Ag-Ligand Ion Complexation: An Alternative Approach for Hemp and Marijuana Differentiation

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ABSTRACT

The 2018 Farm Bill defines marijuana as *Cannabis sativa* L. or any derivative thereof that contains more than 0.3% Δ^9-tetrahydrocannabinol (Δ^9-THC), whereas anything that contains less is considered hemp [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]. This study provides a novel direct mass spectrometry approach for the differentiation of hemp and marijuana using Ag-ligand ion complexation and a semi-quantitative decision-point assay.

INTRODUCTION

The main constituents of hemp and marijuana are the structural isomers cannabidiol (CBD) and Δ^9-THC, which are difficult to distinguish when using soft ionization sources, such as electrospray ionization (ESI), due to their nearly indistinguishable product ion spectra. Therefore, current techniques used to differentiate hemp and marijuana rely on the chromatographic separation of the cannabinoids before mass spectrometry analysis, resulting in long analysis times, increased cost for instrument consumables, and degradation or conversion of cannabinoids in the case of gas chromatography-mass spectrometry (GC-MS) [3].

Ag-ligand ion complexation is a direct mass spectrometry approach for the differentiation of hemp and marijuana. The difference in binding affinity of the cannabinoids to the Ag complex leads to the formation of unique MS/MS product ions, enabling differentiation without chromatography. In this study, Ag-ligand ion complexation was used to characterize 12 cannabinoids in positive ionization mode, as well as the development of a 1% semi-quantitative decision-point assay for the differentiation of hemp and marijuana.

MATERIALS & METHODS

Sample Preparation

The following cannabinoids were analyzed with and without the presence of $[Ag(PPh_3)(OTf)]_2$: Δ^9-THC, CBD, THCA, cannabidiolic acid (CBDA), cannabichromene (CBC), Δ^8-tetrahydrocannabinol (Δ^8-THC), $\Delta^{6a,10a}$-tetrahydrocannabinol ($\Delta^{6a,10a}$-THC), cannabinol (CBN), cannabigerol (CBG), exo-tetrahydrocannabinol (exo-THC), and cannabicitran (CBT). The cannabinoid Ag complexes consisted of 50 ppm of cannabinoid and 225 µM of $[Ag(PPh_3)(OTf)]_2$. Calibration curves were prepared across varying ratios of Δ^9-THC:CBD and THCA:CBDA with a total concentration of 50 ppm per Δ^9-THC:CBD ratio, 50 ppm per THCA:CBDA ratio, and 225 µM of $[Ag(PPh_3)(OTf)]_2$. A THCA:CBDA response ratio calibration curve was prepared in a 1:1 ratio with the total cannabinoid content ranging from 10 ppm to 700 ppm with a constant 225 µM of $[Ag(PPh_3)(OTf)]_2$ and 50 ppm of Δ^9-THC-d_9.

RESULTS & DISCUSSION

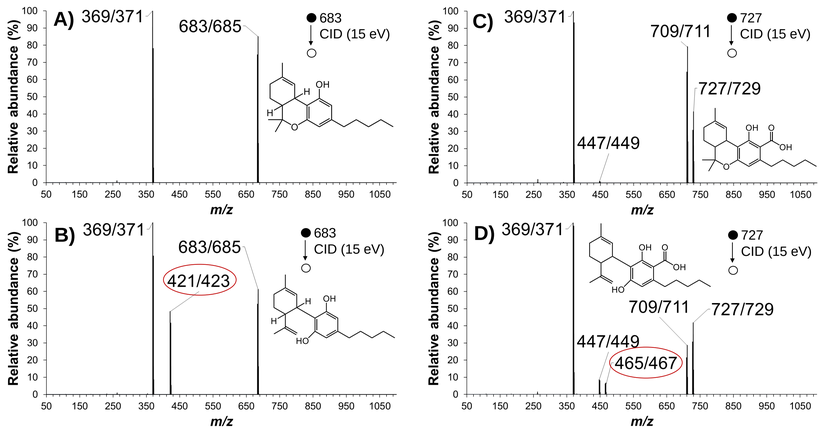

Figure 1. Comparison of MS/MS product ion spectra for the following cannabinoid Ag complexes: A) Δ^9-THC, B) CBD, C) THCA, and D) CBDA.

- Under 15-eV activation conditions, there are unique product ions enabling the differentiation of cannabinoid isomers (i.e., Δ^9-THC/CBD and THCA/CBDA).

Table 1. 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 enabling differentiation of THCA and CBDA.

- The cannabinoids were analyzed and separated into three groups based on their precursor ions and fragmentation patterns.
- THCA and CBDA both have a precursor ion at *m/z* 727/729. However, THCA and CBDA can be differentiated due to a unique product ion in the CBDA spectrum at *m/z* 465/467.

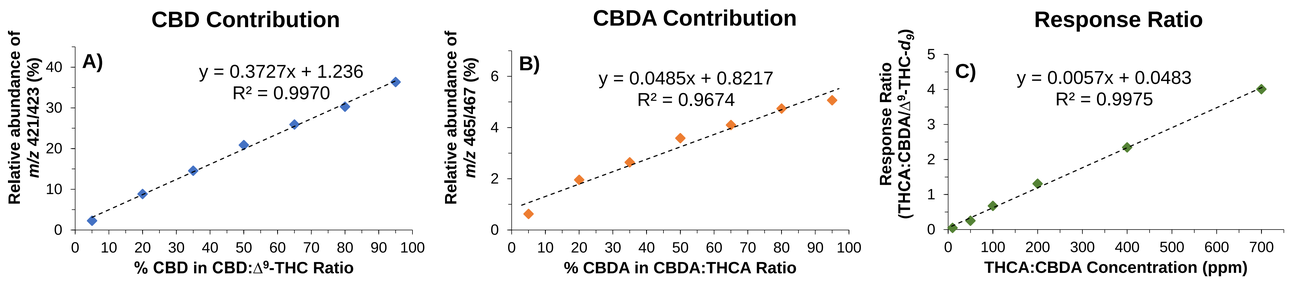

Figure 2. Calibration curves used to calculate the total THC based on the relative abundance of the unique product ions for A) *m/z* 421/423 across varying CBD:Δ^9-THC ratios and B) *m/z* 465/467 across varying CBDA:THCA ratios. Figure 2C shows the response ratio calibration curve for the abundance of the precursor ion of THCA:CBDA at varying concentrations to Δ^9-THC-d_9 at 50 ppm for the 20-eV mass spectra.

- Using a range of CBD:THC and CBDA:THCA ratios, the CBD and CBDA contribution can be calculated based on the relative abundance of the product ions at *m/z* 421/423 and *m/z* 465/467, respectively.
- Using the response ratio calibration curve, the concentration of THCA:CBDA in authentic samples can be calculated.

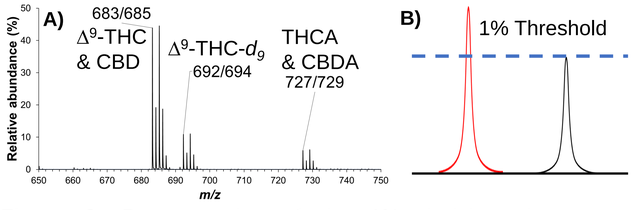

Figure 3. Representation of the 1% decision-point assay highlighting: A) full scan mass spectra and B) visualization of the 1% administrative threshold concept.

- The total THC abundance is normalized to the internal standard abundance with any value greater than 1 indicating marijuana.

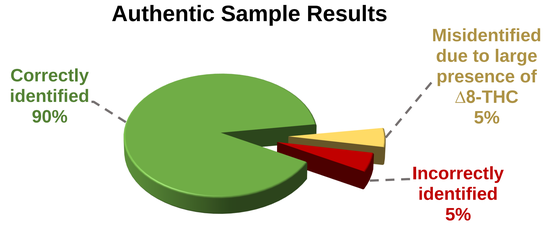

Figure 4. Summary of results when analyzing 20 authentic samples.

- 18/20 authentic samples were correctly identified as marijuana or not marijuana based on the 1% administrative threshold.
- 1 misidentification was a result of a large presence of Δ^8-THC.

MATERIALS & METHODS

Sample Preparation Continued

Authentic samples consisted 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 6530 quadrupole time-of-flight (Q-TOF) mass spectrometer was used for this study. For cannabinoid Ag complex characterization, MS/MS activation was performed with collision energies of 15-45 eV for each precursor ion of interest. Spectral comparisons were used to determine the fragmentation pathways for each cannabinoid-$[Ag(PPh_3)(OTf)]_2$ complex. 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 response ratio was used to determine the combined concentration of THCA:CBDA present in the authentic sample. 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 in positive mode due to the difference in preferential 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 correctly identified 18/20 authentic samples as marijuana or not marijuana.
- Further research is required to address matrix interferences and improve the correct identification rate.

REFERENCES

[1] H.R.2-115th Congress: Agricultural Improvement Act of 2018.
[2] Establishment of a Domestic Hemp Production Program; 84(211).
[3] A. L. Capriotti 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.

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