Weeding Out the Methods to Enable Rapid Differentiation of Fiber-type and Drug-type Varieties of *Cannabis sativa*: A Combined Ambient Ionization Mass Spectrometric and Chemometric Approach

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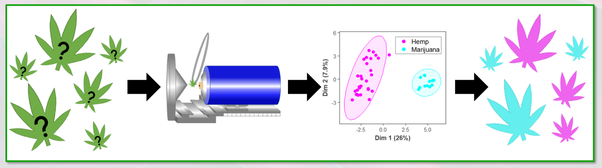
Introduction

- One of the challenges to emerge for U.S. crime laboratories in recent years results from the increased legalization and decriminalization of marijuana at the state level, and the permitted production of hemp
- The 2019 NIJ "Report to Congress: Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices" identified this area as requiring focused attention to improve criminal justice practices in the U.S.
- The challenges that hemp and marijuana present are as follows:
  - Both are varieties of the same species, *Cannabis sativa*
  - While they each contain Δ^9-tetrahydrocannabinol (THC), they differ in the amount of this molecule that is present
  - Current federal guidelines stipulate that *C. sativa* with >0.3% THC is marijuana (a schedule I controlled substance), while plant material that contains ≤0.3% THC is hemp (a legal agricultural commodity)
- Current ID methods and their disadvantages include:
  1) Color tests
     - Presumptive results
     - False positives
  2) Chromatography-based methods
     - Extended run times to separate cannabinoids
     - Chemical derivatization steps
- Proposed approach: Combined ambient ionization mass spectrometry with advanced chemometrics to differentiate between hemp and marijuana

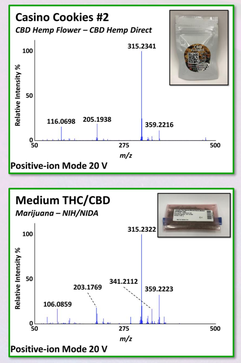


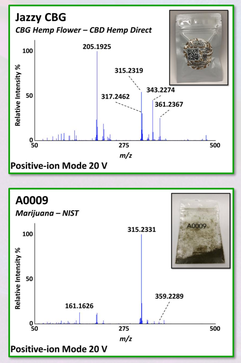
DART-HRMS

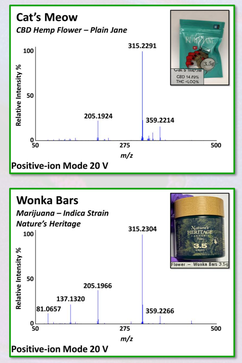
- Direct Analysis in Real Time – High-Resolution Mass Spectrometry
- All *C. sativa* plant materials were analyzed by DART-HRMS in positive-ion mode at 350 °C and an orifice 1 voltage of 20 V
- A capillary tube sampling technique was used for all samples
- Training Set – Model Development:
  - 29 commercial hemp strains
  - 12 marijuana samples from DEA-registered suppliers (8 NIST; 4 NIDA)
  - 10 recreational marijuana strains
- Test Set – External Validation:
  - 12 commercial hemp strains
  - 11 recreational marijuana strains

Sample Sets

DART-HRMS Analysis

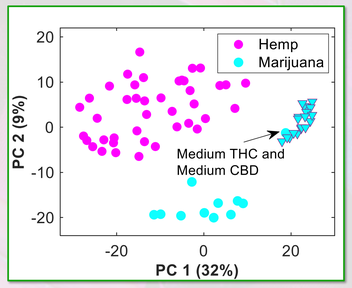


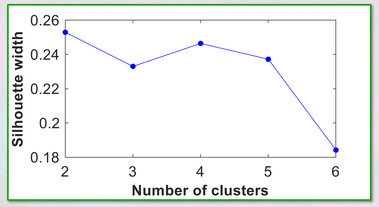


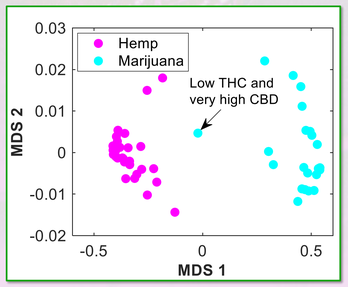


Model Development

Recreational Marijuana Commercial Hemp DEA-registered Marijuana





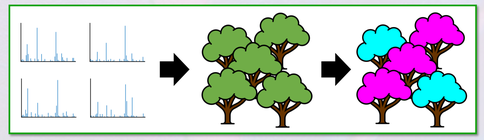


Method Validation

	Out-of-Bag Samples			External Validation Samples		
	Accuracy: 0.98 (98%)			Accuracy: 1.00 (100%)		
	Sensitivity	Specificity	Precision	Sensitivity	Specificity	Precision
Hemp	1.00	0.96	0.97	1.00	1.00	1.00
Marijuana	0.96	1.00	1.00	1.00	1.00	1.00

Model Development

- A 2D score plot was developed from principal component analysis (PCA) of the DART mass spectra of hemp and marijuana samples
- The optimal number of clusters was estimated by computing the average silhouette width
  - This measures the quality of the clustering
- The optimum number of clusters is the number with the maximum average silhouette width, which was determined to be 2
- Random forest: supervised machine learning method consisting of individual tree predictors
- The samples not included in the replicates for a given tree are "out-of-bag", and are used to validate the model
  - Optimal number of variables (*m/z* values): 20
  - Optimal number of trees: 500
- Each tree makes a prediction. The predictions are then tallied to determine the majority vote
- Performance results are reported for the random forest model in prediction of "out-of-bag" and external validation samples



Conclusions

- A combined ambient ionization mass spectrometric and chemometric approach was successfully developed and used to create a prediction model for rapid, high-accuracy differentiation of *C. sativa* hemp and marijuana plant materials
- This method circumvents extensive sample preparation steps, and it does not require chromatography to separate/differentiate cannabinoids for quantifying THC content
- Future work includes identifying the *m/z* values determined to be diagnostic for optimal differentiation

Acknowledgements

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