Cannabis Sativa

Milling Effects on Cannabinoid Content

XD022, XD023 – Cannabis milling comparison experiments

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Abstract

Different milling instruments and conditions were compared to investigate the effect of different milling protocols on cannabinoid recovery. It was found that a Fritsch Pulverisette 11 with a single use container shows comparable to higher recoveries of cannabinoids, while having the added benefits of protecting against cross contamination and reducing cleaning time and costs.

Introduction

Testing of cannabis for safety and consistency is the bedrock of any legal cannabis system. Compared to other agricultural crops, Cannabis testing has specific and unique challenges. Generally, *Cannabis sativa* flower must be comminuted before extraction for optimal analyte recovery. Sample milling is an often-overlooked step in process optimization studies. Studies on milling cannabis flower have investigated optimum particle size for supercritical CO_2 extraction of THC,[1] and the relationship between heat produced from a lengthy milling time and acidic cannabinoid decarboxylation.[2] For example, Caleb Proctor *et al.* showed that up to 120 seconds of homogenization via bead milling would not result in the decarboxylation of CBDA and THCA so long as the sample temperature was maintained below 90 °C.[2] However, so far no studies of milling method effects on analyte recovery for cannabis or related plant extracts have been reported.

Throughout the industry, there are variations in cannabinoid extraction procedures. Electric bladed mills, a mortar and pestle, ball mills, cryogenic mills, bladed mills, and many other methods are available, but most labs use small-scale hand-grinding or electric mills/coffee grinders for plant analysis. Many available electric mills are advertised to the cannabis industry; however, their parameters have not been evaluated for optimal conditions for analyte preservation. Therefore, our goal was to understand how a standard step in a sample preparation workflow can affect quantitative accuracy for cannabinoids and to optimize our method. To the best of our knowledge, no other mill/grinder manufacturers present data on cannabinoid recovery.

To evaluate the effect of milling on measurement accuracy, we measured the cannabinoids content of extracts of cannabis milled under various conditions. Specifically, an electric bladed mill (typically used for coffee grinding) and the Fritsch Pulverisette 11 (P11) equipped with different grinding vessels (single-use and 1.4L vessels) were used to mill THCA and CBDA cannabis flower at different rates.

Experimental

Two *Cannabis sativa* cultivars (THC- and CBD-rich) were acquired from Valens (Kelowna, BC) and stored in the dark under ambient laboratory conditions. An electric bladed mill (advertised as a coffee grinder) and the Fritsch Pulverisette 11 (P11) were used to homogenize two flower cultivars under varied milling conditions (milling speed and time). The particulate was sized and extracted using HPLC grade methanol using sonication assistance. The filtered and diluted extracts were analyzed via HPLC-VWD to quantify cannabinoids.

Sample Preparation

Milling

The procedures below were conducted for THC and CBD cannabis flowers, respectively.

Triplicate samples of approximately 3 g of cannabis flower were weighed and milled according to the parameters listed in Table 1. The electric bladed mill and the P11 grinding vessels were cleaned between each milling time to avoid cross-contamination.

Approximately 0.75 g of each milled flower sample was taken for particle size analysis (see supporting information for details). In addition, three aliquots of each milled sample (250-400 mg) were measured for extractions. 4 mL of MeOH was added to each aliquot, vortexed to mix, then sonicated for 15 minutes. The liquid was then filtered out through a 0.2 µm Nylon filter into an autosampler vial.

Table 1. Cannabis flower milling parameters: Cannabis flower was milled using either an electric bladed mill or the P11. Sample identifications represent the batch and replicate numbers.

Mill Type	Parameters	Sample ID
coffee grinder	20s, continuous	XD022-T-1 (XD022-C-1)
coffee grinder	20s, pulses (3s on, 2s off)	XD022-T-2 (XD022-C-2)
P11 with single-use grinding vessel	2000rpm, 20s	XD022-T-3 (XD022-C-3)
P11 with single-use grinding vessel	4000rpm, 10s	XD022-T-4 (XD022-C-4)
P11 with single-use grinding vessel	2000rpm, 10s	XD022-T-5 (XD022-C-5)

P11 with 1.4L grinding vessel	2000rpm, 20s	XD022-T-6 (XD022-C-6)

Note: for the sample ID - T stands for THCA flower, while C stands for CBDA flower

Analysis

All samples were diluted by a factor of 25 in Methanol for HPLC analysis (40 uL sample in 1.00 mL MeOH). The samples were run on an Agilent 1220 HPLC equipped with a variable wavelength detector set to monitor 230 nm. A full description of the RP-HPLC program used for cannabinoid quantification may be found in the supplementary information.

Results

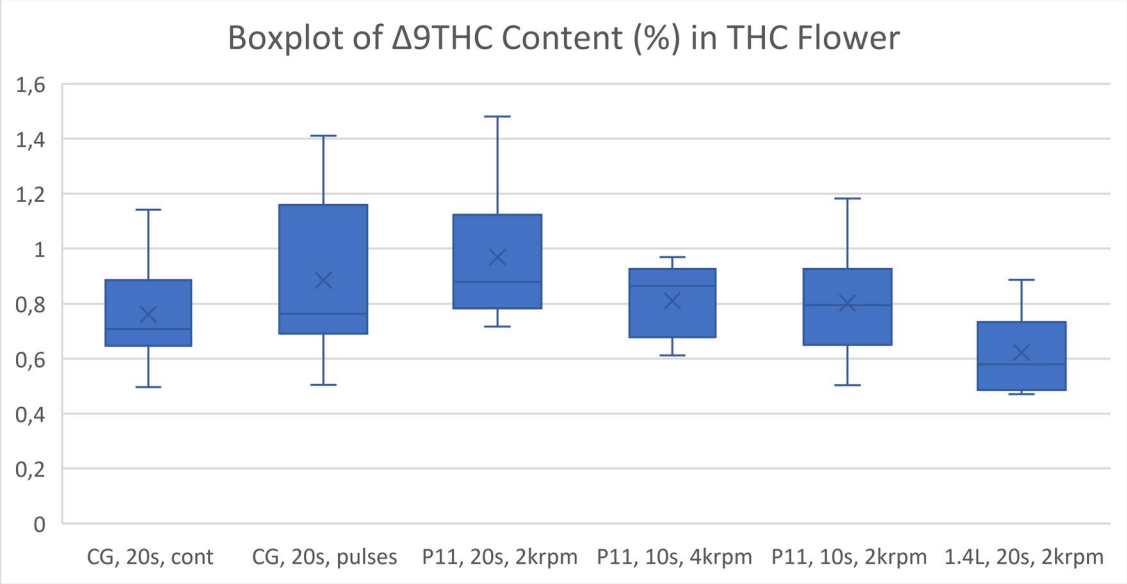


Figure 1: The percent Δ9-THC detected in high THC Cannabis flower that has been milled in the bladed mill (CG), and the P11 mill using either the single use containers (P11) or the 1.4L grinding vessel (1.4L) at varying milling conditions.

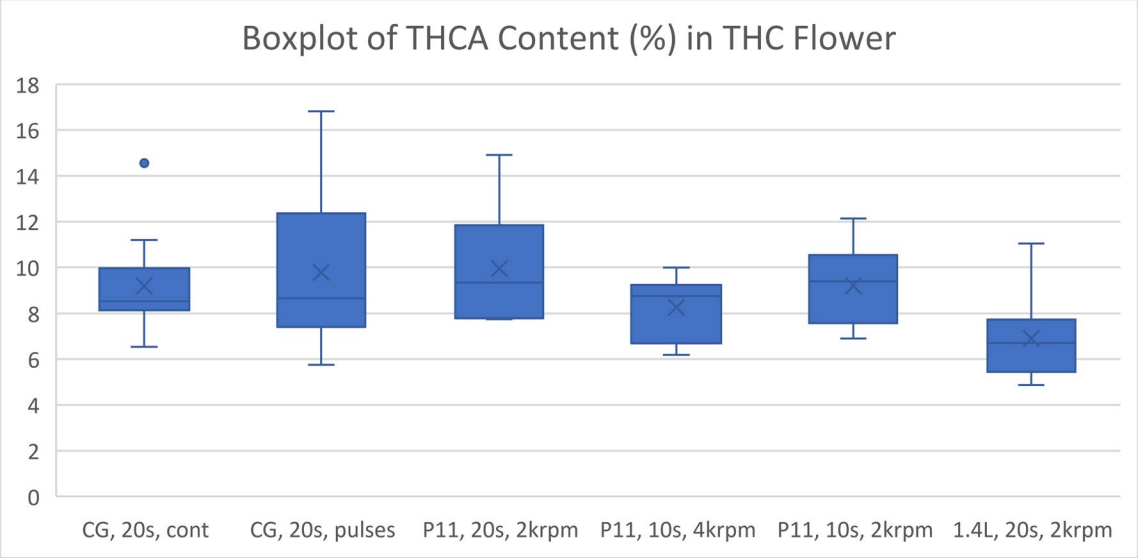


Figure 2: The percent THCA content in high THC cannabis flower that has been milled in the bladed mill (CG), and the P11 mill using either the single use containers (P11) or the 1.4L grinding vessel (1.4L) at varying milling conditions.

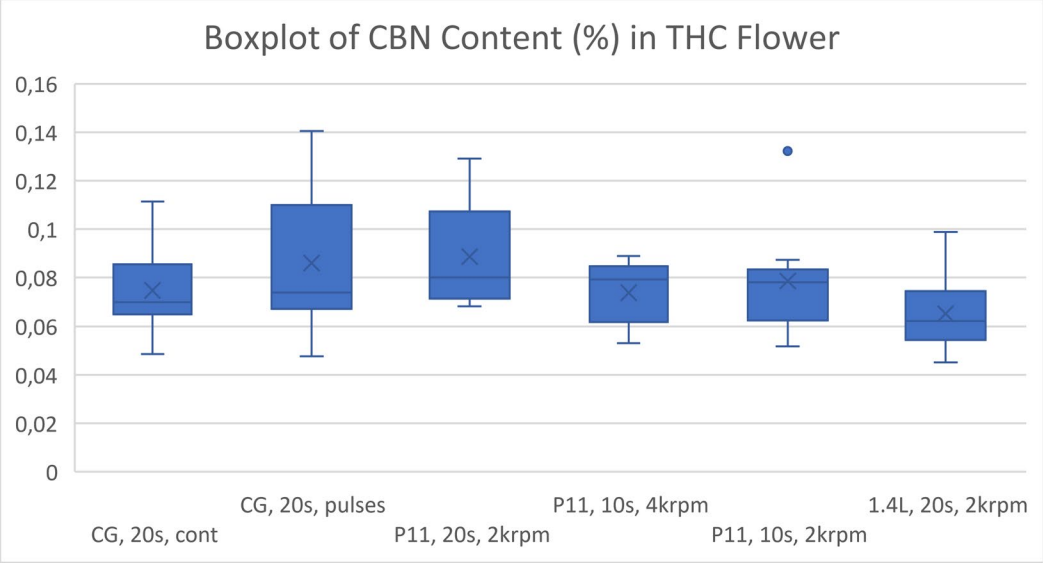


Figure 3: The percent CBN content in high THC cannabis flower that has been milled in the bladed mill (CG), and the P11 mill using either the single use containers (P11) or the 1.4L grinding vessel (1.4L) at varying milling conditions.

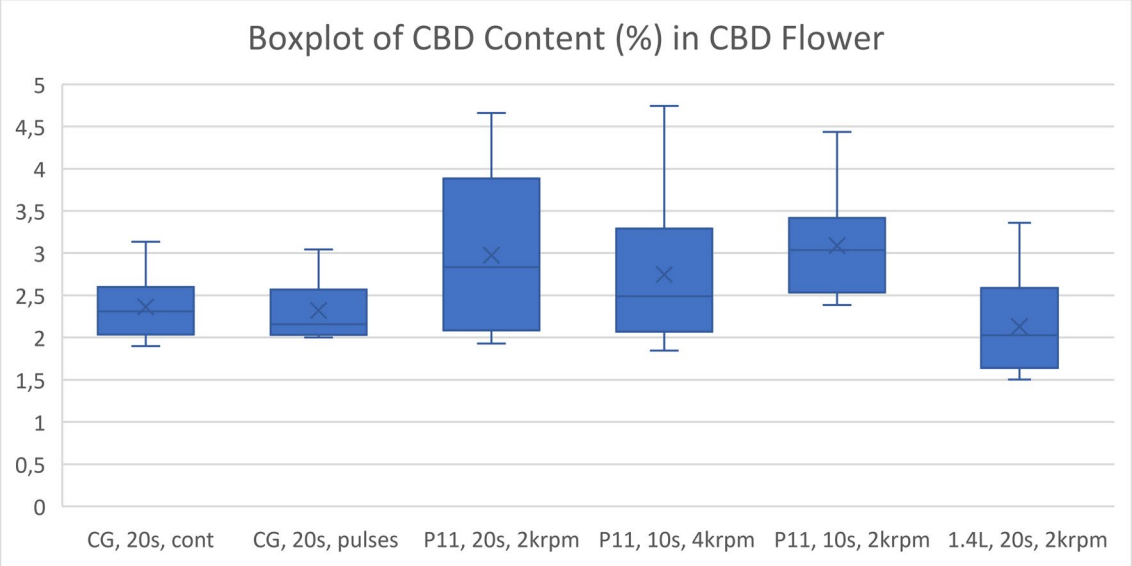


Figure 4: The percent CBD detected in high CBD Cannabis flower that has been milled in the bladed mill (CG), and the P11 mill using either the single use containers (P11) or the 1.4L grinding vessel (1.4L) at varying milling conditions.

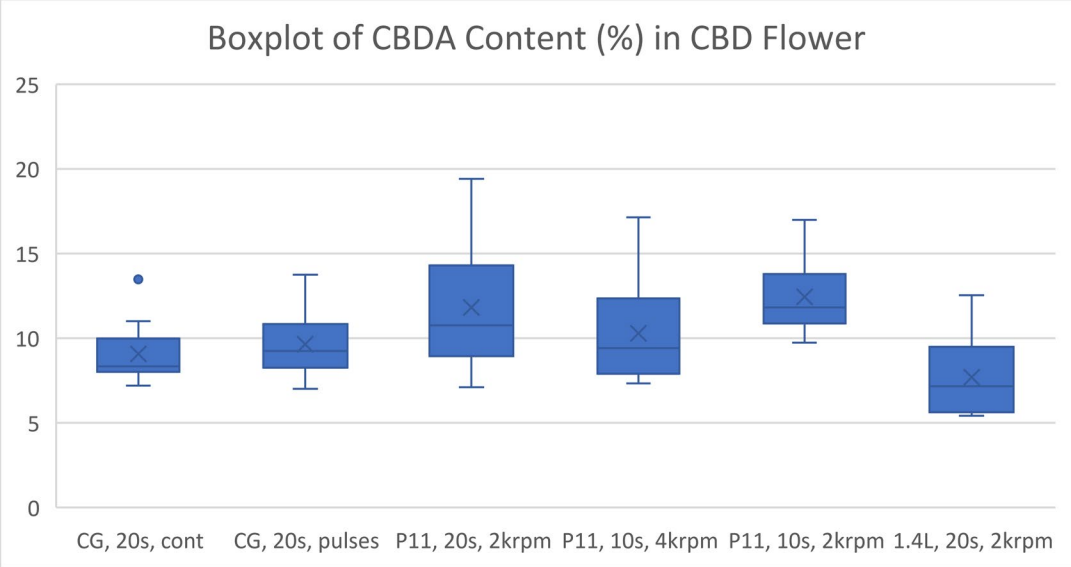


Figure 5: The percent CBDA content in high CBD cannabis flower that has been milled in the bladed mill (CG), and the P11 mill using either the single use containers (P11) or the 1.4L grinding vessel (1.4L) at varying milling conditions.

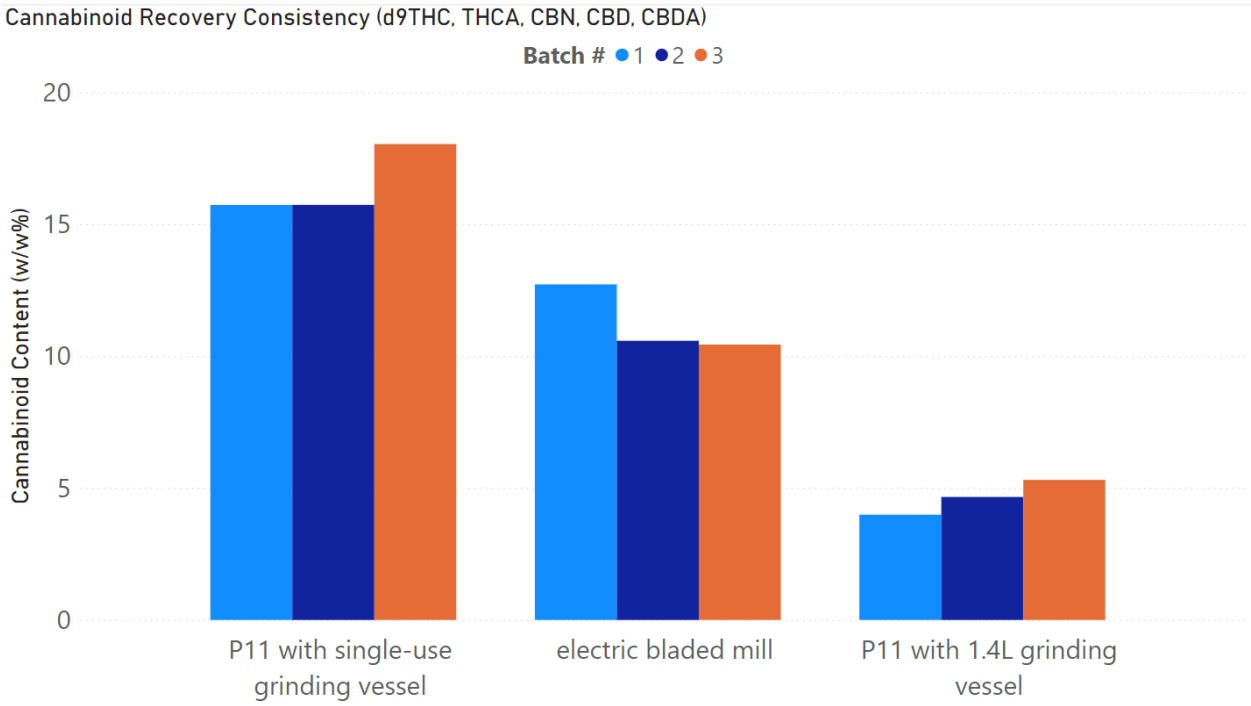


Figure 6: Example of reproducibility between separate millings of same flower batch. Example is THC flower.

Results

Overall, we found more intense milling conditions resulted in a loss of cannabinoids, presumably due to thermal decomposition (Figures 1-5). High THC cannabis that was milled using a bladed mill pulsed for 20 seconds and the P11 with a single use container at 2krpm for 20 seconds yielded the highest concentrations of Δ9-THC (Figure 1). The highest CBD yield from high CBD cannabis came from flower that was milled using the P11 with the single use containers at 2krpm, for 20 seconds (Figure 2). The highest CBN and THCA yields from high THC cannabis was achieved from the bladed mill pulsed for 20 seconds, and the single use containers with the P11 (Figures 3 and 4). Finally, the highest CBDA yield from high CBD cannabis was achieved using the P11 single use containers, at 20 and 10 seconds, at 2krpm (Figure 5).

Our results show the use of the single use containers is not correlated with any loss in cannabinoids. In fact, containers offer an advantage for recovering the cannabinoids detected in the Cannabis Sativa extracts over both an electric bladed mill and the P11 with a 1.4L grinding vessel. The advantage of the Fritsch P11 mill over a bladed mill is the added control of milling parameters, which can be set on the P11. There is a marked advantage of using the P11 with the single use containers, as they prevent the possibility of cross contamination between samples and reduces the time and work required for sanitation between samples. Overall, the P11 Fritsch mill with the single use milling containers is an excellent option for those looking to achieve a high cannabinoid recovery for less effort.

References

Internal data:

1. Eöry, L., Béla Dános & Tibor Veress. SUPERCRITICAL FLUID EXTRACTION OF TETRAHYDROCANNABINOL FROM MARIHUANA STUDY OF THE EFFECT OF PARTICLE SIZE. *Institute for Forensic Sciences, Budapest, Hungary* **47**, 322–327 (2001).

2. Proctor, C., Soldat, S., Easparro, B., Nash, R. & Atwood, J. *The Decarboxylation Myth – Does Cannabis Homogenization by Bead Milling Result in Cannabinoid Decarboxylation?* . https://www.researchgate.net/profile/Brandon-Easparro/publication/329629388_The_Decarboxylation_Myth-Does_Cannabis_Homogenization_by_Bead_Milling_Result_in_Cannabinoid_Decarboxylation/links/5c12ba19299bf139c756bee8/The-Decarboxylation-Myth-Does-Cannabis-Homogenization-by-Bead-Milling-Result-in-Cannabinoid-Decarboxylation.pdf.

Supplementary Information

RP-HPLC method for cannabinoid quantification

Introduction

The following is a gradient RP-HPLC method appropriate for the quantification and separation of cannabinoids. Samples may include purified cannabis extracts or other refined products.

Column

Octadecyl silica (C18, Agilent Poroshell 2.1 um, 50 x 150 x 300 mm)

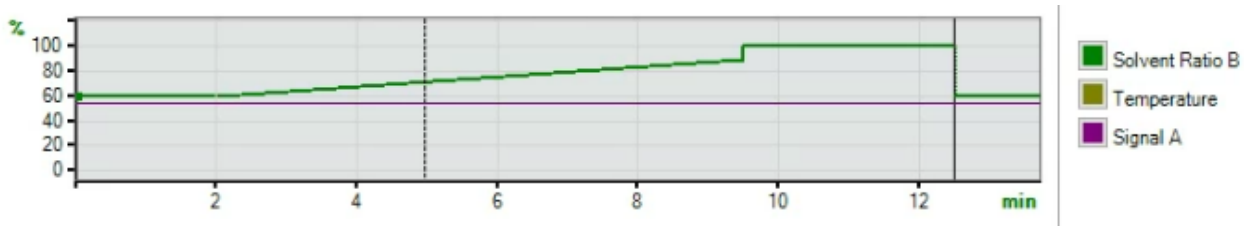
Eluents

In all cases HPLC-grade solvents should be used and sonicated for 30 mins/L to degas.

- A: Deionized water (DI) with 1% (v/v%) MeOH, 0.1% formic acid
- B: MeOH, 0.1% formic acid

Gradient Pump

Time (mins)	A (%)	B(%)	Flow (mL/min)	Max Pressure (bar)
0.00	40	60	1	400
2.00	40	60	1	400
9.50	11	89	1	400
9.51	0	100	1	400
12.50	0	100	1	400
15.00 (posttime)	40	60	1	400



Instrument and Acquisition

Column Oven (°C)	VWD (nm)	Injection Volume (µL)	Needle Wash Pos.
50.0	220	5	100

Calibration

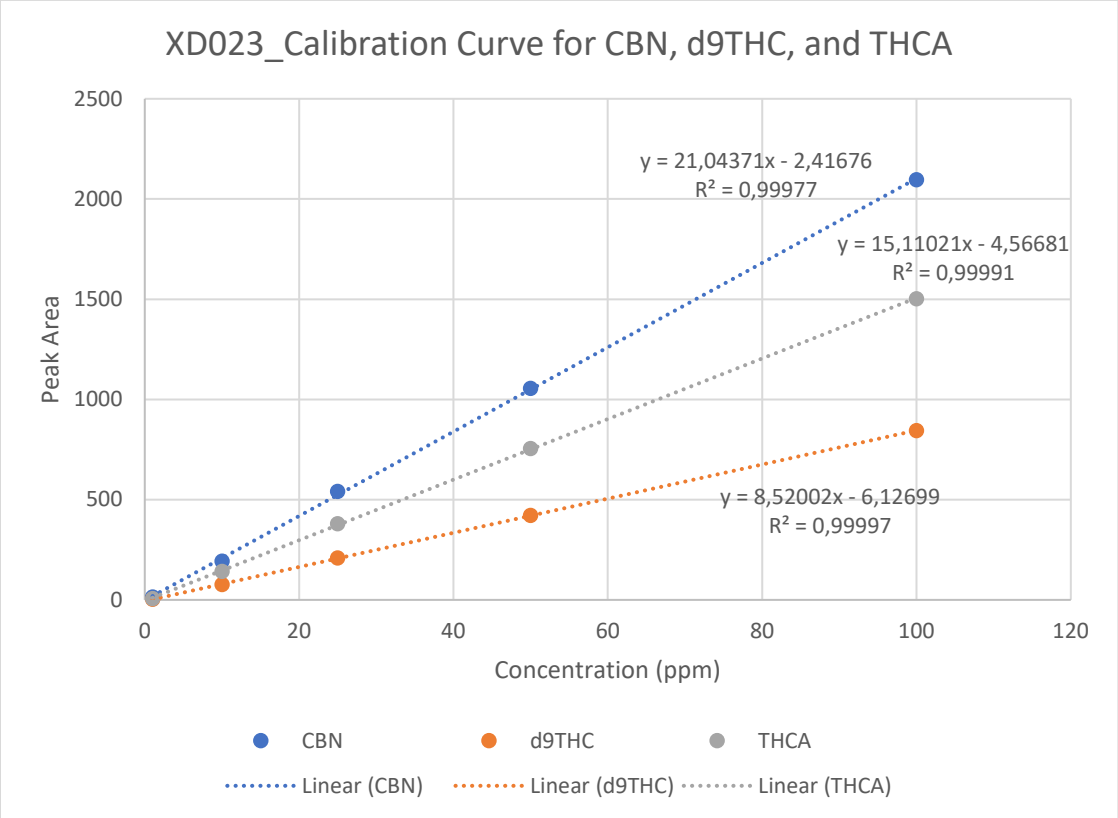


Figure S1: A Calibration curve was created, using pure CBN, Δ9-THC, and THCA cannabinoid certified reference material run on a HPLC.

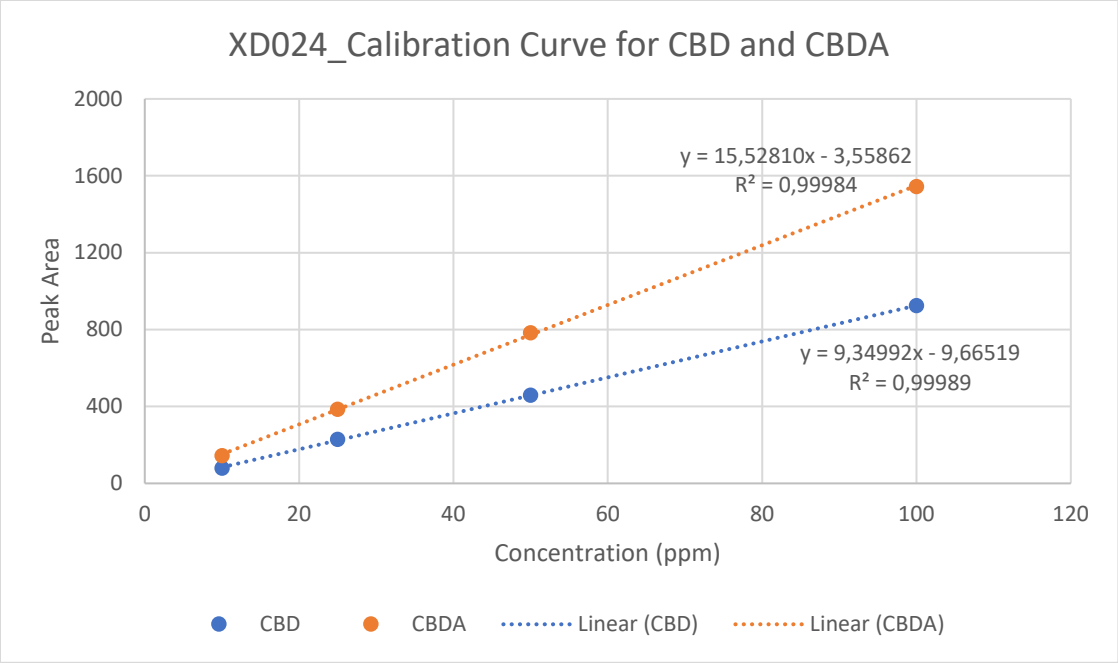
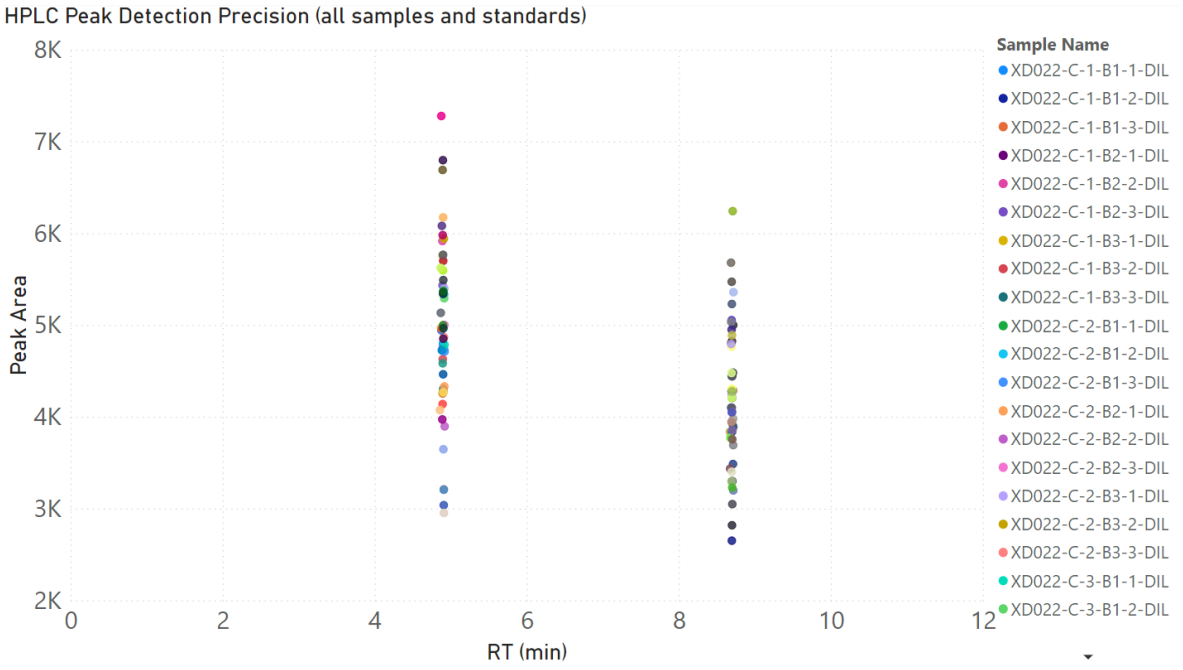
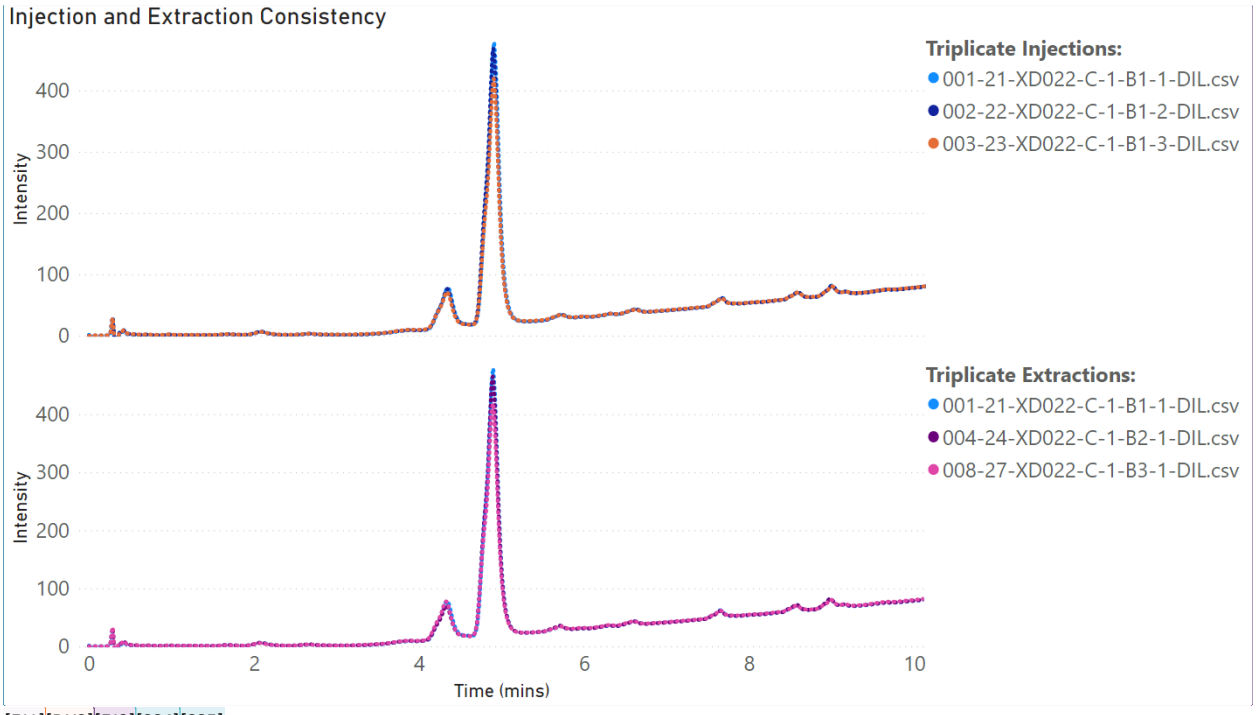


Figure S2: A Calibration curve was created, using pure CBD and CBDA cannabinoid certified reference material run on a HPLC.





[EJ1][DX2][EJ3][SS4][SS5]