Utilization of Copper Salts to Induce the Formation of Cannabinoid Molecular Ions Enabling Isomer Differentiation

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INTRODUCTION

Cannabis sativa L. often contains a multitude of cannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). Accurate identification of these cannabinoids is imperative in forensic casework, particularly when distinguishing between controlled marijuana and legal hemp [1]. Under soft ionization conditions, such as electrospray ionization (ESI), Δ^9-THC isomers have identical precursor ions and nearly identical product ion spectra upon collision-induced dissociation (CID) activation, complicating their identification. In contrast, cannabinoids can be differentiated under electron ionization (EI) conditions based on characteristic ions or ion ratios. However, limitations exist using gas chromatography-mass spectrometry (GC-MS) due to the high temperature of the GC inlet, causing potential cannabinoid conversion or decarboxylation [2].

This study provides the first application of Cu salts to promote the formation of cannabinoid molecular ions under ESI-MS/MS conditions. 13 Cu-cannabinoid solutions were analyzed, and the resulting cannabinoid molecular ions were characterized under a variety of CID activation energies. The generated tandem mass spectrometry (MS/MS) product ion spectra closely resembled traditional EI spectra obtained from GC-MS analysis, enabling the differentiation of Δ^9-THC cannabinoid isomers. NIST MS Search was used to evaluate the similarity of the MS/MS product ion spectra against the NIST EI library. Extracts from ZeroC and authentic cannabis plant material were fortified with cannabinoids and Cu salt to demonstrate the applicability of this method to real-world samples. These findings demonstrate the potential application of Cu assisted ESI-MS/MS for molecular ion generation and isomer differentiation of cannabinoids, offering an alternative to traditional GC-MS analysis.

MATERIALS & METHODS

Sample Preparation

Cannabinoid samples were prepared as a methanol solution with a concentration of 50 ppm of cannabinoid (Table 1) and fortified with 158.9 µM of tetrakis(acetonitrile)copper(I) tetrafluoroborate ($Cu(MeCN)_4BF_4$).

Table 1. Overview of cannabinoids analyzed in this study.

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

RESULTS & DISCUSSION

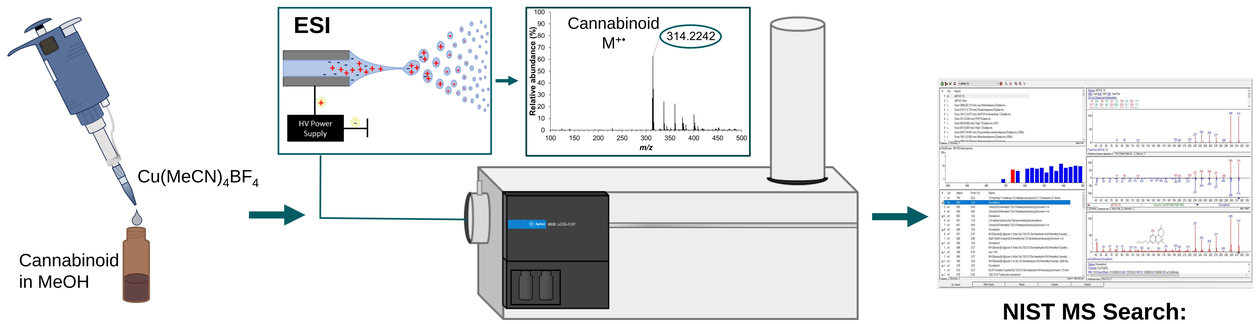

Figure 1. Overview of the developed method for the formation of molecular ions under ESI-MS/MS conditions.

- Cannabinoid molecular ions were formed under ESI conditions due to the addition of copper salts.
- Resulting MS/MS product ion spectra were imported into NIST MS Search and searched against the NIST 20 EI library.

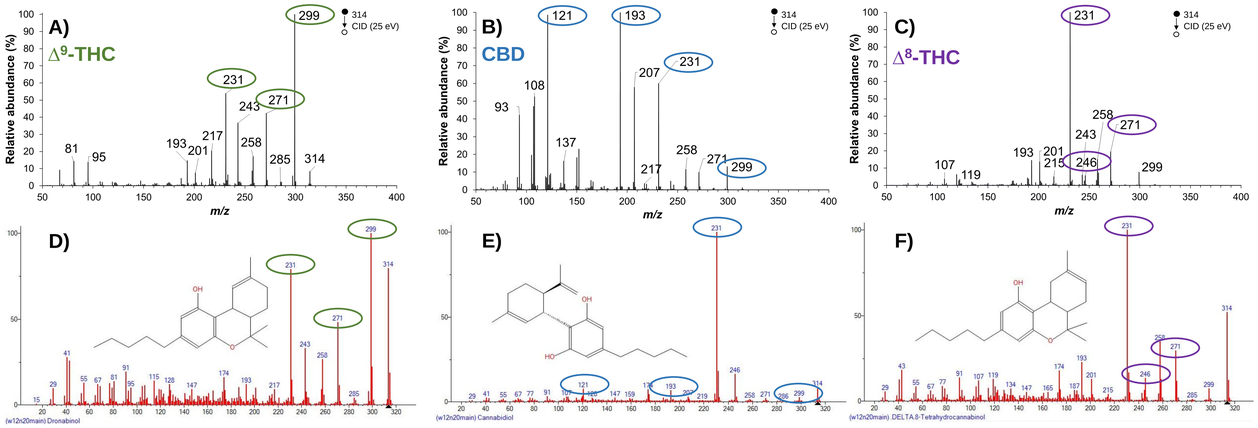

Figure 2. Comparison of the 25-eV product ion spectra of the cannabinoid molecular ions: A) Δ^9-THC, B) CBD, C) Δ^8-THC to the NIST 20 EI Library spectra through NIST MS Search: D) Δ^9-THC, E) CBD, F) Δ^8-THC.

- CID activation of cannabinoid molecular ions resulted in EI-like product ion spectra, enabling isomer differentiation.

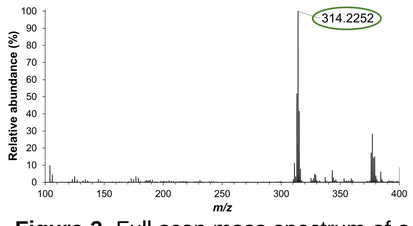

Figure 3. Full scan mass spectrum of a cannabinoid molecular ion in an authentic cannabis extract.

- The cannabinoid molecular ions were formed, even in a complex matrix.

Table 2. Summary of the NIST MS Search library results for product ion spectra of each cannabinoid collected with the optimal collision energy.

Cannabinoid	CE (eV)	Hit #	Match	Cannabinoid	CE (eV)	Hit #	Match
Δ^9-THC	25	2	691	CBC	15	3	754
CBD	35	1	535	CBL*	-	-	-
Δ^8-THC	25	1	643	CBN	35	3	634
Exo-THC	25	2	623	CBG	15	1	633
Δ^{10}-THC*	Not present in library			THCA	35	7	474
$\Delta^{6a,10a}$-THC*	Not present in library			CBDA*	-	-	-
CBT*	15	18	673				

*Library results matched for a cannabinoid isomer rather than the correct cannabinoid.

MATERIALS & METHODS

Sample Preparation Continued

The Δ^9-THC, CBD, and Δ^8-THC Cu cannabinoid solutions were also prepared in methanolic extracts of ZeroC, a cannabis free plant matrix purchased from Cayman Chemical, and an authentic cannabis plant material purchased online. However, with the complexity of the plant matrices, the concentration of $Cu(MeCN)_4BF_4$ needed to be increased to 635.6 µM (i.e., 200 ppm).

Instrumentation and Data Analysis

An Agilent Technologies 6530 quadrupole time-of-flight was used for analysis. Instrumental parameters are as follows: a nozzle voltage of 1500 V, a capillary voltage 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. CID activation was performed from 15-45 eV for the cannabinoid molecular ions. Spectra were extracted as .CSV file from MassHunter Qualitative Analysis 10.0, imported into NIST MS Search 2.4, and searched against the NIST 20 EI library. Data was visualized using Microsoft Excel.

CONCLUSIONS

- The copper salt, $Cu(MeCN)_4BF_4$, can induce the formation of cannabinoid molecular ions under ESI conditions.
- The cannabinoid molecular ion MS/MS product ion spectra provided EI-like mass spectra, enabling cannabinoid isomer differentiation.
- Cannabinoid molecular ion product ion spectra imported into NIST MS Search for cannabinoid isomer identification.
- The rank and match score changed as a function of collision energy
- Even cannabinoids absent from the EI library still returned hits for cannabinoid isomers.
- Potential solution to differentiate cannabinoid isomers that otherwise cannot be differentiated under ESI-MS/MS conditions.

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 Δ^9-THC from cannabidiol during GC–MS analysis. *Drug Test Anal*, **2023**. 16(9) DOI: 10.1002/dta.3617.

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