Differentiation of Δ9-THC and CBD Using Silver-Ligand Ion Complexation and Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS)

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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% Δ9-tetrahydrocannabinol (Δ9-THC), with anything containing 0.3% or less being considered hemp [1]. Due to the classification of marijuana, or Δ9-THC-rich cannabis, as a Scheduled I controlled substance, the differentiation between hemp and marijuana has become crucial within the seized drug community. This study provides a method for the differentiation of Δ9-THC and cannabidiol (CBD) using Ag-ligand ion complexation with electrospray ionization tandem mass spectrometry (ESI-MS/MS) through the production of unique MS/MS product ion spectra.

INTRODUCTION

Cannabis sativa L. has long been used as a source for medicinal, recreational, and manufacturing purposes [2]. The enactment of the 2018 Farm Bill provided guidelines on the differentiation of hemp versus marijuana based on the total Δ9-THC content, therefore altering the way forensic analysts must examine potential marijuana samples [1]. The qualitative identification of Δ9-THC is complicated by the presence of other cannabinoids in cannabis. For example, CBD, which is the main cannabinoid present in hemp, has the exact same elemental composition as Δ9-THC (i.e., isobaric), with only a subtle difference in molecular structure (i.e., structural isomers).

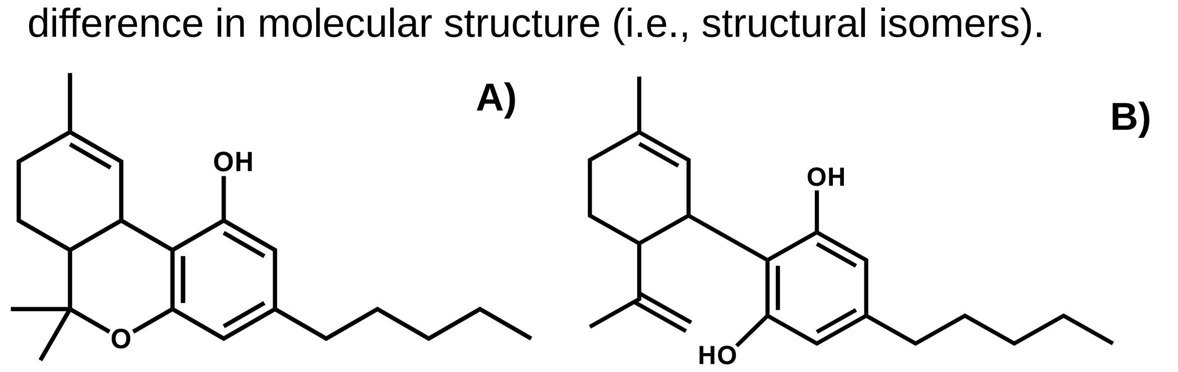

Figure 1. Comparison between the structure of A) Δ9-THC and B) CBD, the main constituents of marijuana and hemp, respectively.

Current techniques used for the differentiation of marijuana and hemp require chromatographic separation prior to mass spectrometry detection. As a result, these methods require lengthy analysis times, derivatization, and additional costs due to instrumental consumables and solvents. These limitations and the increasing number of suspected marijuana casework submissions have necessitated research into alternative techniques. Ag-ligand ion complexation is an alternative approach for the differentiation of Δ9-THC and CBD based on the formation of unique MS/MS product ion spectra caused by differences in metal-ligand ion binding affinities. The presence of unique MS/MS product ion spectra enables the differentiation of the two main chemical constituents of cannabis, without the need for chromatographic separation, as well as provides a mechanism for the differentiation of Δ9-THC-rich versus CBD-rich cannabis. Incorporation of ligands in addition to the Ag ions increases the selectivity, solubility, and stability improving upon previous Ag ion complexation approaches [3]. This research provides a first step towards the incorporation of Ag-ligand ion complexation for the differentiation of hemp and marijuana.

RESULTS & DISCUSSION

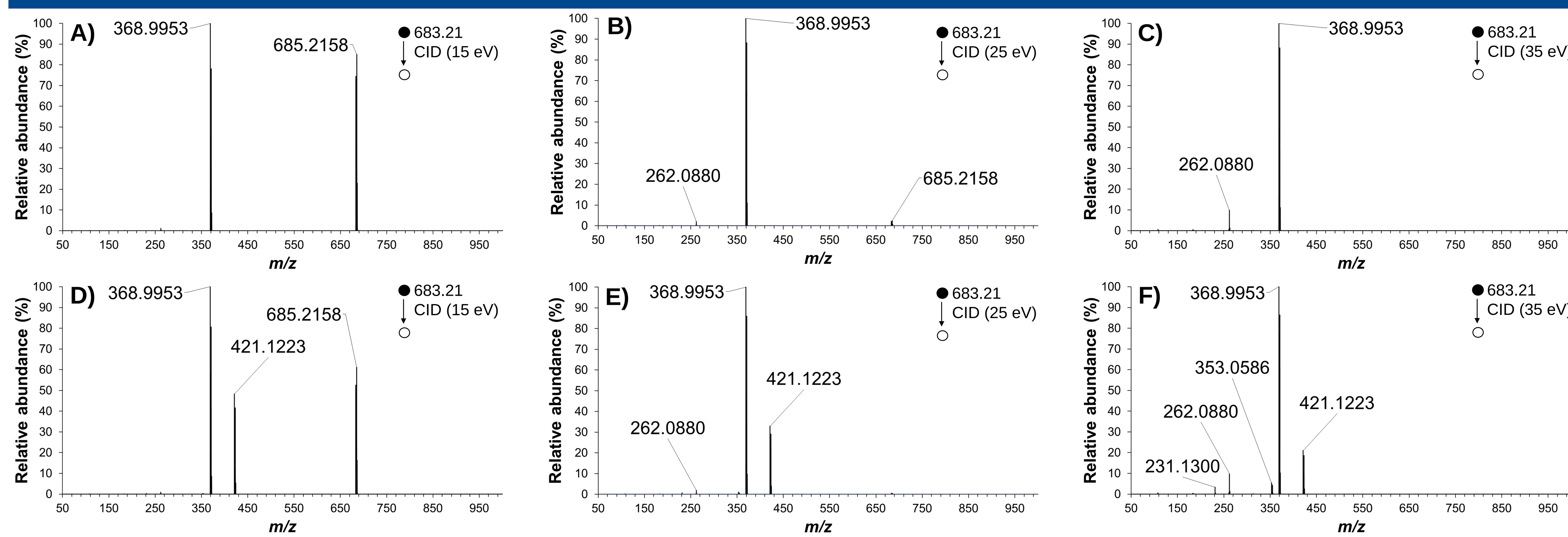

Figure 2. Comparison of MS/MS spectra for Δ9-THC:Ag-ligand at A) 15 eV, B) 25 eV, and C) 35 eV and CBD:Ag-ligand at D) 15 eV, E) 25 eV, and F) 35 eV.

- Under 15 eV activation conditions, there is a unique product ion observed at *m/z* 421 for CBD that enables differentiation from Δ9-THC.
- Additional product ions are observed at *m/z* 353 and *m/z* 231 at higher collision energies (i.e., 35 eV) that provide further confirmation.

Table 1. Investigated Ag-ligand complexes.

Ag-Ligand Complexes	Ability to Differentiate Δ9-THC and CBD?
$[Ag(PPh_3)(OTf)]_2$	✓
$[Ag(PPh_3)_2(OTf)]_2$	✓
$[Ag(dppe)(OTf)]_2$	✓
$[Ag(dppp)(OTf)]_2$	X
$[Ag(2\text{-}pyr)(OTf)]_2$	X
$[Ag(dppm)(OTf)]_2$	X

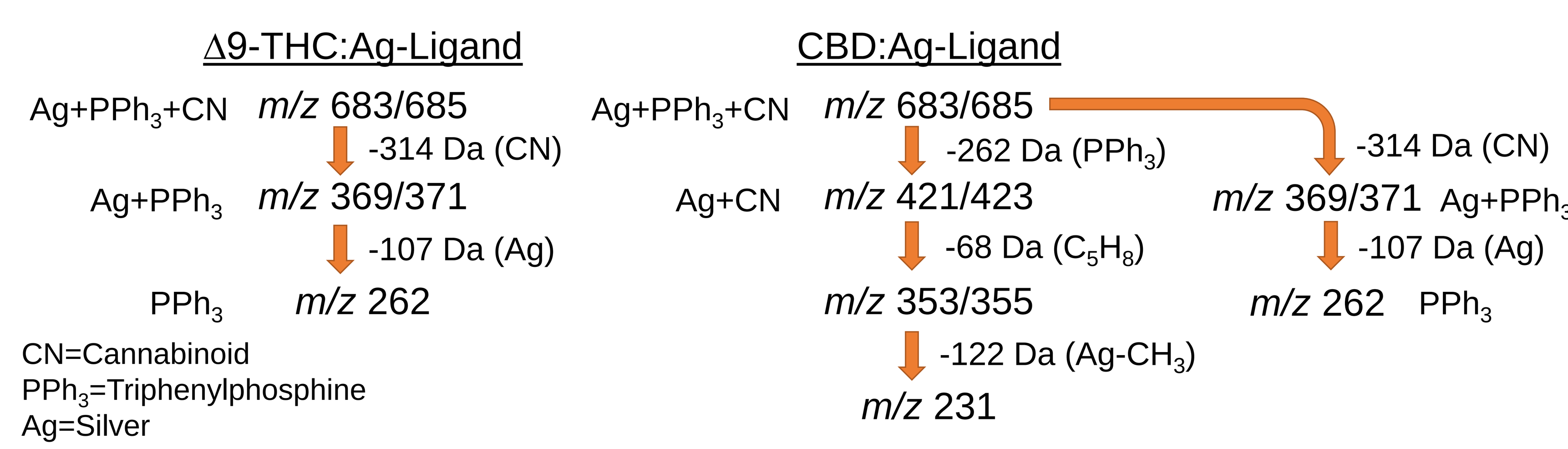

Figure 3. Proposed fragmentation pathways for cannabinoid:Ag-ligand ion complexes.

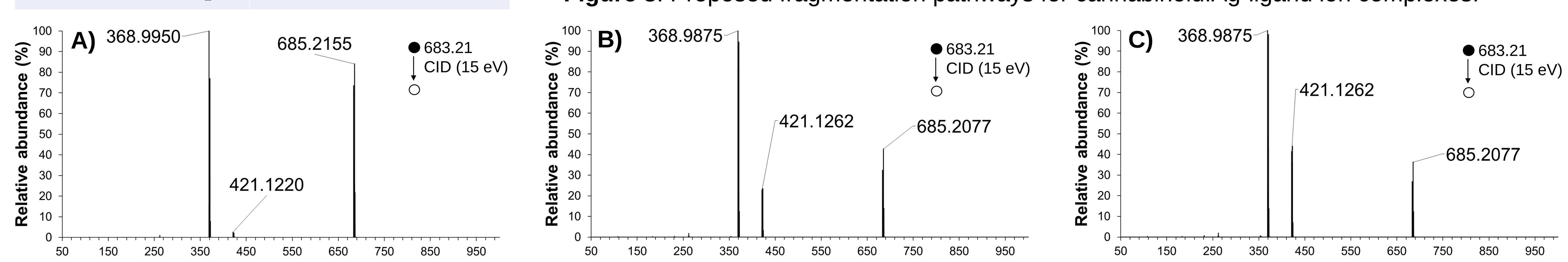

Figure 4. Comparison of MS/MS product ion spectra for varying ratios of Δ9-THC:CBD demonstrating the difference in abundance of the product ion at *m/z* 421 in A) Δ9-THC-rich cannabis (95:5), B) Δ9-THC-rich cannabis (50:50), and C) CBD-rich cannabis (5:95).

- The product ion at *m/z* 421 increases as a function of the amount of CBD present, even with excessive Δ9-THC:CBD ratios.

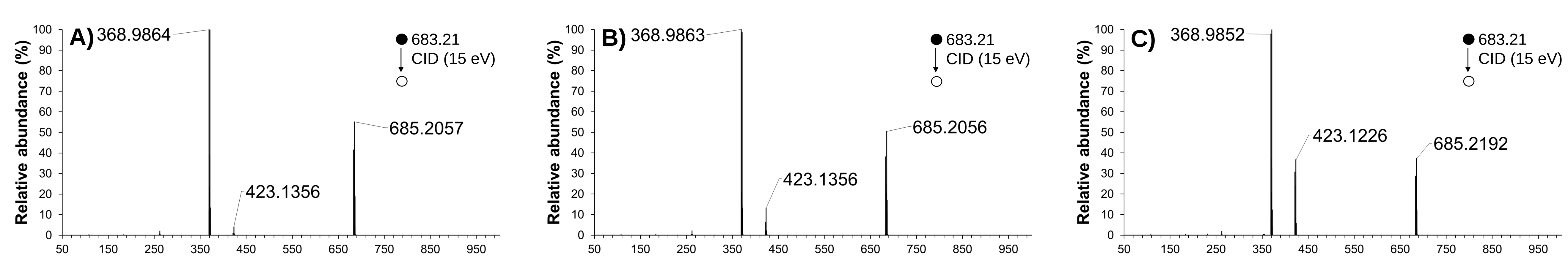

Figure 5. Comparison of MS/MS product ion spectra for authentic samples; A) marijuana, B) marijuana with increased CBD content, and C) hemp.

- This method enables the differentiation of Δ9-THC-rich cannabis and CBD-rich cannabis in authentic cannabis samples.

MATERIALS & METHODS

Sample Preparation

Δ9-THC and CBD were analyzed individually and as a mixture, both without the presence of Ag complexes and with pre-formed Ag-ligand complexes. The mixtures were composed of the cannabinoid at a concentration of 50 ppm and the Ag-ligand at a concentration of 225 µM. The Ag-ligand complexes were synthesized in simple 1-2 step reactions between silver salts and the desired ligand, followed by purification through recrystallization. The effectiveness of the differentiation of THC-rich and CBD-rich samples was performed with Δ9-THC to CBD in ratios of 95:5, 80:20, 65:35, 50:50, 35:65, 20:80, 5:95. Authentic samples were prepared by combining marijuana and hemp sample extracts of known concentrations with $[Ag(PPh_3)(OTf)]_2$.

Instrumentation and Data Analysis

An Agilent Technologies 6530 quadrupole time-of-flight (Q-TOF) mass spectrometer was used to analyze the pure cannabinoids and Ag-ligand ion cannabinoid complexes. MS/MS activation was performed with collision energies of 15 eV, 25 eV, 35 eV, and 45 eV for each precursor ion of interest. Resulting mass spectral data was extracted through MassHunter Qualitative Analysis version 10.0 and exported to Microsoft Excel. Spectral comparisons were used to identify unique product ions enabling the differentiation of Δ9-THC to CBD. MS/MS product ion spectra were used to understand the binding of the Ag-ligand to the target cannabinoid.

CONCLUSIONS

- Ag-ligand ion complexation can be used to differentiate Δ9-THC and CBD due to the difference in the binding affinity between the Ag-ligand and the cannabinoids.
- $[Ag(PPh_3)(OTf)]_2$ was determined to be the optimal Ag-ligand for the differentiation of Δ9-THC to CBD.
- With the addition of the ligand, there is enhanced selectivity, solubility, and stability compared to the use of simple silver salts.
- The developed method enables the differentiation of Δ9-THC-rich and CBD-rich cannabis.

REFERENCES

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ACKNOWLEDGEMENTS

This project was made possible by funding received from the Sam Houston State University Office of Research and Sponsored Programs.