APPLICATION NOTE

High-throughput extraction of cannabinoids from cannabis analog on the Omni Prep 96 automated homogenizer.

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Omni Prep 96 automated homogenizer

Introduction

Cannabinoid quantification is the most common analytical method performed by cannabis producers and testing facilities. Producers are required to define the quantity of cannabinoids in cannabis-based products before release to the market. While there are regional variations in potency testing requirements and more than 60 cannabinoids present in cannabis, producers are typically required to define the quantity of the abundant and psychoactive cannabinoids, Tetrahydrocannabinol (THC), Tetrahydrocannabinolic Acid (THCA), Cannabidiol (CBD), Cannabidiolic Acid (CBDA), Cannabigerol (CBG), and Cannabinol (CBN) (Figure 1). [1-2]

The most common method for cannabis potency testing is to mill the flower or edibles to create a homogenous mixture in the presence of an organic solvent such as methanol. Following centrifugation to pellet debris, the supernatant is further diluted prior to analysis by reverse phase HPLC or mass spectrometry. While the analytical methods are well defined and easily automated, the sample disaggregation process is low-throughput and often tedious. Herein, we evaluate the utility of the Omni Prep 96 automated homogenizer for the extraction of cannabinoids from a spiked cannabis analog.

Materials and methods

Equipment

- Omni Prep 96 automated homogenizer (Cat # 51-02A-1)
- Deck Adapter for Clean Probe/Tip Rack (Cat # 51-BRA-1006-J02) & Rack for 7 mm Clean Probes/Tips (Cat # 23-HWA-22)
- 14/15 mL x 96 Round Bottom Tubes Standard Sample Rack (Cat # 23-HWR-14) & Deck Adapter for Standard Sample Racks (Cat # 23-HWR-11-JA)
- Deck Adapter for Dirty Probe/Tip Disposal Rack (Cat # 23-HWA-13B-JA) & Disposal Rack for Dirty Probes/Tips (Cat # 23-HWA-13B)
- 7 mm hard tissue Omni Tip™ plastic homogenizing probes (Cat # 30750H)
- 14 mL Round Bottom Tubes with Snap Caps (Cat # 19-6614-500)

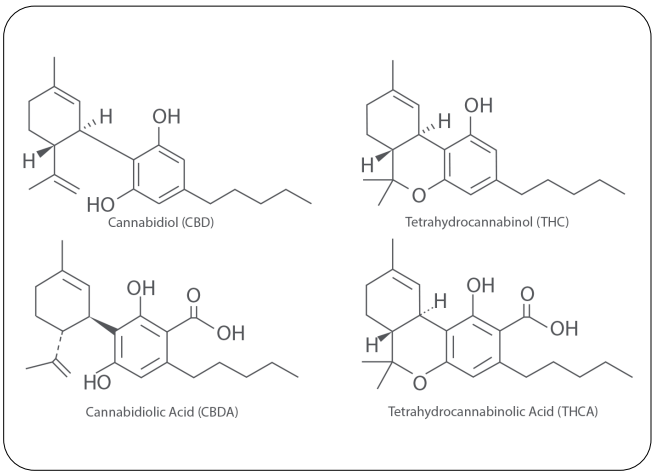

Figure 1: CBD, CBDA, THC, and THCA structure. The four cannabinoids were purchased as 1 mg/mL standards and used in this analysis.

Cannabinoid standards

THC, THCA, CBD and CBDA, 1 mg/mL standards were obtained from Cerilliant. All standards were combined to create a stock solution with a concentration of 250 ppm for each cannabinoid. Serial dilutions were created in 100 % methanol following the dilution series in Table 1. Fifteen µL of each dilution was diluted further with 35 µL of 100 % methanol and analyzed by reverse phase HPLC.

Table 1: Cannabinoid serial dilution strategy. Each parent solution was diluted 2X in methanol.

Concentration	Amount of parent solution	Methanol
250 ppm	Stock Solution	0
125 ppm	500 µL	500 µL
62.5 ppm	500 µL	500 µL
31.25 ppm	500 µL	500 µL
15.625 ppm	500 µL	500 µL
7.81 ppm	500 µL	500 µL

Sample preparation

In many states, obtaining cannabis samples for analysis, is not yet legal. As a cannabis analog, fresh hops were obtained. Ten grams of fresh hops were milled in a coffee grinder to a fine powder. Seven, 1 gram samples of hops powder was placed in 14 mL round bottom tubes. As a control, 5 mL of 100 % HPLC grade methanol was added to the ground hops. The sample was incubated with gentle manual mixing for two minutes then 250 µL was extracted for centrifugation. Six 1 g powdered hops samples were spiked with 1 mL of 250 ppm cannabinoid standard mixture. The standards were allowed to absorb into the hop powder for 2 minutes. Four mL of 100 % methanol was then added to create a cannabinoid standard concentration of 50 ppm. The samples were then processed on the Omni Prep 96 homogenization workstation equipped with 7 mm Hard Tissue Omni Tip plastic homogenizing probes at the speeds and durations listed in Table 2. Following homogenization, 250 µL was extracted and placed in a 1.5 mL microcentrifuge tube. The samples were then centrifuged at 10,000 g for 10 minutes to pellet insoluble debris and the supernatant was transferred to the bed of a 0.2 µm spin filter. The supernatant was filtered over the 0.2 µm filter, at 1,000 g, for 1 minute. Fifteen µL filtrate was further diluted with 35 µL of 100 % methanol. The samples were then analyzed by HPLC.

Table 2: Omni Prep 96 parameters for CBD and THC quantification. The control was not homogenized and was gently swirled. 250 µL was extracted after processing.

Processing	Duration	Extraction
No homogenization (Control)	2 minutes of incubation	0
25,000 rpm	10 sec	250 µL
25,000 rpm	20 sec	250 µL
25,000 rpm	30 sec	250 µL
25,000 rpm	60 sec	250 µL
25,000 rpm	120 sec	250 µL

Reverse phase HPLC

Cannabinoid separation and quantification was performed on a Waters 1525 HPLC equipped with a binary pump and photodiode array detector. Buffer A was ddH_2O and formic acid (0.2 % v/v) and buffer B was acetonitrile and formic acid (0.2 % v/v). Fifteen µL of each sample was separated on a Raptor ARC-18 150 mm x 4.6mm, 2.7 µm column over a 20-minute linear gradient from 60 % B to 100 % B at a flow rate of 1.5 mL/min. Absorbance was measured at 280 nM.

Results

Four pure cannabinoids (THC, THCA, CBD and CBDA) were combined, serially diluted and analyzed by reverse phase HPLC as shown in Figures 2-3. The HPLC method was able to quantify the cannabinoids from a concentration range of 7 ppm to 250 ppm at an RSD of 0.99. The order of elution was CBDA, CBD, THC, and THCA respectively.

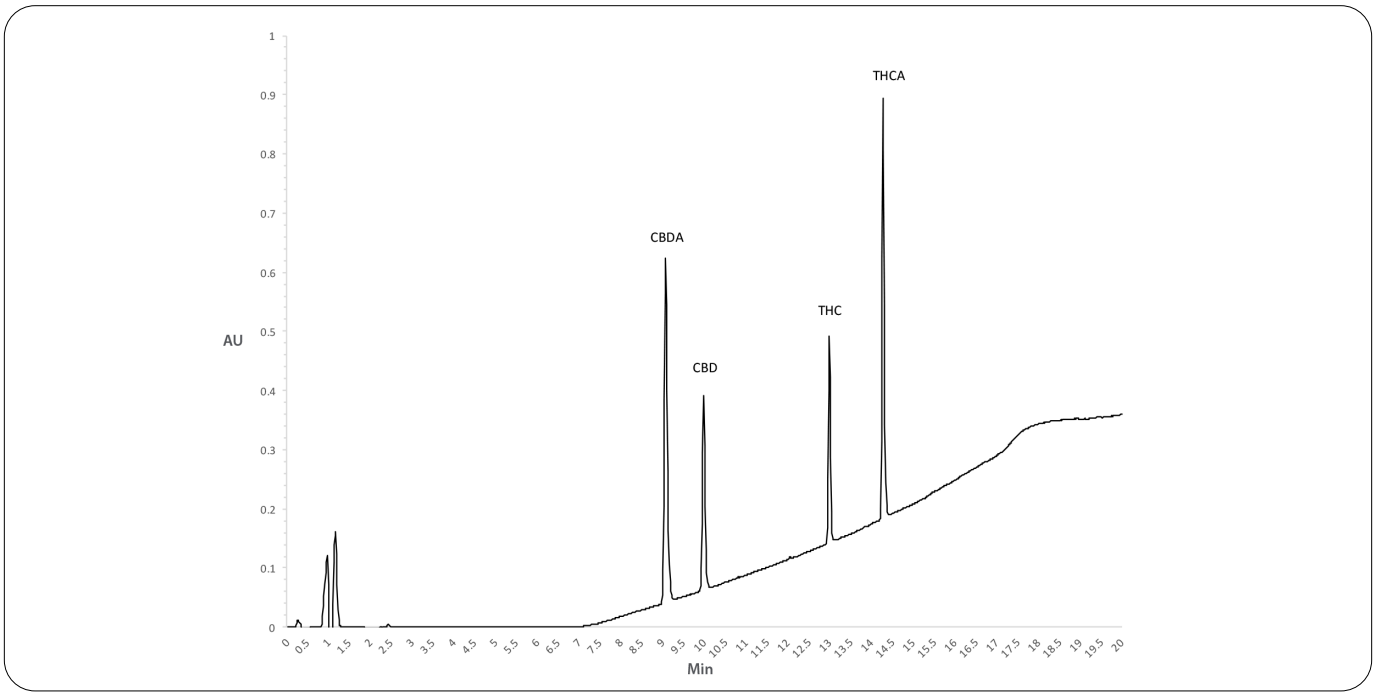

Figure 2: Reverse phase HPLC analysis of CBDA, CBD, THC, and THCA at a concentration of 250 ppm. Quantification was performed by peak integration at 228 nm.

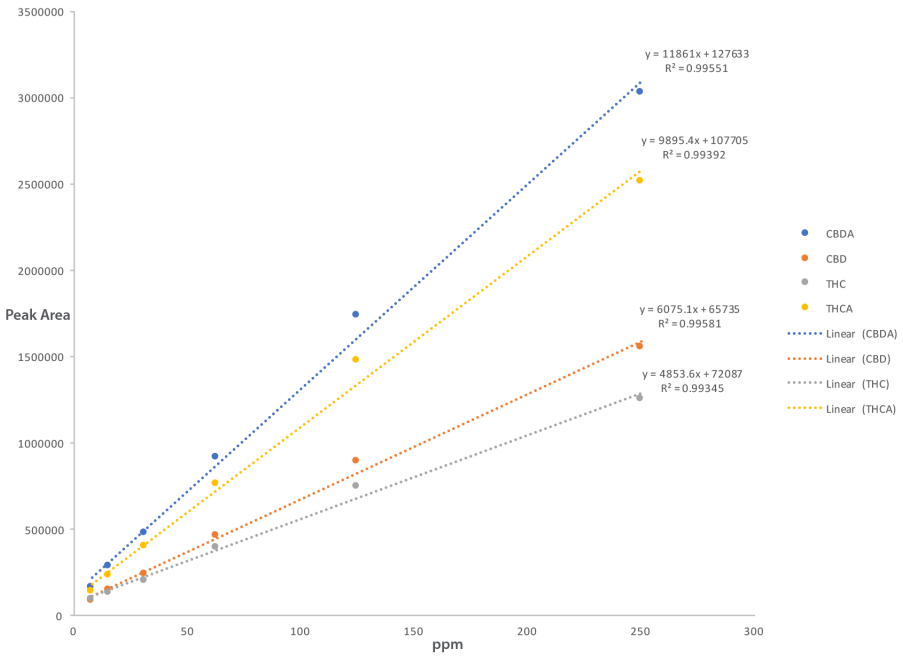

Figure 3: Calibration curves of cannabinoids with linear fit applied. Concentration of unknowns were determined by comparing the peak area to the calibration curve.

As a control, an extract of powdered hops was analyzed to determine if there were compounds originating from the plant material that would co-elute with the spiked cannabinoid standards. Two abundant peaks were present from native plant compounds eluting at 9 mins and 12 minutes that co-eluted with CBDA and THCA (Figures 4A and B).

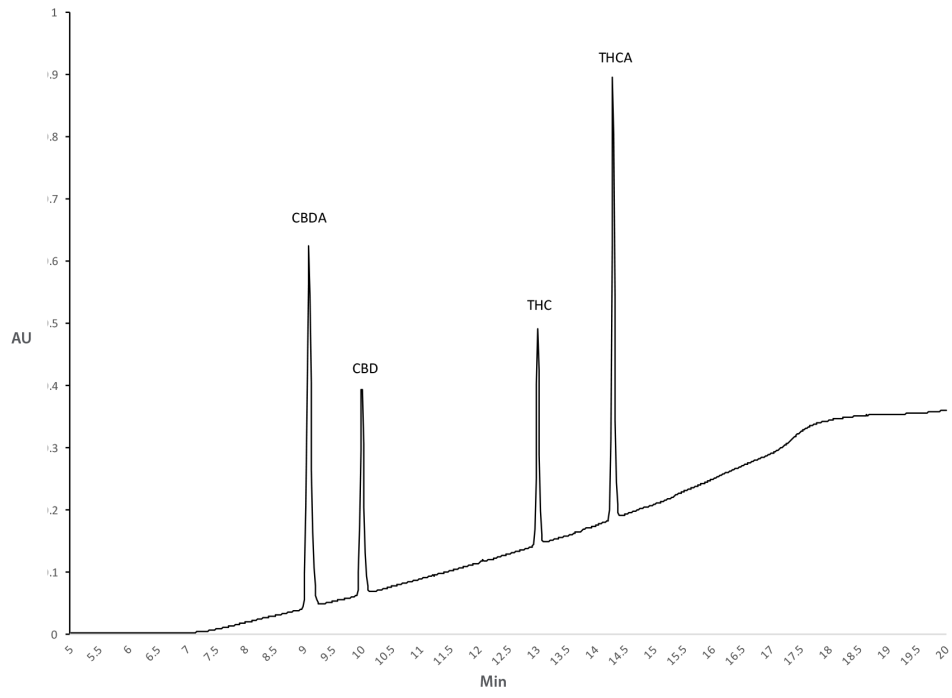

Figure 4: A. Cannabinoid 250 ppm standard showing elution of CBDA (9.039 min), CBD (9.979 min), THC (12.988 min), and THCA (14.264 min).

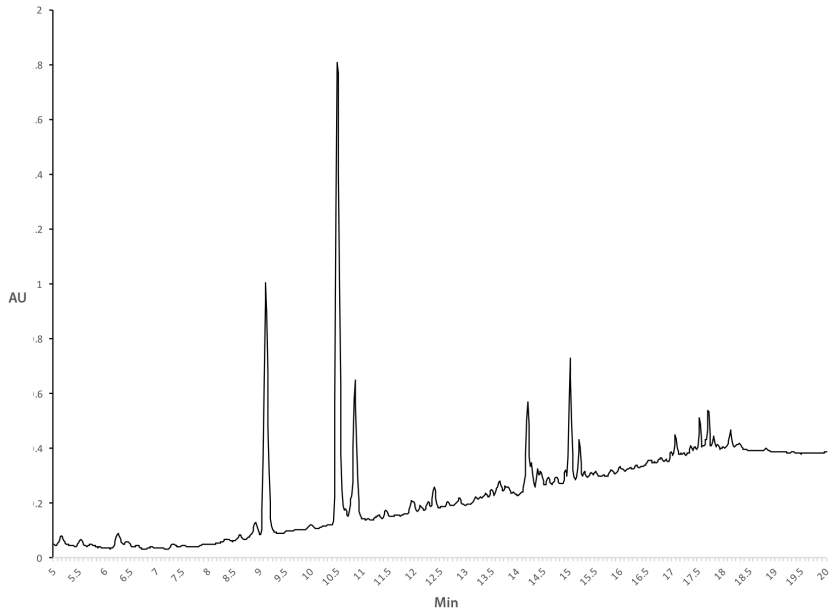

Figure 4: B. UV chromatogram of methanol extracted hops without cannabinoids. Abundant peaks at 9-9.2 min and 14-14.5 min were observed that would co-elute with CBDA and THCA.

Based on these findings, it was determined that extraction efficiency would be measured for CBD and THC only (eluting at 9.9 min and 13 min, respectively). Peak areas from the controls were recorded at times 9.9 min and 13 min and used as a background subtraction to obtain peak areas from the spiked samples.

Reverse phase HPLC analysis of the spiked hops indicated that a 16-20 % recovery was obtained from the gently manual mixing. As homogenization times were extended, recovery was increased, with 89-100 % recovery being obtained after 120 seconds of sample disaggregation (Figure 5-6 and Table 3).

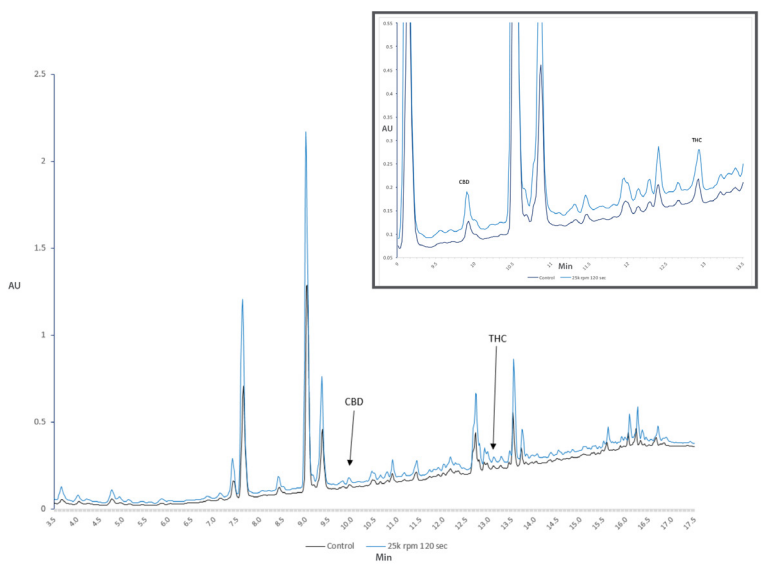

Figure 5: Reverse phase HPLC UV chromatogram of spiked hops with no homogenization and 120 secs of homogenization at 25k rpm. The insert shows the chromatogram from 9-13.5 minutes. Increased peak areas for CBD and THC are observed for the 120 seconds of homogenization time as compared to the methanol extraction only.

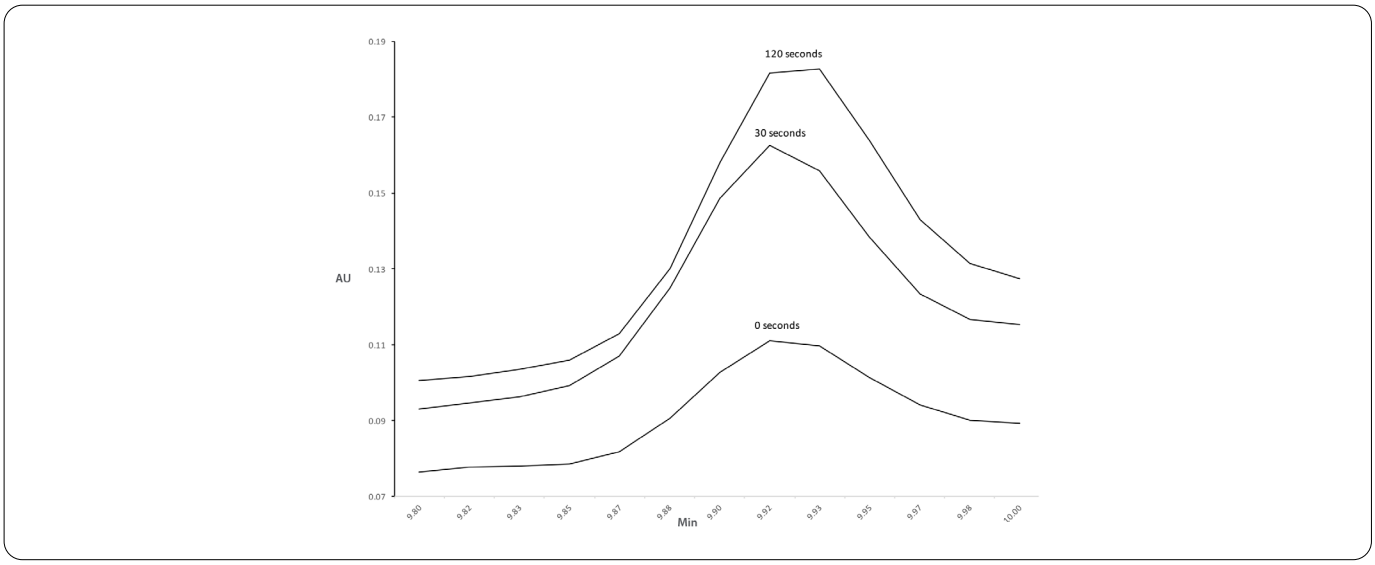

Figure 6: HPLC UV chromatograms for CBD at 0, 30 sec and 120 sec of homogenization time from spiked hops.

Table 3: CBD and THC recovery as a function of homogenization time.

	Calculated concentration		% Recovery	
Homogenization Time (sec)	CBD (ppm)	THC (ppm)	CBD	THC
0	12.4	8.1	24.8	16.2
10	42.8	30.7	85.6	61.4
20	42.9	33.4	85.9	66.8
30	47.3	37.1	94.5	74.3
60	42.5	44.5	90.0	89.0
120	53.4	44.7	106.9	89.5

Conclusions

Ground hops was used as a cannabis analog to evaluate CBA and THC recovery using purchased standards at a concentration of 50 ppm. 89 to 100 % recovery was obtained using a rotor-stator homogenization method as deployed in an automated method using the Omni Prep 96 automated homogenizer as compared to 16 to 25 % recovery with a manual mixing method.

References

1. Leghissa A, Hildenbrand Z, Foss F, Schug K. Determination of cannabinoids from a surrogate hops matrix using multiple reaction monitoring gas chromatography with triple quadrupole mass spectrometry. J Sep Sci. 2018, 41; 459-468.

2. Giese M, Lewis M, Giese L, Smith K. Development and validation of a reliable and robust method for the analysis of cannabinoids and terpenes in cannabis. J AOAC Inter. 2015, 98; 1503-1522.

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