Differentiation of Hemp and Marijuana Using Ag-Ligand Ion Complexation and a Semi-Quantitative Decision-Point Assay

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INTRODUCTION

The Agricultural Improvement Act of 2018, commonly known as the Farm Bill, defines marijuana as *Cannabis sativa* L. or any derivative thereof that contains greater than 0.3% Δ^9-tetrahydrocannabinol (Δ^9-THC) on a dry weight basis, whereas hemp contains no more than 0.3% Δ^9-THC[1]. As a result, seized drug analysts have altered the way potential marijuana samples are examined to include both the qualitative identification and quantitative or semi-quantitative analysis of the total THC content, which includes Δ^9-THC and its acidic precursor tetrahydrocannabinolic acid (THCA)[2].

The main two constituent of cannabis, Δ^9-THC and cannabidiol (CBD), are structural isomers and difficult to distinguish using soft ionization sources, such as ESI, due to their nearly indistinguishable product ion spectra. Hemp and marijuana differentiation is further complicated by the presence of additional cannabinoids. Therefore, current techniques used to differentiate hemp and marijuana require chromatographic seperation of the cannabinoids prior to mass spectrometry analysis, leading to longer analysis times, degradation or conversion of cannabinoids, and increased costs due to instrument consumables and solvents[3].

Ag-ligand ion complexation is an alternative approach that enables the differentiation of Δ^9-THC and CBD due to the formation of unique MS/MS product ions based on the difference in preferential binding of the cannabinoids to the Ag complex. Therefore, Δ^9-THC and CBD can be differentiated without chromatographic seperation. This research characterizes 12 cannabinoid Ag complexes, and the resulting fragmentation pathways were classified as Δ^9-THC-like, CBD-like, or unique. Additionally, this research provides the first method for the differentiation of hemp and marijuana using Ag-ligand ion complexation and a semi-quantitative decision-point assay, in the presence of other cannabinoid interferences.

MATERIALS & METHODS

Sample Preparation

All cannabinoids in Table 1 were analyzed with and without the presence of the Ag-phosphine complex, $[Ag(PPh_3)(OTf)]_2$. The cannabinoid Ag complexes were composed of the cannabinoids at a concentration of 50 ppm and $[Ag(PPh_3)(OTf)]_2$ at a concentration of 225 µM. $[Ag(PPh_3)(OTf)]_2$ was synthesized in a simple reaction between Ag tetrafluoroborate and triphenylphosphine, with purification by recrystallization. Calibration curves were prepared across varying ratios of Δ^9-THC:CBD and THCA:CBDA with a total cannabinoid content of 50 ppm and 225 µM of $[Ag(PPh_3)(OTf)]_2$.

Table 1. Cannabinoids analyzed in this study.

Δ^9-THC	Δ^8-THC	CBG
CBD	Exo-THC	CBL
THCA	$\Delta^{6a,10a}$-THC	CBC
CBDA	CBL	CBT

RESULTS & DISCUSSION

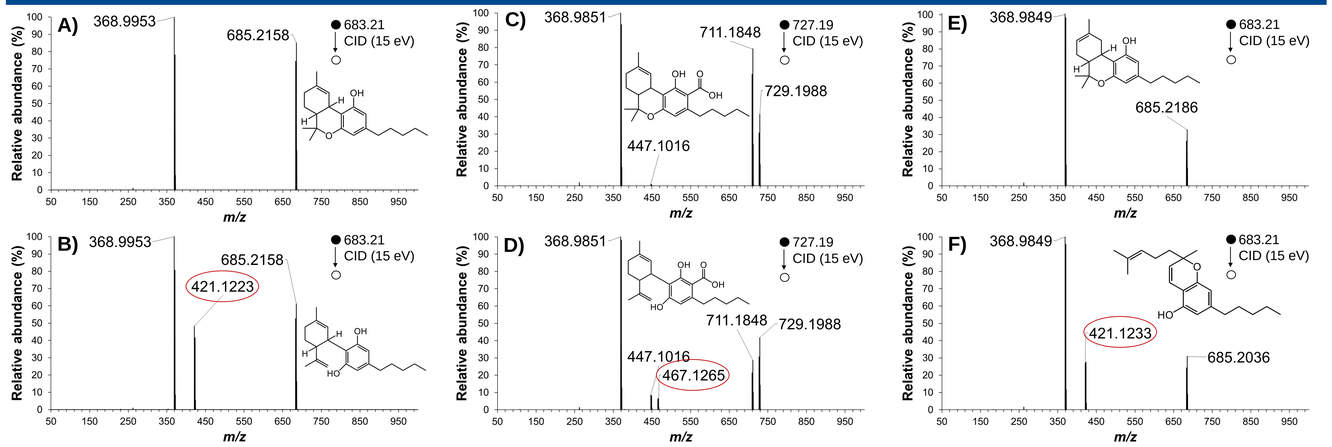

Figure 1. Comparison of MS/MS spectra for cannabinoid Ag complexes for A) Δ^9-THC, B) CBD, C) THCA, D) CBDA, E) Δ^8-THC and F) CBC.

- Under 15 eV activation conditions, there are unique product ions capable of differentiating cannabinoid isomers such as Δ^9-THC and CBD, and THCA and CBDA.
- The Δ^8-THC fragmentation pattern is similar to Δ^9-THC, whereas CBC fragmentation pattern is similar to CBD.

Table 2. Classification of cannabinoid Ag complexes based on observed fragmentation pathways.

Δ^9-THC-Like Pathway	CBD-Like Pathway	Different Precursor Ion
CBL	CBC	CBN
CBT		CBG
Δ^8-THC		THCA
Exo-THC		CBDA*
$\Delta^{6a,10a}$-THC		

*Presence of unique MS/MS product ion capable of differentiating THCA and CBDA.

- The cannabinoids were analyzed and separated into three groups based their precursor ions and fragmentation patterns.

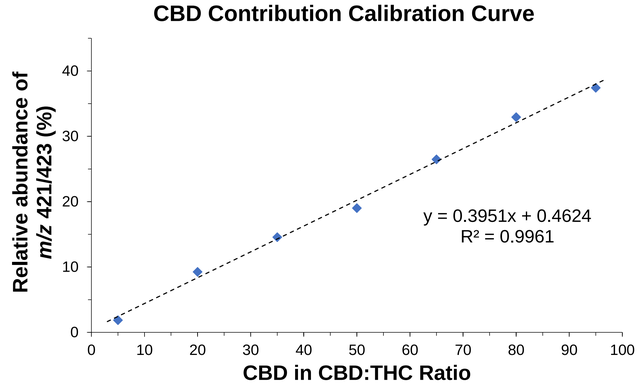

Figure 3.Calibration curve for the resulting relative abundance of the product ions at *m/z* 421/423 across varying CBD:THC ratios.

- Using a range of CBD:THC ratios, the CBD contribution can be calculated based on the relative abundance of *m/z* 421/423.

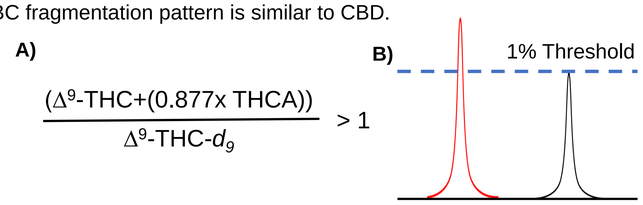

Figure 2. Representation of the1% decision-point assay highlighting A) the numerical assessment and B) visualization of 1% administrative threshold concept.

- The total THC abundance is normalized to the internal standard abundance. Any value greater than 1 indicates the sample is marijuana.

Table 3. 1% Decision-point assay results for 10 authentic samples.

Unknown Sample	Marijuana or Not Marijuana	Classification Result
1	Marijuana	✓
2	Marijuana	✓
3	Not Marijuana	✓
4	Marijuana	✓
5	Marijuana	✓
6	Marijuana	✓
7	Marijuana	✓
8	Marijuana	✓
9	Not Marijuana	✓
10	Marijuana	✓

- All authentic samples were correctly identified as marijuana or not marijuana based on the 1% administrative threshold.

MATERIALS & METHODS

Sample Preparation Continued

Authentic samples were composed of marijuana and hemp extracts with known concentrations of Δ^9-THC and CBD, 225 µM of $[Ag(PPh_3)(OTf)]_2$, and Δ^9-THC-d_9 (ISTD) at concentration 1% by weight for the administrative threshold.

Instrumentation and Data Analysis

An Agilent Technologies 6530 quadrupole time-of-flight (Q-TOF) mass spectrometer was used to analyze all samples. For cannabinoid Ag complex characterization, MS/MS activation was performed with collision energies of 15 eV-45 eV for each precursor ion of interest. Calibration curves and authentic samples were analyzed with a 20 eV collision energy. Spectral comparisons were used to determine if the cannabinoid $[Ag(PPh_3)(OTf)]_2$ complexes provided unique fragmentation pathways or fragmented similarly to the Δ^9-THC or CBD. The calibration curves were used to determine the contribution of Δ^9-THC/CBD and THCA/CBDA in the authentic samples based on the relative abundance of unique product ions at *m/z* 421/423 and *m/z* 465/467, respectively. The total THC abundance was normalized to the Δ^9-THC-d_9 abundance. If the resulting value was above 1, the unknown sample was determined to be marijuana.

CONCLUSIONS

- Ag-ligand ion complexation can be used to differentiate Δ^9-THC/CBD and THCA/CBDA due to the difference in the binding affinity between the Ag complex and the cannabinoids.
- CBL, CBT, Δ^8-THC, exo-THC, and $\Delta^{6a,10a}$-THC fragment similarly to Δ^9-THC, whereas CBC fragments similarly to CBD. CBN, CBG, THCA, and CBDA have unique precursor ions.
- The developed semi-quantitative 1% decision-point assay method enables the differentiation of hemp and marijuana in authentic cannabis samples with a direct mass spectrometry approach, even in the presence of other cannabinoid interferences.

REFERENCES

[1] H.R.2-115th Congress: Agricultural Improvement Act of 2018.
[2] Establishment of a Domestic Hemp Production Program; 84(211).
[3] Capriotti, A. L., et al., Recent applications of mass spectrometry for the characterization of cannabis and hemp phytocannabinoids: From targeted to untargeted analysis. *J Chromatogr A*, 2021. 1655: 462492.

ACKNOWLEDGEMENTS

This project was made possible by funding received from the Sam Houston State University Office of Research and Sponsored Programs. The authors declare no competing financial interest.