APPLICATION NOTE

The Decarboxylation Myth – Does Cannabis Homogenization by Bead Milling Result in Cannabinoid Decarboxylation?

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Introduction

Cannabinoid quantification ("Potency Testing") 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 psychoactive and non-psychoactive cannabinoids, Tetrahydrocannabinol (THC), Tetrahydrocannabinolic Acid (THCA), Cannabidiol (CBD), Cannabidiolic Acid (CBDA), Cannabigerol (CBG), and Cannabinol (CBN) (Figure 1). [1-2] Potency is typically expressed as the sum of acidic (CBDA and THCA) and neutral forms (CBD and THC) of cannabinoids. This is due to the fact that neutral cannabinoids do not occur in significant concentrations in plants as the plant primarily synthesizes the carboxylic acid forms, including Δ9 -tetrahydrocannabinolic acid A (THCA-A) and cannabidiolic acid (CBDA). Since the carboxylic acid forms are thermally unstable, they convert to their neutral forms when exposed to substantial heat, in excess of 90°C, which can occur via smoking or in the production of food-based cannabinoid products (Figure 1).[3-4]

Maintaining the ratio of neutral/acid cannabinoid forms is critical in the sample preparation process as the goal is the accurately quantify the amount of both forms in the cannabis product. Sample disaggregation is the first step in a potency analysis and can be performed via manual hand grinding, rotor-stator homogenization, bead milling or cryo-milling. Herein we evaluate the effect of bead milling on sample temperature and acidic cannabinoid decarboxylation.

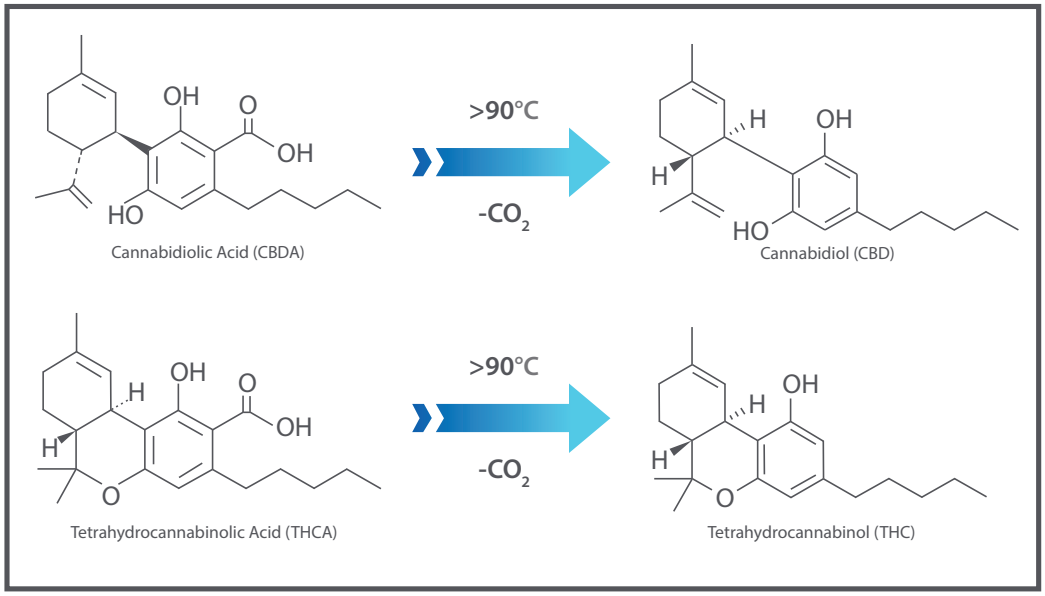

Figure 1. CBD, CBDA, THC and THCA structure. Acid forms (CBDA and THCA) are converted to neutral forms when exposed to temperatures in excess of 90°C.

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Materials and Methods

Equipment

- **Bead Ruptor Elite** (Cat# 19-040E)
- **2 mL pre-filled bead beating tubes** (Cat # 19-628)
- **1 mg/mL THC** (Cerilliant Cat# T-005)
- **1 mg/mL THCA** (Cerilliant Cat# T-093)
- **1 mg/mL CBD** (Cerilliant Cat# C-045)
- **1 mg/mL CBDA** (Cerilliant Cat# C-144)
- **Raptor ARC-18 150mmx4.6mm, 2.7 um** (Restek Cat# 9314A65)
- **Waters 1525 HPLC** with a 2996 photodiode array detector

Bead Ruptor Elite (Cat# 19-040E)

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.

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

Table 1. Cannabinoid standard serial dilution strategy

Sample Preparation

Six 1 mL samples of the 250 ppm cannabinoid standard were added to 2 mL reinforced tubes that were pre-filled with 2.8 mm ceramic beads (Omni International, Cat. # 19-628). The tubes were processed on the Bead Ruptor Elite bead mill homogenizer at 5 m/s for increasing durations of 10, 20, 30, 60, and 120 seconds. After each time point, 250 µL was removed to a new 1.5 mL microcentrifuge tube and placed on ice. As a positive control, 500 µL of the 250 ppm cannabinoid standard was placed in a 1.5 mL microcentrifuge tube and heated, at 90°C, for 180 minutes. All samples were filtered through a 0.2 µm spin filter and 15 µL of the filtrate was combined with 35 µL of 100% HPLC grade methanol for reverse phase HPLC analysis.

Reverse Phase HPLC

Cannabinoid separation and quantification was performed on a Waters 1525 HPLC equipped with a binary pump and 2996 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 4.6 mm, 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.

Data Analysis

Peak areas were integrated for each cannabinoid standard and compared against the known concentrations. Peak areas were plotted and fitted using a linear regression. To evaluate the degree of decarboxylation, the relative analyte concentrations were compared between CBD/CBDA and THC/THCA. The relative amount of decarboxylation was evaluated as a percentage.

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 accurately 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.

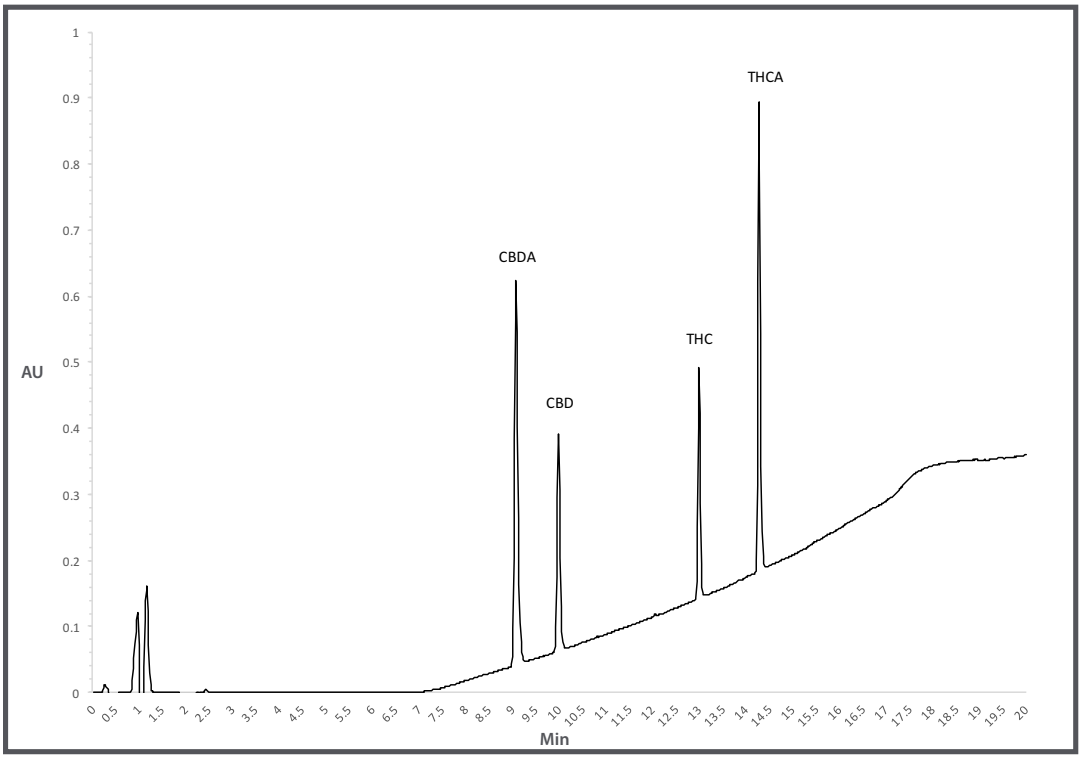

Figure 2. Reverse phase HPLC analysis of CBDA, CBD, THC and THCA at a concentration of 250 ppm. Quantification was performed by peak integration at 228 nm.

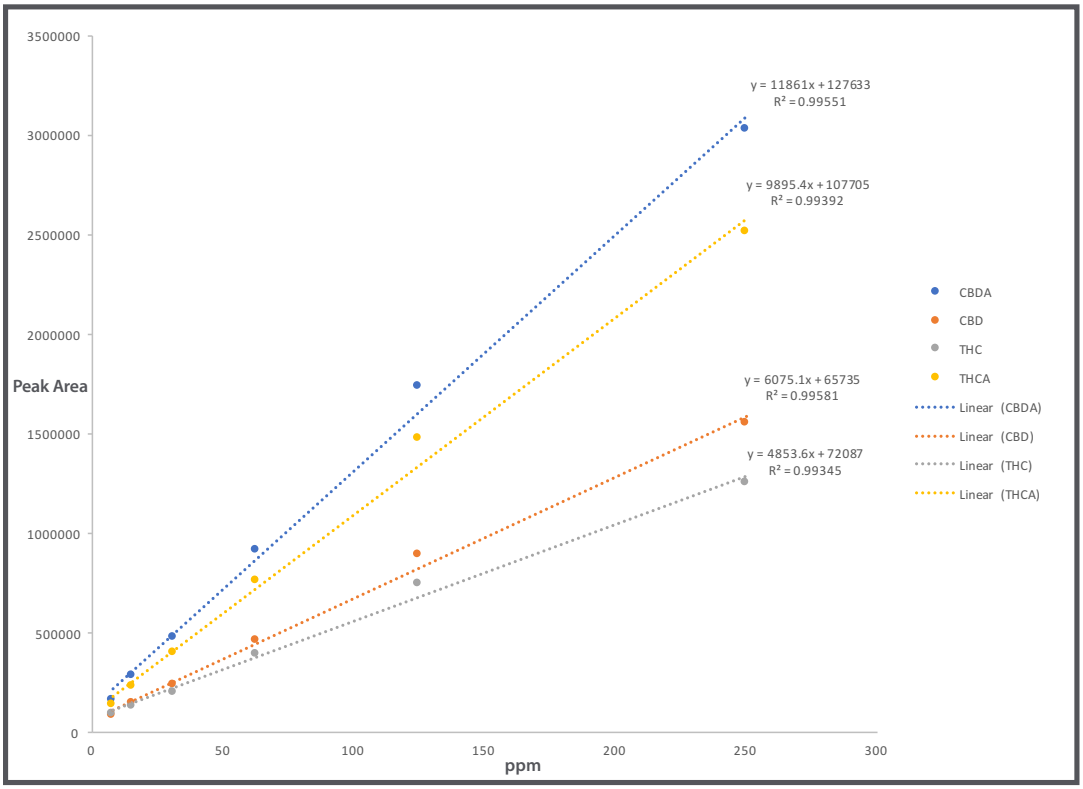

Figure 3. Calibration curves of cannabinoids with linear fit applied. Concentration of each neutral and acidic cannabinoid standard from the bead milling experiment were determined by comparing the peak area to the calibration curve.

Four cannabinoids were sequentially processed for increasing periods of time at 5 m/s on the Bead Ruptor Elite bead mill homogenizer. A positive control was created by heating the cannabinoids at 90°C for 90 minutes to initiate decarboxylation of CBDA and THCA. All samples were analyzed by reverse phase HPLC and peak areas were compared to a serial dilution as shown in figures 2-3. Figures 4A and 4B compare the peak areas for the cannabinoids without homogenization, after 120 seconds of bead mill homogenization and following heating at 90°C for 90 minutes. Notably, the peak areas for CBDA and THCA were reduced by 24.2% and 50.6% respectively after heating for 90 minutes, indicating decarboxylation had occurred. The reduction in peak intensity for CBDA and THCA correlated with an increase in peak area by 13% and 35% for the neutral cannabinoids CBD and THC, as expected for the heated sample. No change in peak area was observed for the cannabinoids processed on the Bead Ruptor Elite. Even after 120 seconds of continuous processing (Figure 4). It's important to note that typical processing on the Bead Ruptor Elite involves sample disaggregation over a period of less than thirty seconds. Thus no decarboxylation will occur when processing is limited in duration such to maintain and intra-tube temperature of less than 90°C.

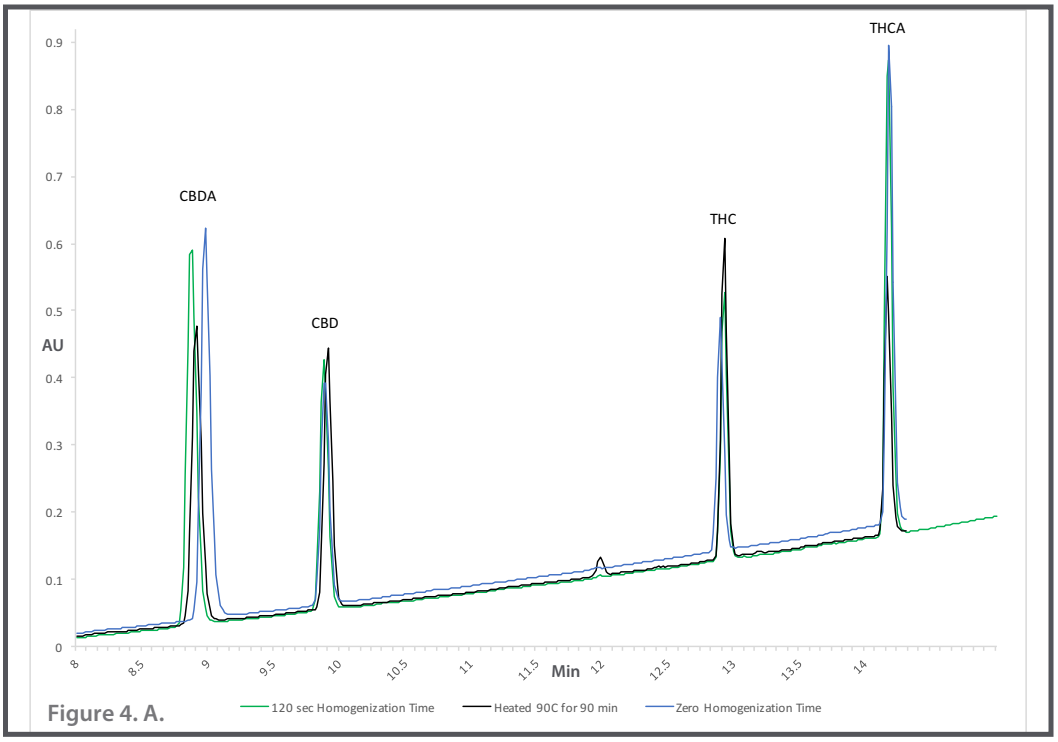

Figure 4. A.

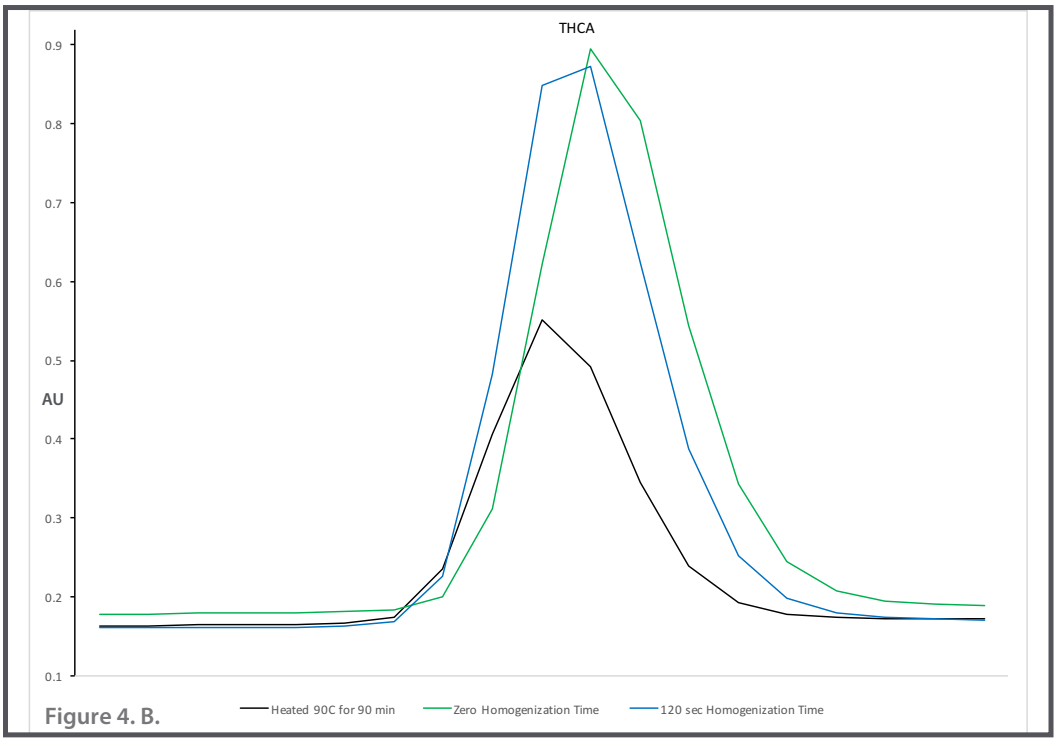

Figure 4. B.

Figure 4A. Unprocessed cannabinoid 250 ppm standard showing elution of CBDA (9.039 min), CBD (9.979 min), THC (12.988 min) and THCA (14.264 min), cannabinoid standard processed on the Bead Ruptor Elite at 5 m/s for 120 sec, cannabinoid standard heated at 90°C for 90 minutes. All samples were analyzed by reverse phase HPLC.

4B. UV chromatogram of THCA comparing the peak areas for THCA with no homogenization, bead mill homogenization at 5 m/s for 120 sec and after heating at 90°C for 90 minutes.

References

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