Development of a Field Method for the Identification of the Hallucinogenic Herb *Salvia divinorum* using ATR-FTIR

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FSF Emerging Forensic Scientist Award Paper Presentation

Abstract

Salvia divinorum is a hallucinogenic plant that is increasing in popularity as a 'legal' alternative to marijuana. In response, however, thirty states have legislation concerning either *S. divinorum* or its psychoactive component, salvinorin A. Salvinorin A is unique to *S. divinorum*. Current analytical methods, including gas chromatography/mass spectrometry and thin layer chromatography, require the extraction of salvinorin A, and use it's presence to identify *S. divinorum* rather than identifying the plant. No presumptive or field tests exist to analyze *S. divinorum*. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) has been shown to be able to distinguish between species of plant genera, and so is being explored as a possible field test for *S. divinorum* that would not require sample preparation.

Introduction

Salvia divinorum is a hallucinogenic plant that has recently increased in popularity as a 'legal' alternative to commonly used hallucinogens like marijuana. *S. divinorum* is the only hallucinogen of the Lamiaceae (mint) family, which contains more than 900 documented species. The short-lived 'high' experienced by users of *S. divinorum* is a result of salvinorin A, a compound unique to *S. divinorum*. In response to the increased recreational usage of *S. divinorum*, several countries and twenty-two states have banned either *S. divinorum* or salvinorin A[1]. Eight additional states have regulations in place for *S. divinorum* and/or salvinorin A[1].

Current analytical methods do not directly identify *S. divinorum*[2]. Instead, the salvinorin A is extracted from the plant material and is analyzed using either gas chromatography/mass spectrometry or thin layer chromatography[2]. No presumptive or field tests exist for the preliminary identification of *S. divinorum* prior to submission to the laboratory[2,3]. Once in the laboratory, there is currently no method for identifying *S. divinorum* without the extraction of salvinorin A.

Fourier transform infrared spectroscopy (FTIR) has been shown to be able to produce a 'biochemical' profile indicative of individual species of plants that is capable of definitively differentiate species within plant genera[4,5]. With the addition of an attenuated total reflectance (ATR) accessory, FTIR could be used to identify *S. divinorum* without any sample preparation. Portable ATR-FTIR units are available that would permit the identification of *S. divinorum* in the field.

Materials and Methods

Materials

Analysis was completed using:

- 13 *Salvia divinorum* samples
- 30 non-*divinorum* species of *Salvia*
- *Cannabis sativa L*
- 11 non-controlled herbs
- salvinorin A

Materials were obtained from CCNET, DOV Collection of Dried Botanical and Woods from the Herbarium collection at the Delaware State University, "eXperience" brand purchased from a local head shop in Huntington, WV, "Goddard Collection," San Bernardino County (CA) Sherriff's Department's Scientific Investigations Division, Gold of Sunshine Ethnobotanicals, culinary sage grown by JGR, "Private Selection" brand purchased from a local grocery store, Mazatec Garden, live plants purchased from World of Salvias (Candor, NC), and a collection of training samples used in the Forensic Science Program at Marshall University. The salvinorin A standard was a generous gift from Daniel Siebert of The *Salvia divinorum* Research and Information Center in June 2011.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

Nicolet Nexus 670 FTIR with a diamond ATR smart accessory:

- 128 scans
- 4 cm^{-1} resolution
- 4000-400 cm^{-1} range
- ATR correction

Smiths Detection Travel IR II (used with a subset of samples):

- 32 scans
- 4 cm^{-1} resolution
- 4000-650 cm^{-1} range
- ATR correction

Each sample of plant material was analyzed three or six times.

Leaves that were obtained fresh were analyzed fresh, air dried, and analyzed again to compare fresh and dried material.

OMNIC software was used to view the spectra.

Results

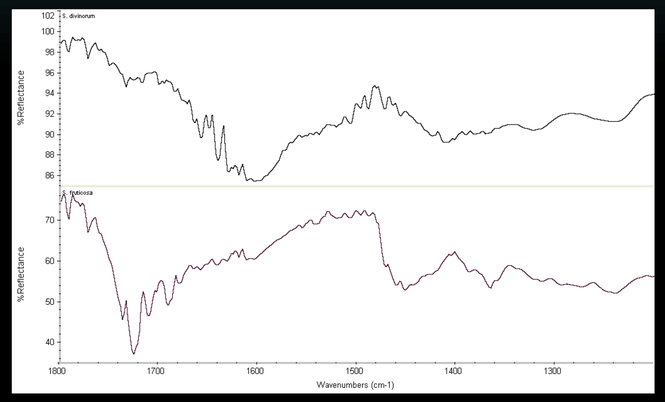

Figure 1: This is a comparison of the FTIR spectra from *S. divinorum* and *S. fruticosa* between 1800 cm^{-1} and 1200 cm^{-1}.

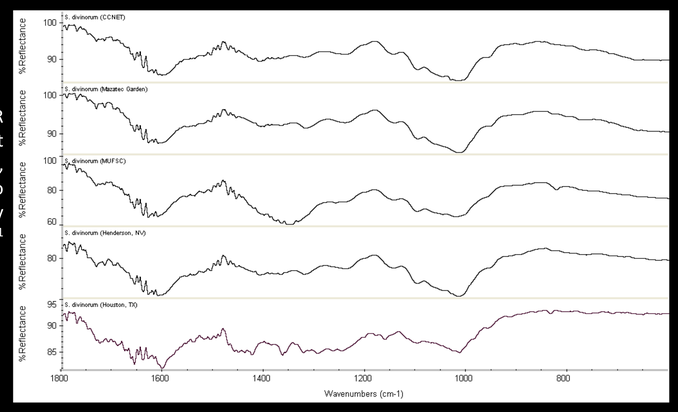

Figure 2: This is a comparison of the FTIR spectra from *S. divinorum* from five different sources (CCNET, MUFSC, Mazatec Garden, Henderson, NV, and Houston, TX) used to explore the reproducibility of this study within the same species between 1800 cm^{-1} and 600 cm^{-1}.

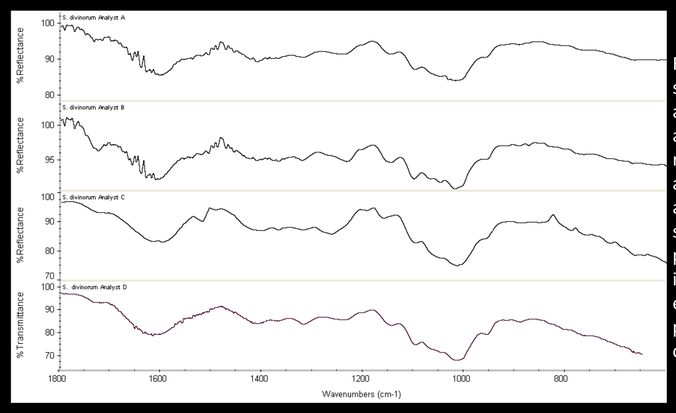

Figure 3: This is a comparison of the FTIR spectra from *S. divinorum* from CCNET, analyzed by four different analysts (listed alphabetically) used to explore the reproducibility of this study between analysts and instruments between 1800 cm^{-1} and 600 cm^{-1}. Analysts A and B used the same instrument with the same experiment program, while Analyst C used the same instrument with a slightly different experiment program. Analyst D used a portable FTIR with similar experiment conditions.

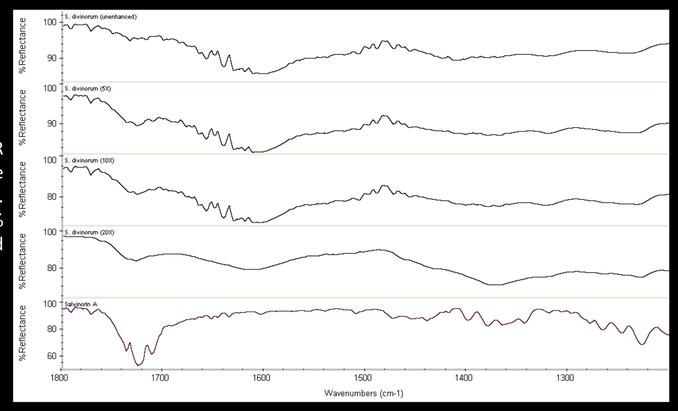

Figure 4: This is a comparison of the FTIR spectra from unenhanced *S. divinorum*, three purchased extract enhanced samples of S. divinorum of increasing potencies, and 98+% pure salvinorin A between 1800 cm^{-1} and 1200 cm^{-1}.

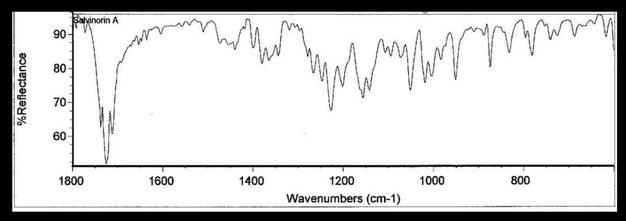

Figure 5: This is the FTIR spectrum of 98+% pure salvinorin A between 1800 cm^{-1} and 600 cm^{-1}.

Discussion

Forty-one species were able to be distinguished from *S. divinorum* visually using the method described. Six species were distinguished from *S. divinorum* in the 1800 to 1200 cm^{-1} range, one species was distinguished using the 1200 to 600 cm^{-1} range, and thirty-four species were distinguished between 1800 and 600 cm^{-1}. The final eight species varied only slightly from *S. divinorum* visually. Multivariate analysis comparing *S. divinorum* to each of the other species shows promise for distinguishing the remaining visually similar spectra. Purchased extract enhanced *S. divinorum* samples showed a trend concerning the band located at approximately 1725 cm^{-1}. As the reported concentration of salvinorin A increased, the magnitude of the band at 1725 cm^{-1} also increased. This trend continued into the spectrum of salvinorin A.

The orientation of the leaf (top or bottom) was determined not to be a factor in the resulting spectra, and dried materials provided slightly more detailed spectra than fresh materials. These spectra were shown to be reproducible within individual plants, within species, and between analysts and instruments.

Figure 6: The *Salvia divinorum* plant living in the forensic chemistry lab at Marshall University.

Conclusion

S. divinorum could be differentiated from forty-one of the forty-nine other species analyzed using ATR-FTIR. The band located at 1725 cm^{-1} in unenhanced *S. divinorum* appears to increase in magnitude with increased potency of salvinorin A in extract enhanced *S. divinorum*. ATR-FTIR is close to becoming a viable technique for the field identification of *S. divinorum*.

Continuing Work

Multivariate analysis of the eight species that were not visually differentiated from *S. divinorum* shows promise in distinguishing each of these species. More species of *Salvia* and non-*Salvia* are continually being analyzed and differentiated from *S. divinorum*. A manuscript of this work is expected to be submitted for publication within 90 days.

Acknowledgements

The authors would like to thank Hiram Evans for providing samples from the Goddard Collection maintained by the San Bernardino County (CA) Sherriff's Department's Scientific Investigations Division, Daniel Siebert of The *Salvia divinorum* Research and Information Center for his generous gift of salvinorin A for analysis, and Linda Black and Amanda Heeren for their assistance in analyzing samples as part of the reproducibility study. RM would also like to thank Marshall University Forensic Science Program for summer support.

References

1. Siebert D. (June 26, 2011). The legal status of *Salvia divinorum*. *Sagewisdom.org*. retrieved July 12, 2011, - http://www.sagewisdom.org/legalstatus.html.
2. Jermain JD, Evans HK, Analyzing *Salvia divinorum* and its active ingredient salvinorin A utilizing thin layer chromatography and gas chromatography/mass spectrometry. J For Sci, 2009; 54(3): 612-616.
3. Tozier LR, Evaluation of commercially available presumptive drug testing kits for the forensic analysis of *Salvia divinorum*. Master's thesis for Boston University, 2010.
4. Kim SW, Ban SH, Chung H, Cho S, Chung HJ, Choi PS, Yoo OJ, Liu JR, Taxonomic discrimination of flowering plants by multivariate analysis of Fourier transform infrared spectroscopy data. *Plant Cell Rep*, 2004; 23: 246-250.
5. Holt C, Hirst D, Sutherland A, MacDonald F, Discrimination of species in the genus *Listeria* by Fourier transform infrared spectroscopy and canonical variate analysis. *Applied and Environmental Microbiology*, 1995; 61(1): 377-378.