOVERY	TOTAL CBD RECOVERY
S1	35	3	93.55%	100.99%
S2	35	6	95.39%	104.93%
S3	35	9	96.54%	106.10%
S4	90	3	91.24%	98.33%
S5	90	6	91.24%	97.14%
S6	90	9	102.76%	134.43%

We expected to see higher conversion of THCA to THC and CBDA to CBD at the 90 °C temperature given the sensitivity of these constituents to transform at elevated temperatures. Table 1 shows that, even at the lower temperature extraction, a portion of CBDA had already converted to CBD and the same with THCA and THC. The total CBD and total THC values obtained are very close to the Certificate of Analysis values indicating the extraction was efficient and complete. It is theorized that either the certificate of analysis supplied at the time of purchase was not representative of the product purchased or that the drying process prior to packaging may have forced conversion after the initial COA analysis was performed. It is also unknown how the samples were handled post packaging. If they were exposed to an atmosphere that was not climate controlled THC and CBD conversion may have occurred in transit or in the final retail location. Further work would need to be performed on a “fresh” sample obtained directly from a grower to confirm these hypotheses.