A84 Development of a Chemical Fingerprint for *Salvia Divinorum* Using Liquid Chromatography-Mass Spectrometry for Association and Discrimination from Related Salvia Species

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After attending this presentation, attendees will learn how *Salvia divinorum* can be differentiated from related *Salvia* species using liquid chromatography-mass spectrometry (LC-MS) and multivariate statistical procedures. Differentiation of *S. divinorum* from other *Salvia* species will be demonstrated based on their non-volatile compounds, using principal components analysis (PCA) and hierarchical cluster analysis (HCA).

This presentation will impact the forensic science community by developing a LC-MS method for the analysis of non-volatile compounds in *S. divinorum*. Most forensic laboratories analyze *S. divinorum* submissions using gas chromatography-mass spectrometry (GC-MS); however, GC-MS is limited to the analysis of volatile compounds. Analysis of non-volatile polar compounds using LC-MS potentially offers more discriminating information to differentiate *S. divinorum* from other *Salvia* species.

Salvia divinorum is a hallucinogenic plant that has recently gained legislative attention due to an increase in its recreational use. *S. divinorum* and/or its active constituent, salvinorin A, are currently regulated in several states. Hence, the ability to identify *S. divinorum* and differentiate it from more than 900 other *Salvia* species is imperative in a forensic context. Salvinorin A is known to be found only in *S. divinorum* thus far. Therefore, the presence of salvinorin A, detected using GC-MS, serves as the current method for identifying *S. divinorum.* However, salvinorin A can be extracted from *S. divinorum* to prepare liquid extracts for recreational use. This can result in low levels of salvinorin A in the residual plant material that may or may not be detectable, causing possible difficulty with identification. Hence, an alternative procedure for identifying *S. divinorum* would be beneficial. Using LC-MS, non- volatile polar compounds in plant material can be determined, generating a chemical "fingerprint" of *S. divinorum*. This fingerprint should be unique and complementary to that already available by using GC-MS.

The purpose of this research was to generate a chemical fingerprint of *S. divinorum* using LC-MS. This chemical fingerprint was then used to investigate differentiation of *S. divinorum* from other related *Salvia* species based on the non-volatile polar compounds. *S. divinorum* was extracted in triplicate using different solvents (e.g., acetonitrile, acetonitrile/water, and acetonitrile/water/isopropanol) and all extracts were analyzed by LC-MS. The optimal extraction solvent was determined based on the number of compounds extracted and the precision of the extraction, which was determined using Pearson product moment correlation (PPMC) coefficients. Using the optimal solvent, an additional four *Salvia* species (e.g., *S. guaranitica, S. nemorosa, S. officinalis,* and *S. splendens*) were then extracted in triplicate and analyzed by LC-MS. Resulting chromatograms were subjected to data pretreatment procedures (e.g., retention time alignment and normalization) to limit any sources of non-chemical variance. Principal component analysis was then used to visually associate and discriminate extracts. Extracts that were chemically similar, such as replicates of the same species, were clustered closely in the scores plot and separately from those extracts that were chemically different. In addition, the chemical compounds contributing to the variance described by the principal components were identified in the loadings plot. Hierarchical

cluster analysis (HCA) was performed based on the scores plot and used to statistically measure the extent of association and discrimination of the different *Salvia* species. The combination of PCA and HCA results in a statistical evaluation of association and discrimination, in accordance with recommendations put forward in the recent National Academy of Sciences Report, "*Strengthening Forensic Science in the United States: A Path Forward*."

***Salvia Divinorum*, Liquid Chromatography-Mass Spectrometry, Multivariate Statistical Procedures**

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