The Development of an Optimized Extraction Protocol for Cannabinoids From Cannabis Plant Material

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ABSTRACT

Due to changing legislation concerning hemp and marijuana, forensic laboratories and regulatory agencies require analytical methods for the quantitative determination of the total Δ^9-tetrahydrocannabinol (Δ^9-THC) content, including Δ^9-THC and its acidic precursor, Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA). Efficient extraction of cannabinoids from cannabis plant material is a crucial step that requires appropriate optimization. This study investigates optimized cannabinoid extraction from cannabis plant material, including solvent selection, mixing method and duration, and the use of centrifugation versus sample settling.

INTRODUCTION

The 2018 Agriculture Improvement Act defined marijuana as Cannabis sativa L. containing greater than 0.3% Δ^9-THC on a dry-weight basis, whereas hemp contains no more than 0.3% Δ^9-THC [1]. In 2019, the U.S. Department of Agriculture clarified that regulatory compliance is based on total THC, which includes Δ^9-THC and its acidic precursor, Δ^9-THCA, to account for potential decarboxylation [2]. This clarification necessitated analytical methods capable of accurately quantifying both neutral and acidic cannabinoids and increased the importance of robust and reproducible extraction procedures. Inefficient extraction can directly affect the measured total THC and lead to incorrect identification of legal hemp versus illegal marijuana.

Cannabis plant material contains over 120 phytocannabinoids, along with matrix components that may influence extraction efficiency and downstream detection. Thus, optimized extraction is essential to ensure representative recovery, minimal matrix effects, and prevent under- or overestimation of Δ^9-THC and Δ^9-THCA. This study investigates optimized cannabinoid extraction from cannabis plant material, including solvent selection, mixing method and duration, and the use of centrifugation versus sample settling, to improve the recovery of 12 targeted cannabinoids while reducing matrix effects. The optimized protocol was incorporated into a rapid ultra-performance liquid chromatography-photodiode array-single quadrupole (UPLC-PDA-QDa) workflow to support accurate total THC determination and reliable differentiation of hemp and marijuana.

MATERIALS & METHODS

Twelve cannabinoid CRMs were purchased from Cayman Chemical. The standards included: CBL, CBG, CBC, CBD, CBDA, CBT, CBN, exo-THC, Δ^8-THC, Δ^9-THC, Δ^{10}-THC, and Δ^9-THCA. All samples were analyzed using a Waters Acquity UPLC-PDA-QDa system fitted with a quaternary pump, sample manager, and heated column compartment. An InfinityLab Poroshell 120 EC-C18 column (100 x 2.1 mm, 2.7 μm) was used to separate the 12 cannabinoids. The calibration range was from 1-50 μg/mL. The column and autosampler were maintained at 22 °C and 5 °C, respectively.

RESULTS & DISCUSSION

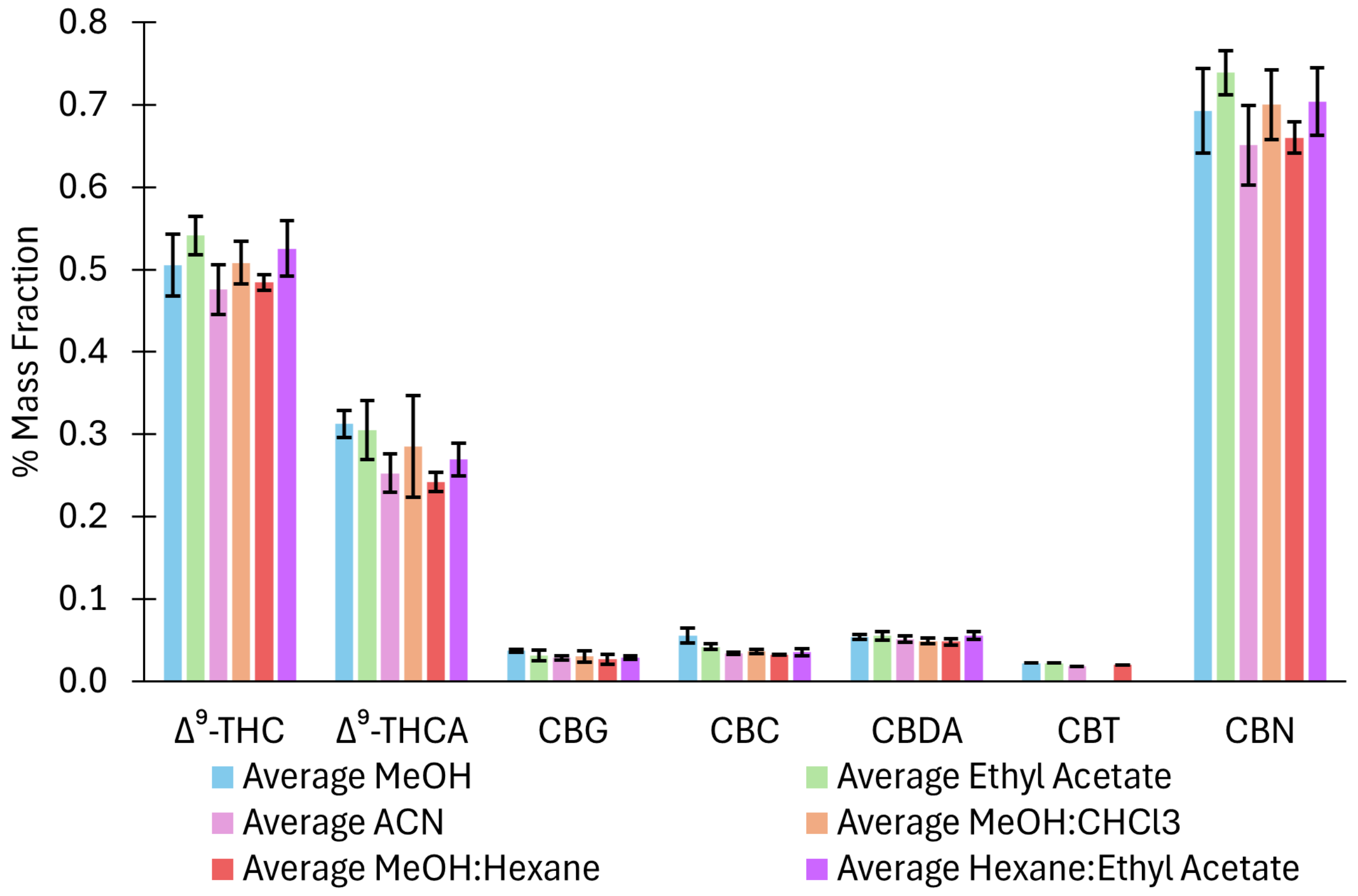

Figure 1. The average percent mass fraction for a known marijuana sample, using six different solvents. Error bars represent ± 1 standard deviation.

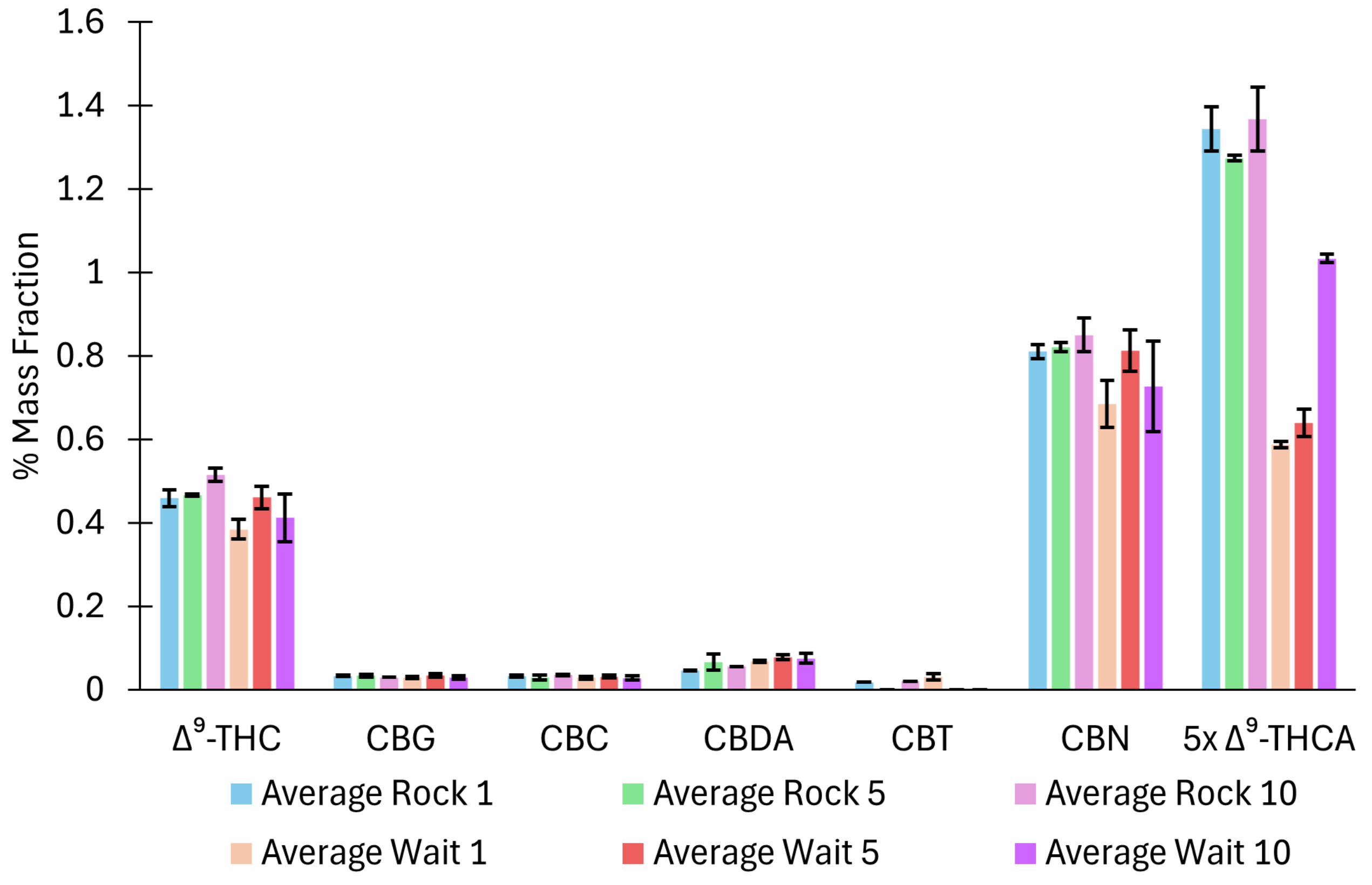

Figure 2. The average percent mass fraction for a known marijuana sample, using two different mixing methods at three time points each. Error bars represent ± 1 standard deviation.

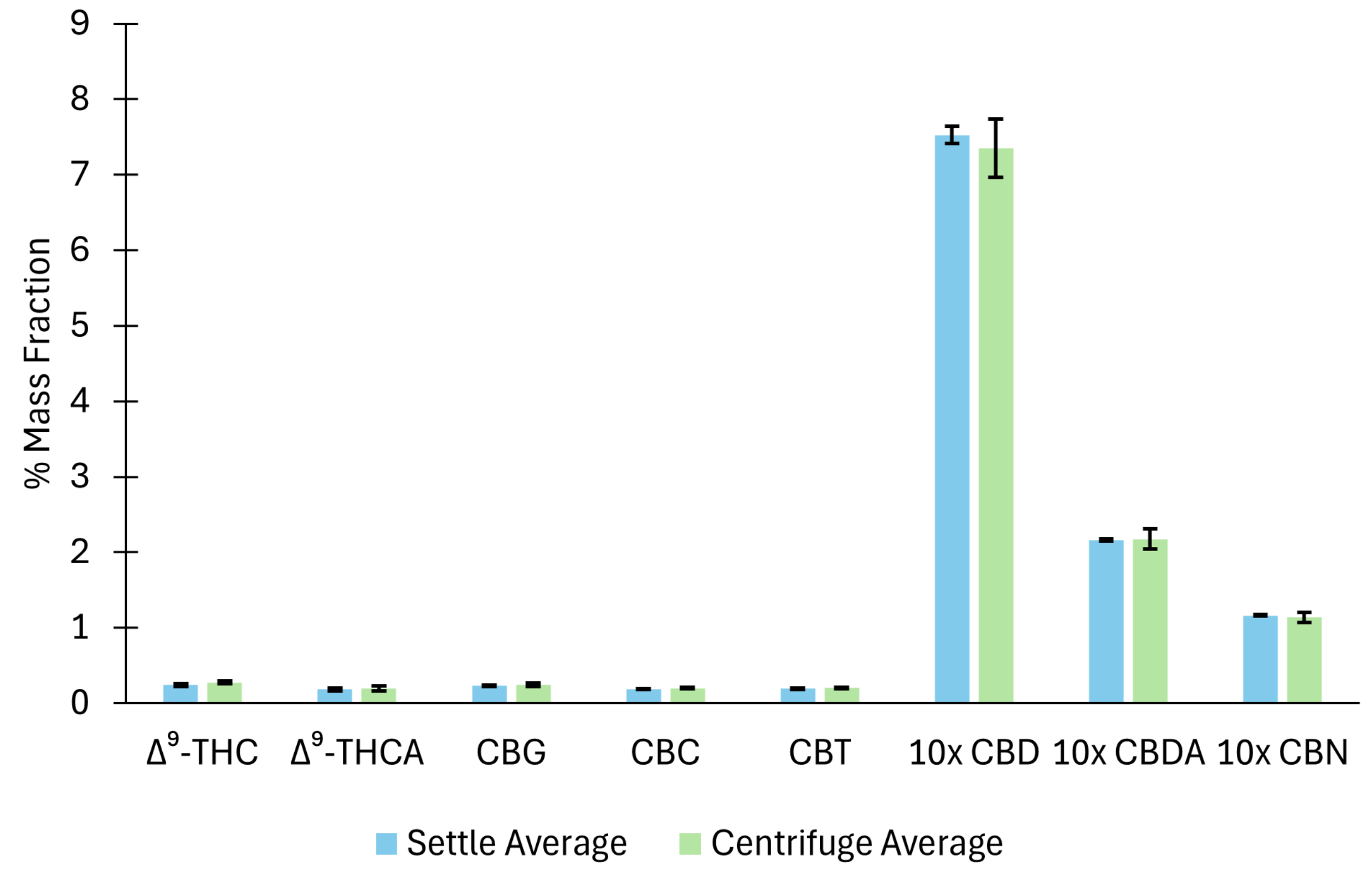

Figure 3. The average percent mass fraction for a known marijuana sample, comparing centrifugation versus settling. Error bars represent ± 1 standard deviation.

Table 1. Average percent recovery for each cannabinoid in the recovery study using the optimized extraction protocol.

	Hemp % Recovery	**Marijuana % Recovery**
Δ^9-THC	100.0	100.0
Δ^9-THCA	100.0	*100.0
CBG	97.8	100.0
CBC	100.0	100.0
CBD	#97.7	100.0
CBDA	#96.7	100.0
CBT	100.0	100.0
CBN	100.0	*98.3
Average % Recovery	99.0 ± 1.4	99.8 ± 0.6
Total Average	**99.4 ± 1.0**	

#Indicates samples diluted 5x   *Indicates samples diluted 10x

- Methanol was selected as the extraction solvent due to its consistent performance across all cannabinoids and its ability to achieve optimal recoveries without requiring dry-down and reconstitution.
- Although mixing time had minimal impact on overall recovery, 10 minutes of rocking provided the most reproducible results, indicating sufficient solvent-matrix interaction without unnecessary processing time.
- Passive settling was shown to be equivalent to centrifugation in terms of extraction efficiency, demonstrating that effective cannabinoid recovery can be achieved without specialized equipment, which improves method accessibility and throughput.
- Sequential extraction results of >99% total recovery confirm that the optimized protocol enables near-complete cannabinoid extraction, supporting the reliability of the method for accurate total THC determination.

ACKNOWLEDGEMENTS

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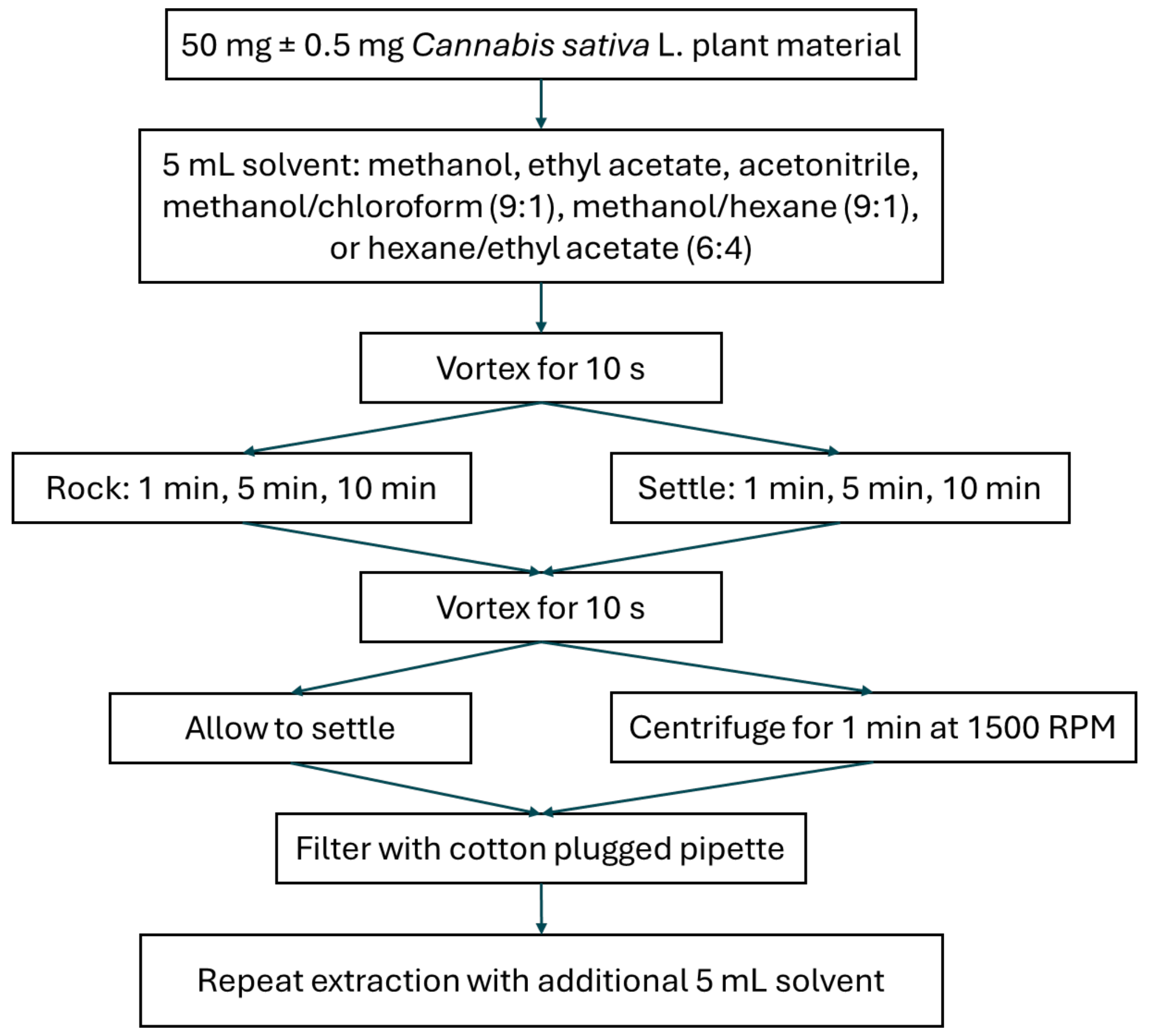
MATERIALS & METHODS

The mobile phase compositions were (A) 0.1% formic acid in H_2O and (B) 0.1% formic acid in ACN. Separation employed a 0.5 mL/min gradient: 25:75 A:B for 5 min, increased to 85% B at 5.1 min (2 min hold), then to 95% B at 7.1 min (2 min hold), followed by 2 min re-equilibration. The injection volume was 5 μL. PDA data were collected from 190-700 nm with quantitation at 220 nm. The ESI source was operated in positive mode. Cone voltages ranged from 5-35 V in 5 V increments, and the scan range was *m/z* 40-400.



50 mg ± 0.5 mg *Cannabis sativa* L. plant material

5 mL solvent: methanol, ethyl acetate, acetonitrile, methanol/chloroform (9:1), methanol/hexane (9:1), or hexane/ethyl acetate (6:4)

Vortex for 10 s

Rock: 1 min, 5 min, 10 min

Settle: 1 min, 5 min, 10 min

Vortex for 10 s

Allow to settle

Centrifuge for 1 min at 1500 RPM

Filter with cotton plugged pipette

Repeat extraction with additional 5 mL solvent

Overall extraction efficiency was evaluated through a sequential recovery study in which two *Cannabis sativa* L. sources (extracted in triplicate) underwent three consecutive extractions analyzed by UPLC-PDA-QDa, enabling the calculation of individual and overall percent recovery for each cannabinoid.

Equation 1. $\%\text{Recovery}=[(\text{Extract }1)\div(\sum \text{all Extracts})]\cdot 100\%$

CONCLUSIONS

- ❖ Extraction procedure:
  - ❖ 50 mg ± 0.5 mg dried, homogenized plant material with 5 mL methanol.
  - ❖ Vortex 10 s → rock 10 min → vortex 10 s → settle → filter through cotton-plugged Pasteur pipette.
  - ❖ Repeat to obtain a total extract volume of 10 mL.
- ❖ Sequential extractions achieved >99% recovery, confirming extraction efficiency without specialized equipment.
- ❖ Integration with a validated UPLC-PDA-QDa method enables accurate total THC quantification and reliable differentiation of hemp and marijuana.

REFERENCES

[1] H.R.2-115th Congress: Agriculture Improvement Act of 2018.
[2] Establishment of a Domestic Hemp Production Program. 2019, Agricultural Marketing Service, United States Department of Agriculture. 58522-58564

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