Quantitative Analysis of 12 Cannabinoids from Cannabis Samples Using UPLC-UV-MS

Fiza Tajdin, BS*; Britni N. Skillman, PhD; J. Tyler Davidson, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

Sam Houston State University

ABSTRACT

The legal distinction between hemp and marijuana based on total Δ^9-tetrahydrocannabinol (Δ^9-THC) content has created a need for rapid and reliable analytical methods to differentiate hemp and marijuana. This study evaluates an ultra-high-performance liquid chromatography-ultraviolet spectroscopy-mass spectrometry (UPLC-UV-MS) method for the identification and quantification of 12 cannabinoids in cannabis plant material. The method was validated through the assessment of linearity, limit of detection, precision, bias, matrix effects, and carryover, prior to analysis of smokeable hemp samples.

INTRODUCTION

The 2018 Agriculture Improvement Act established a legal distinction between hemp and marijuana based on a 0.3% Δ^9-THC threshold, creating new analytical challenges for forensic and regulatory laboratories [1]. The legalization of hemp has also contributed to an unregulated cannabis market in which products are frequently unlabeled or mislabeled, increasing the need for reliable methods to assess cannabinoid content [2]. Accurate differentiation of hemp and marijuana requires sensitive and selective analytical techniques capable of quantifying the total Δ^9-THC content in cannabis plant material. Although gas chromatography–mass spectrometry (GC-MS) is widely used, thermal decarboxylation and cannabinoid interconversion may bias Δ^9-THC measurements [3]. Liquid chromatography-based methods avoid these issues but remain limited by challenges in differentiating THC isomers and maintaining analytical throughput.

This study focuses on the development and validation of a rapid UPLC-UV-MS method for the quantification of 12 cannabinoids in cannabis plant material. The coupling of liquid chromatography with ultraviolet spectroscopy and mass spectrometric detection offers complementary advantages for quantitative accuracy and compound identification. Once validated, the UPLC-UV-MS method was used to analyze 15 authentic smokable hemp samples to evaluate its efficacy for differentiating legal hemp and illicit marijuana in forensic and cannabis regulatory applications.

MATERIALS & METHODS

Instrumentation

Analyses were performed on a Waters Acquity UPLC system with PDA and single quadrupole detection. Separation was achieved using an InfinityLab Poroshell 120 EC-C18 column (100 × 2.1 mm, 2.7 µm). Column and autosampler temperatures were 22 °C and 5 °C, respectively. Chromatographic separation was performed using mobile phases consisting of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.5 mL/min, with the gradient detailed in Table 1. PDA data were collected from 190-700 nm with the quantitation wavelength at 220 nm. ESI-MS was operated in positive mode (*m/z* 40-400), with cone voltages from 5-35 V.

RESULTS & DISCUSSION

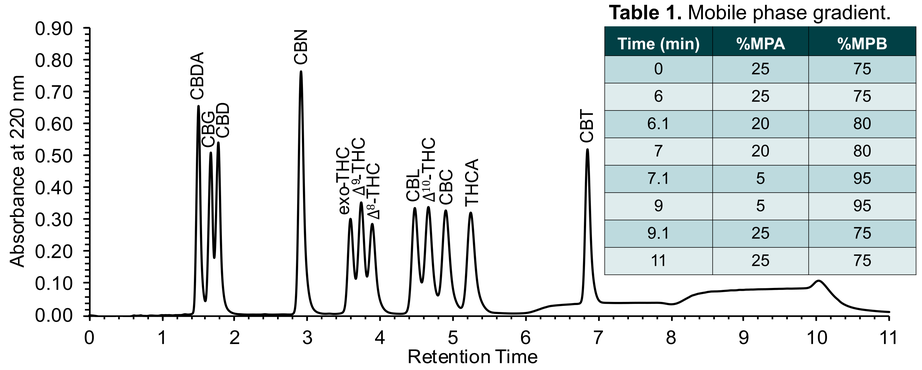


Table 1. Mobile phase gradient.

Time (min)	%MPA	%MPB
0	25	75
6	25	75
6.1	20	80
7	20	80
7.1	5	95
9	5	95
9.1	25	75
11	25	75

- Lower column temperature increased cannabinoid retention and enhanced analyte-stationary phase interactions, improving separation of late-eluting cannabinoids.
- Method validation met all study acceptance criteria, confirming that the optimized chromatographic method provides reliable quantitative performance despite partial chromatographic co-elution of cannabinoids.

Figure 1. LC-UV chromatogram at 220 nm for the 12 cannabinoids using a column temperature of 22 °C

Table 2. Validation criteria and results based on guidance from ANSI/ASB 036 [4].

Parameter	Validation Procedure	Results (for all cannabinoids)
Linearity	$R^2 \geq 0.995$ with 6 non-zero calibrators over 5 days; residuals within ± 3σ	$R^2 \geq 0.995$ for all calibrators; 1-50 µg/mL with 1/x linear model
Limit of detection (LOD)	Estimated using linear calibration curve over 5 days; LOD < 1 µg/mL = cannot be quantified	Sub-µg/mL LODs (0.03-0.30 µg/mL)
Within-run precision	% CV within 20%; 50, 25, 2.5 µg/mL in triplicate over 5 days	Within-run < 8% CV at high, medium, and low QC
Between-run precision	% CV within 20%; pooled replicates at 50, 25, 2.5 µg/mL (n=15)	Between-run < 5% CV at high, medium, and low QC
Bias	% bias within ±20%; 50, 25, 2.5 µg/mL in triplicate over 5 days	Within ±2%
Matrix effects	Suppression/enhancement (%) within ±25%; Post extraction addition at 50 and 2.5 µg/mL	< 25% suppression/enhancement of signal
Carryover	Blank injections following 50 µg/mL over 3 runs	No carryover detected

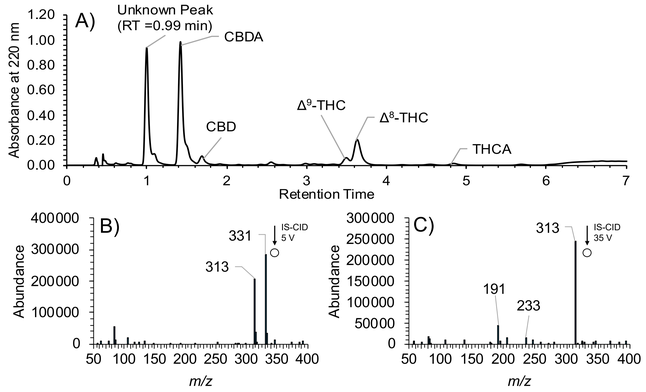


Figure 2. A) LC-UV chromatogram at 220 nm for authentic sample #8 and the mass spectra of an unknown peak at 0.99 min with cone voltages of B) 5 V and C) 35 V.

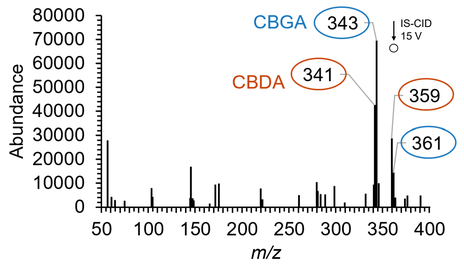


Figure 3. Exemplar mass spectrum for an unknown peak partially co-eluting with CBDA at a retention time of 1.48 min in authentic sample #1, collected with a cone voltage of 15 V, highlighting the presence of CBGA.

- Total Δ^9-THC content was determined for all 15 authentic smokable hemp samples, resulting in the classification of 9 samples as marijuana, indicating these samples were mislabeled.
- Increasing cone voltage enhanced in-source fragmentation (from *m/z* 331 → *m/z* 313, *m/z* 233, and *m/z* 191), supporting the identification of cannabidivarinic acid (CBDVA) in authentic sample #8.
- CBGA was identified as co-eluting with CBDA in authentic sample #1 based on diagnostic ions (*m/z* 361/343), enabling distinction from CBDA (*m/z* 359/341) and preventing inaccurate CBDA quantitation.
- Δ^8-THC was identified and quantified in sample #8, demonstrating the method can distinguish between Δ^9-THC and Δ^8-THC isomers.

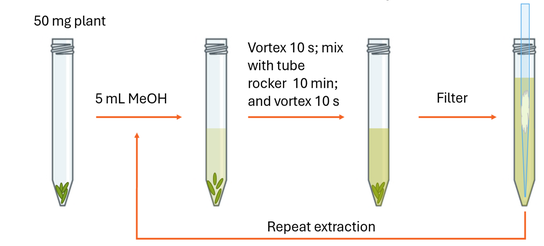
MATERIALS & METHODS

Validation Study

Method validation was conducted with guidance from ANSI/ASB Standard 036 [4], assessing linearity, limit of detection, precision, bias, matrix effects, and carryover (Table 2).

Authentic Samples

15 authentic smokable hemp samples were analyzed using an optimized extraction (shown below) and the validated UPLC-UV-MS method to evaluate method efficacy.



CONCLUSIONS

- ❖ Validated UPLC-UV-MS method provides rapid and reliable quantitative analysis of 12 cannabinoids in cannabis extracts within an 11-minute analysis time.
- ❖ Combined UV quantitation and MS selectivity enabled confident identification and quantification of cannabinoids in 15 authentic cannabis samples, including qualitative support for unknown cannabinoids.
- ❖ Practical solution for forensic marijuana casework and hemp compliance testing, supporting differentiation of hemp and marijuana under federal regulatory requirements.

REFERENCES

[1] Agriculture Improvement Act of 2018, Pub. L. No. 115-334 (2018).
[2] A.E. Outhous, A.K. Holt, J.L. Poklis, M.R. Peace, Evaluation of cannabis product mislabeling: The development of a unified cannabinoid LC-MS/MS method to analyze e-liquids and edible products, Talanta Open 10 (2024).
[3] K. Tsujikawa, Y. Okada, H. Segawa, T. Yamamuro, K. Kuwayama, T. Kanamori, Y.T. Iwata, Thermal decomposition of CBD to Δ^9-THC during GC-MS analysis: A potential cause of Δ^9-THC misidentification, Forensic Sci Int 337 (2022).
[4] American Academy of Forensic Science, Standard Practices for Method Validation in Forensic Toxicology (first ed.) (2019).

ACKNOWLEDGEMENTS

This project was supported by Award No. 15PNIJ-24-GG-03858-RESD, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice. The authors would also like to thank Dr. Walter B. Wilson from the National Institute of Standards and Technology (NIST) for providing the authentic cannabis samples.

DAVIDSON GROUP

FSF FORENSIC SCIENCES FOUNDATION
EMERGING FORENSIC SCIENTIST AWARD

Interested in a copy of this poster?

Department of Forensic Science
College of Criminal Justice
forensics.shsu.edu

SAM HOUSTON STATE UNIVERSITY FORENSIC SCIENCE