Differentiation of CBD and Δ⁹-THC Isomers Using Copper Ion Complexation and Electrospray Ionization Tandem Mass Spectrometry

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ABSTRACT

The 2018 Farm Bill redefined marijuana as *Cannabis sativa* L. or any derivative thereof that contains greater than 0.3% Δ⁹-tetrahydrocannabinol (Δ⁹-THC), whereas hemp contains no more than 0.3% Δ⁹-THC [1]. The analysis required for marijuana identification is further complicated by the presence of cannabidiol (CBD) and Δ⁹-THC isomers. This study provides the first application of copper ion complexation and electrospray ionization-tandem mass spectrometry (ESI-MS/MS) for differentiating CBD and Δ⁹-THC Isomers.

INTRODUCTION

The structural isomers Δ⁹-THC and CBD are the main components of marijuana and hemp, respectively. Although they can be readily differentiated using gas chromatography-electron ionization-mass spectrometry (GC-EI-MS), there are concerns with thermal degradation and interconversion between cannabinoid isomers [2]. Alternative techniques such as liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) have been explored. However, the product ion spectra of CBD and Δ⁹-THC isomers are nearly identical. In addition, there are many other cannabinoids present in *cannabis sativa* L. that complicate the identification of Δ⁹-THC. As a result, current techniques used to differentiate hemp and marijuana rely on chromatographic separation of the cannabinoids for cannabinoid differentiation.

Copper ion complexation is a novel approach to differentiate CBD and Δ⁹-THC isomers. Subtle differences in preferential binding affinity between each cannabinoid and Cu^+ enable the formation of characteristic ions in either full scan or the product ion spectra generated upon MS/MS analysis [3]. In this study, 13 copper-cannabinoid complexes were analyzed to identify characteristic ions, in either the full scan or product ion spectra, that enable cannabinoid differentiation. Extracts from authentic cannabis plant material were fortified with cannabinoids and analyzed to demonstrate the applicability of this approach to real-world samples. This study demonstrates the potential for copper ion complexation with ESI-MS/MS to be an alternative method for the differentiation of CBD and Δ⁹-THC isomers, which is necessary for the differentiation of hemp and marijuana.

MATERIALS & METHODS

Sample Preparation

The following cannabinoids were analyzed as Cu complexes: Δ⁹-THC, CBD, Δ⁸-tetrahydrocannabinol (Δ⁸-THC), exo-tetrahydrocannabinol (exo-THC), Δ¹⁰-tetrahydrocannabinol (Δ¹⁰-THC), Δ⁶ᵃ,¹⁰ᵃ-tetrahydrocannabinol (Δ⁶ᵃ,¹⁰ᵃ-THC), cannabichromene (CBC), cannabicitran (CBT), cannabicyclol (CBL), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA), and cannabidiolic acid (CBDA). Solvent optimization studies were completed with acetonitrile and methanol. The copper complexes were prepared in acetonitrile with a cannabinoid concentration of 50 ppm and 158.9 µM of tetrakis(acetonitrile)copper(I) tetrafluoroborate ($Cu(MeCN)_4BF_4$).

RESULTS & DISCUSSION

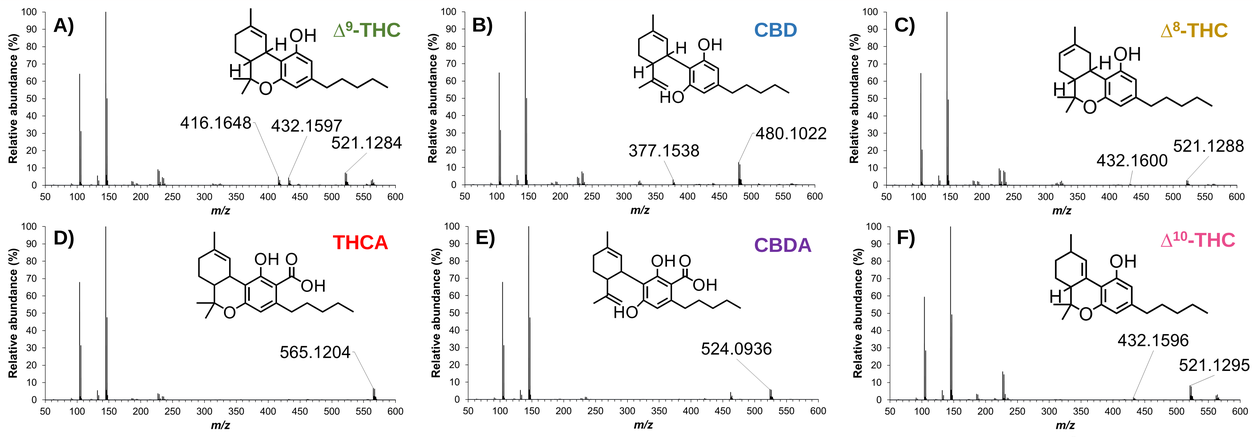

Figure 1. Comparison of the full scan mass spectra of the copper complexes for the following cannabinoids: A) Δ⁹-THC, B) CBD, C) Δ⁸-THC, D) THCA, E) CBDA, and F) Δ¹⁰-THC.

- The presence and/or absence of characteristic ions enables the differentiation of cannabinoid isomers (Table 1).
- Δ⁹-THC and THCA can be differentiated from CBD and CBDA, respectively, which is imperative for marijuana identification.

Table 1. Characteristic ions for the cannabinoid copper complexes.

Cannabinoid	Ions of Interest (*m/z*)
Δ⁹-THC	416, 432, 521
CBD	377, 480
Δ⁸-THC	432, 521
Exo-THC	521
Δ¹⁰-THC	432, 521
Δ⁶ᵃ,¹⁰ᵃ-THC	416, 432, 521
CBC	377, 418, 480
CBT	447, 511
CBL	432, 521
CBN	517, 559
CBG	379, 482
THCA	565
CBDA	524

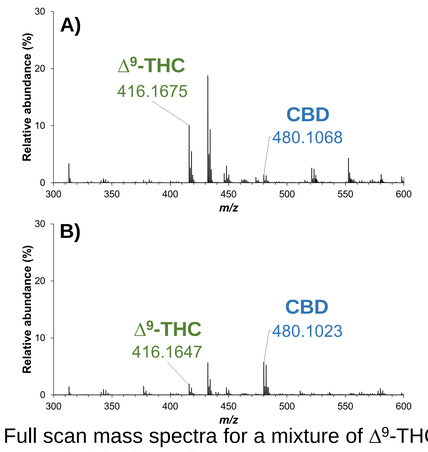

Figure 2. Full scan mass spectra for a mixture of Δ⁹-THC and CBD in ratios of A) 80:20 and B) 20:80.

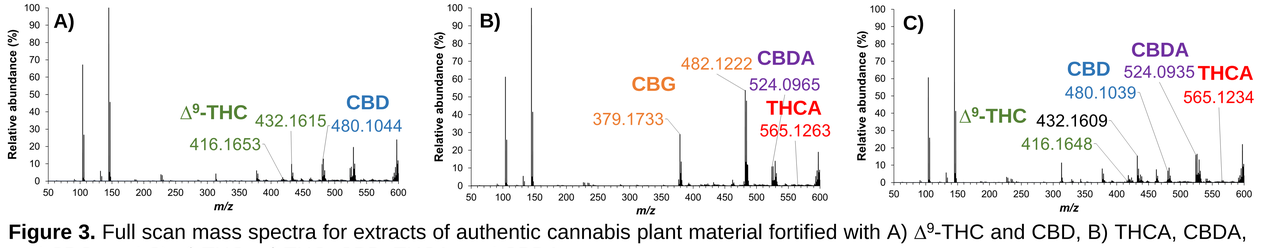

Figure 3. Full scan mass spectra for extracts of authentic cannabis plant material fortified with A) Δ⁹-THC and CBD, B) THCA, CBDA, and CBG, and C) Δ⁹-THC, Δ⁸-THC, CBD, THCA, and CBDA.

- Characteristic ions were identified in both the ZeroC and authentic cannabis plant material extracts.
- MS/MS required for differentiation of copper-cannabinoid complexes that share a common precursor ion. For example, the precursor ion at *m/z* 432 produces product ions at *m/z* 295 for Δ⁹-THC and *m/z* 230 for Δ¹⁰-THC, enabling differentiation.

MATERIALS & METHODS

Sample Preparation Continued

Two-, three-, four-, and five-component mixtures were prepared with a total cannabinoid concentration of 50 ppm and 635.8 µM of $Cu(MeCN)_4BF_4$ in both acetonitrile, as well as a methanolic extract of ZeroC, which is a cannabis plant material matrix purchased from Cayman Chemical. Representative mixtures were analyzed in varying ratios in the ZeroC cannabis plant matrix. Methanolic extracts of authentic cannabis plant material were also prepared for 10 different authentic cannabis samples and fortified with cannabinoids at a concentration of 50 ppm per cannabinoid and a concentration of 635.8 µM of $Cu(MeCN)_4BF_4$.

Instrumentation and Data Analysis

An Agilent Technologies 6530 quadrupole time-of-flight was used to analyze the copper complexes. Instrumental parameters are as follows: a nozzle voltage of 1500 V, a capillary voltages of 3500 V, a 350 °C sheath gas with an 8 L/min flow rate, a nebulizer pressure of 40 psi, and a 300 °C drying gas with a flow rate of 8 L/min. MS/MS activation was performed under collision energies of 15-45 eV, with an isolation width of 4 Da, for all precursor ions of interest.

CONCLUSIONS

- Cu ion complexation can be used to differentiate CBD and Δ⁹-THC isomers due to slight differences in the preferential binding affinity between the Cu^+ and the cannabinoids.
- Acetonitrile was determined to be the optimal solvent to differentiate the Cu-cannabinoid adducts.
- Cannabinoid isomers can be identified in mixtures based on the presence or absence of characteristic ions.
- The developed method enables the differentiation of CBD and Δ⁹-THC isomers in extracts of authentic cannabis plant material.
- Potential alternative solution to address marijuana identification.

REFERENCES

[1] H.R.2-115th Congress: Agricultural Improvement Act of 2018

[2] Cheng, Y.-C. and Kerrigan, S. Factors influencing the in situ formation of Δ⁹-THC from cannabidiol during GC–MS analysis. *Drug Test Anal*, **2023**. 16(9) DOI: 10.1002/dta.3617.

[3] Mehara, J., Watson, B. T., Noonikara-Poyil, A., Zacharias, A. O., Roithova, J., and Rasika Dias, H. V. Binding Interactions in Copper, Silver and Gold pi-Complexes. *Chem.*, **2022**. 28(13): e202103984. DOI: 10.1002/chem.202103984.

ACKNOWLEDGEMENTS

This project was made possible by funding received from the 2023-2024 Forensic Sciences Foundation Lucas Research Grant.

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