Analysis of Major Cannabinoids using Raman Microscopy, Chemometrics, and a Novel Artificial Intelligence Approach

John Laetsch III, BS; Jose Grijalva, MS; Ting-Yu Huang, MS; Patrick Buzzini, PhD; Jorn Chi-Chung Yu, PhD, ABC-CC; J. Tyler Davidson, PhD; Geraldine Monjardez, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

ABSTRACT

The present study evaluates the ability of Raman microscopy to differentiate between cannabinoids by collecting high-quality spectra of seven cannabinoid analytical standards and classifying them using linear discriminant analysis (LDA) and a novel transfer learning approach. Raman microscopy enabled the determination of optimal sampling areas for acidic and nonacidic cannabinoids. A traditional chemometric approach using LDA resulted in a classification accuracy of 99.83%. For the transfer learning approach, Raman spectra were transformed into scalograms and introduced to transfer learning-based artificial intelligence (AI) models for sample classification. The convolutional neural network (CNN) achieved a classification accuracy of 100% for the test dataset.

INTRODUCTION

Recent discussion surrounding the legalization of cannabis in the United States has driven a growing interest in the detection of cannabinoids and the characterization of cannabis samples. In recent years, numerous studies have been reported regarding the development of cannabinoid prediction models using spectroscopic methods including Fourier transform infrared (FTIR) and Raman spectroscopy[1]. As computer science has advanced, supervised learning algorithms such as LDA and k-nearest neighbor (KNN) have routinely been applied to Raman spectra for classification and identification. However, the limitations of these models, including poor prediction performance when dealing with complex spectral data in certain circumstances, have led to the recent development of CNN approaches. Many CNN methods have been successfully applied to binary classification problems and the classification of several types of samples. The present study aims to expand on current research by visualizing and classifying Raman spectra of seven common cannabinoids using the traditional chemometric approaches of principal component analysis (PCA) and LDA, as well as a novel transfer learning approach[2].

MATERIALS & METHODS

Sample Preparation – Seven cannabinoids were purchased as 1 mg/mL certified reference materials from Cayman Chemical and stored at -20°C: Δ9-THC, CBD, CBG, CBN, and CBC were prepared in methanol, while THCA and CBDA were prepared in acetonitrile. 10 µL of each cannabinoid standard was pipetted onto an aluminum foil-covered microscope slide. The solvent was evaporated at room temperature, and the laser was focused on the optimal sampling area (**Figure 1**).

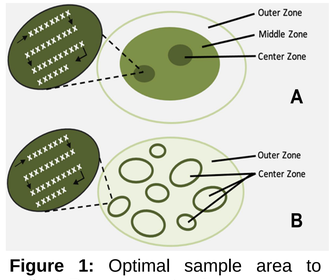

Figure 1: Optimal sample area to collect Raman spectra from nonacidic (A) and acidic (B) cannabinoids based on solvent drying pattern.

Instrumentation – A Renishaw inVia™ InSpect confocal Raman microscope was used to collect 34 replicate Raman spectra for each cannabinoid. Spectra were collected using a 785 nm laser, spectral range of 400-2200 cm^{-1}, exposure time of 10 seconds, 3 accumulations, and laser power of 100 mW. Raman spectra were extracted using WiRE 5.1 software and baseline corrected using Microsoft Excel.

Chemometrics – A visual assessment of the Raman data to determine potential groupings was carried out using PCA, followed by a supervised classification using LDA. Analyses were performed using R statistical software and the MASS package.

Artificial Intelligence – Each Raman spectrum was converted into a scalogram (**Figure 2**) using a continuous wavelet transform (CWT) filter bank**.** The scalograms were randomly divided into training and validation data and fed into a modified version of the CNN architecture GoogLeNet. An additional ten Raman spectra were collected for each cannabinoid and used as a test dataset for the AI model.

RESULTS & DISCUSSION

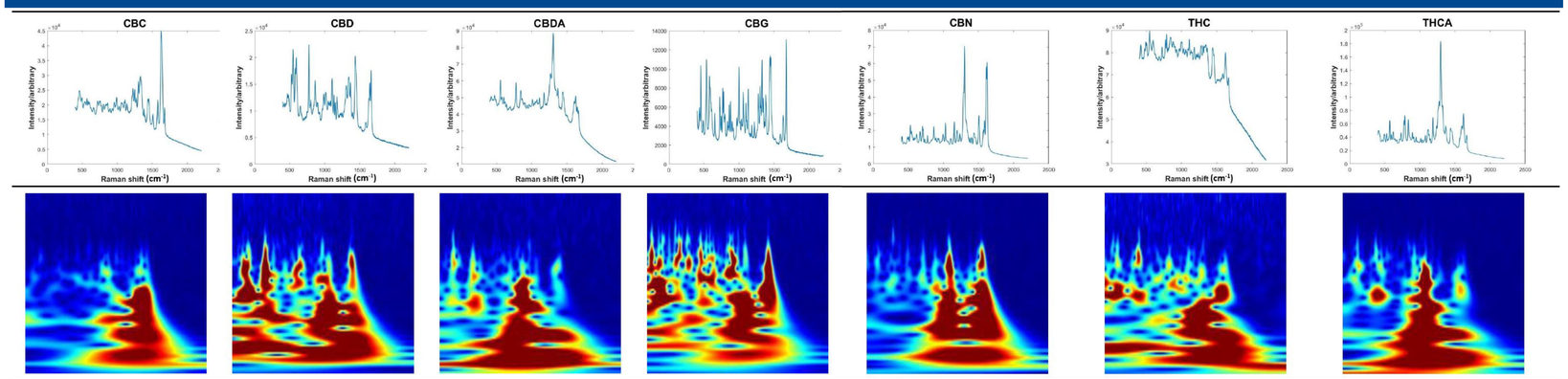

Figure 2: Examples of Raman spectra obtained for each cannabinoid and their corresponding scalograms.

- As seen in **Figure 3**, several identified Raman peaks were shared across all cannabinoids, generally associated with vibrations in the shared core structure.

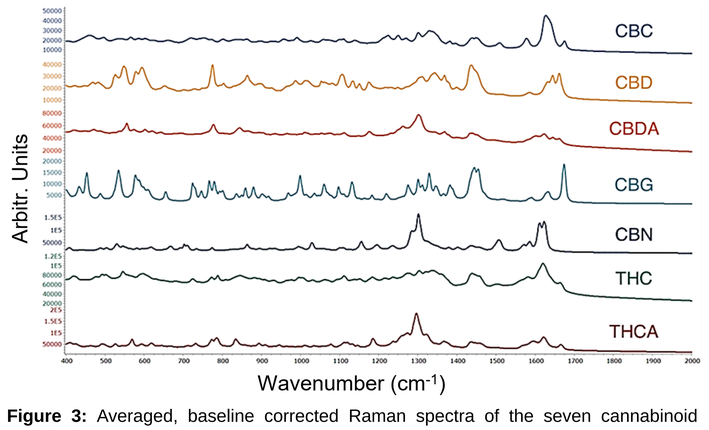

Figure 3: Averaged, baseline corrected Raman spectra of the seven cannabinoid standards.

- Observable differences in the spectra of each cannabinoid were noted, such as peak shifts, different peak shapes, and the presence of unique peaks.
- The PCA score plot of PC1 and PC2 (**Figure 4**) showed clustering for each cannabinoid, as well as separation between cannabinoids.
- Following a 5-fold cross-validation, the application of LDA yielded an accuracy level of 99.83%. Four Δ9-THC spectra were misclassified, while all other spectra were classified correctly.
- The training process for the CNN resulted in a validation accuracy of 100%. Average prediction probability for correct classification was calculated to be 99.31%. The CNN achieved a classification accuracy of 100% for the test dataset.

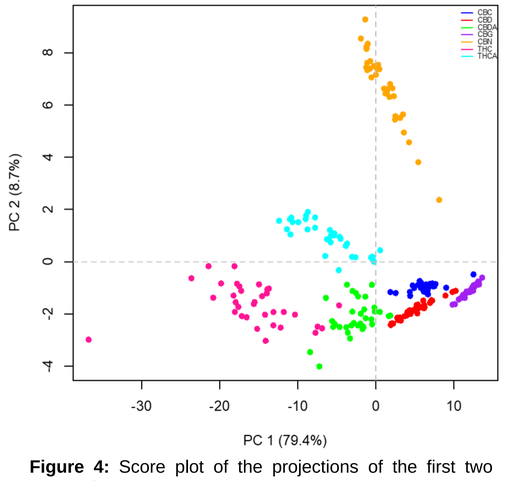

Figure 4: Score plot of the projections of the first two principal components of the Raman spectra of the seven cannabinoids evaluated in this study.

CONCLUSIONS

- Coupling Raman microscopy with chemometrics and machine learning enabled robust and reliable results to be obtained, with correct classification rates exceeding 99% for both approaches investigated in this study.
- A traditional data analysis approach based on PCA and LDA resulted in a high classification accuracy (99.83%), demonstrating its suitability for this purpose.
- The traditional chemometrics approach yielded a slightly lower performance than the AI approach in this study, highlighting the potential impact of CNN classification without the necessity for large-scale data collection.

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[2] Huang T.Y, Yu J.C.C. Development of Crime Scene Intelligence Using a Hand-Held Raman Spectrometer and Transfer Learning. *Analytical Chemistry*. **2021**. 93(25) 8889-8896.

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