Rapid Differentiation of Hemp and Marijuana Using Cu-Phosphine Ion Complexation and Commercialized Paper Spray Ionization-Mass Spectrometry

Alleigh N. Couch[1*], Christopher M. Zall[2], J. Tyler Davidson[1]

[1]*Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340*
[2]*Department of Chemistry, Sam Houston State University, Huntsville, TX 77340*

Sam Houston State University

INTRODUCTION

The differentiation of legal hemp and illicit marijuana is imperative for the seized drug community. While the 2018 Agriculture Improvement Act delineated hemp and marijuana based on a 0.3% Δ^9-tetrahydrocannabinol (Δ^9-THC) dry-weight threshold [1], many laboratories have implemented semi-quantitative analyses using a more conservative 1% administrative threshold [2]. Although gas chromatography-mass spectrometry (GC-MS) is the gold standard for seized drug analysis, high temperatures of the GC inlet induce decarboxylation and potential cannabinoid conversion. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) avoids these limitations but produces indistinguishable product ion spectra for common cannabinoids, including Δ^9-THC and cannabidiol (CBD).

In this study, Cu-phosphine ion complexation was utilized to generate characteristic copper-bound, oxidized cannabinoid species, enabling the full scan differentiation of cannabinoid isomers. A semi-quantitative 1% decision-point assay was developed and validated using commercialized paper spray ionization-mass spectrometry (PSI-MS) instrumentation, enabling the rapid differentiation of hemp and marijuana.

MATERIALS & METHODS

Sample Preparation

Cannabinoids were prepared at 10 ppm in acetonitrile (ACN). The rewetting and spray solvents were composed of 5 µM of $[Cu(PPh_3)_2(ACN)_2]BF_4$ in ACN. Δ^9-THC-d_9 was fortified into cannabinoid samples at a 1% (w/w) threshold for the semi-quantitative decision-point assay.

Table 1. Overview of cannabinoids analyzed.

Cannabinoids Analyzed in this Study		
Δ^9-THC	Exo-THC	CBG
CBD	CBC	THCA
Δ^8-THC	CBT	CBDA
Δ^{10}-THC	CBL	Δ^9-THC-d_9
$\Delta^{6a,10a}$-THC	CBN	

RESULTS & DISCUSSION

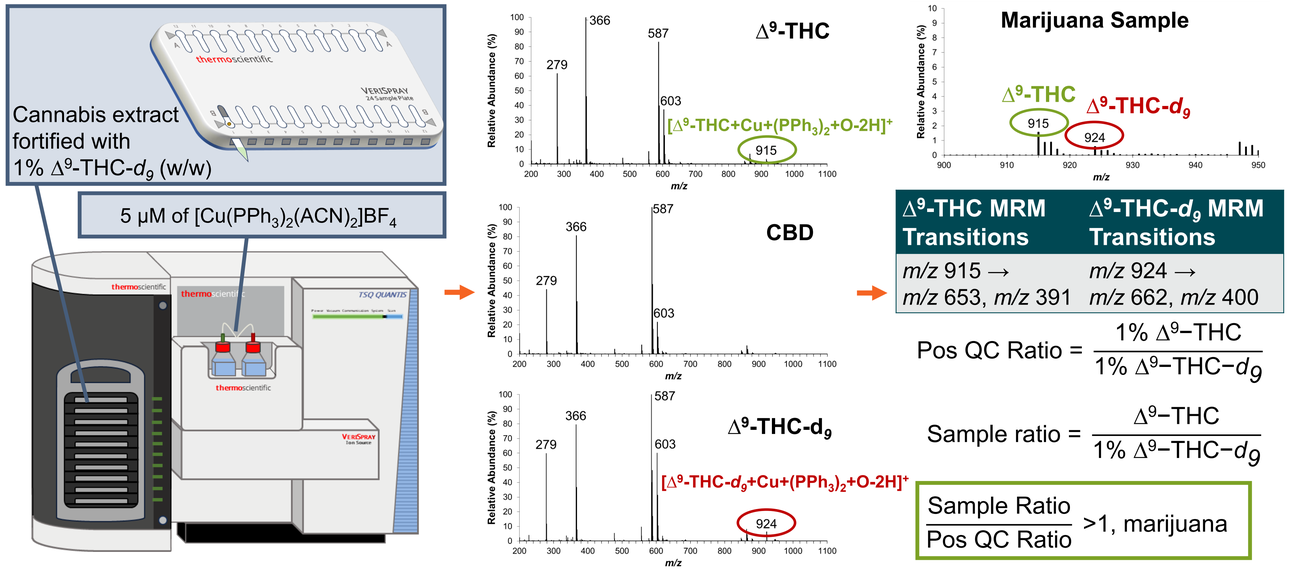

Figure 1. Overview of novel method for the rapid differentiation of hemp and marijuana using Cu-phosphine ion complexation, a semi-quantitative 1% decision-point assay, and a commercialized PSI-MS platform.

- Differentiation of hemp and marijuana based on the full scan mass spectra in less than 4 mins.

Table 2. Overview of validation studies and results.

Study	Parameter	Results
Selectivity	Pure cannabinoids in ACN and ZeroC extracts	All cannabinoids, except CBD, formed complexes
Accuracy	Known cannabis (7 positive/7 negative), n=3	100% accurate hemp and marijuana identification
Precision (%CV)	Repeatability (intra-day), n=10 Reproducibility (inter-day), n=50	7.75% at the 1.0% threshold 1.51 to 6.59% at the 1.0% threshold
Measurement Uncertainty	Fortified 1.0% Δ^9-THC in plant matrix and estimated with a 95% confidence interval (k=2)	1.06 ± 0.15% at the 1.0% threshold
Carryover	High-concentration of Δ^9-THC, followed by a blank	No carryover
LOD	Serial dilutions: 3x background & MRM transitions present	0.5 ppm

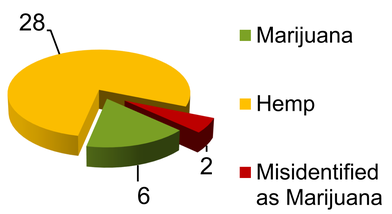

Figure 2. Authentic sample results.

- 34/36 authentic samples were correctly identified.

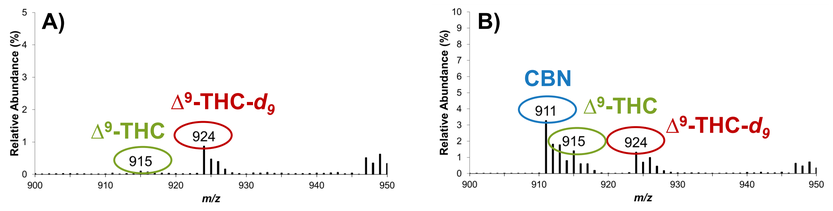

Figure 3. Authentic samples showing A) correct identification of hemp and B) incorrect identification of hemp.

- Misidentification due to contributions of the CBN isotopic envelope.

MATERIALS & METHODS

Sample Preparation Continued

ZeroC was used as a blank plant matrix for the validation studies. Authentic cannabis plant material was decarboxylated at 140 °C for 15 minutes.

Instrumentation and Data Analysis

A Thermo Scientific TSQ Quantis Plus QqQ mass spectrometer was coupled to a VeriSpray ionization source, with the high voltage probe set to 4,300 V. Cu-phosphine cannabinoid complexes were characterized in full scan and with activation energies of 15-65 V. MRM transitions were selected for Δ^9-THC and Δ^9-THC-d_9 for additional confirmation. Samples were classified as marijuana if the normalized total THC abundance was above 1, and the MRM transition ions were present.

CONCLUSIONS

- $[Cu(PPh_3)_2(ACN)_2]BF_4$ enables full scan differentiation of Δ^9-THC and CBD.
- Cu-phosphine ion complexation paired with a semi-quantitative 1% decision-point assay and commercialized PSI-MS enables the rapid differentiation of hemp and marijuana.
- 34/36 authentic cannabis samples were correctly identified as hemp or marijuana
- Incorrect identification was due to overlapping isotopic envelopes with CBN.
- Alternative approach for the rapid differentiation of hemp and marijuana, with analyses in less than 4 minutes.

REFERENCES

[1] H.R.2-115th Congress: Agriculture Improvement Act of 2018
[2] Cheng, Y.-C.; Kerrigan, S. Differentiation of hemp from marijuana using a qualitative decision-point assay. *Forensic Chem.* **2024**, *37*. DOI: 10.1016/j.forc.2023.100541.

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