A179 Objective Discrimination of *Salvia divinorum* From Related Salvia Species Using Chromatographic Techniques and Multivariate Statistical Procedures

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The goal of this presentation is to demonstrate statistical differentiation of the hallucinogenic plant, *Salvia divinorum*, from related Salvia species based on chemical profiles obtained using both gas chromatography-mass spectrometry (GC/MS), and liquid chromatography-mass spectrometry (LC/MS).

This presentation will impact the forensic science community by providing an objective methodology for the differentiation of S. divinorum from four related Salvia species using a combination of chromatographic techniques and multivariate statistical procedures. The application of such statistical procedures directly addresses one of the recommendations highlighted in the 2009 *National Academy of Sciences Report*; that is, the need for statistical evaluation of forensic comparisons.

Salvia divinorum is a hallucinogenic herb that is currently regulated in 25 states in the United States. Although there are over 900 Salvia species, *S. divinorum* is the only one known to contain the hallucinogen, salvinorin A. As such, forensic identification of *S. divinorum* currently relies on the extraction and identification of salvinorin A in the plant, typically using GC/MS. Despite being more widely available in forensic laboratories, GC/MS is limited to the analysis of sufficiently volatile compounds that do not thermally degrade at the high operating temperatures used during analysis. In contrast, LC/MS is more suited to the analysis of non-volatile compounds and hence, may offer additional discriminatory information.

In this research, five different Salvia species were investigated: *S. divinorum*, *S. guaranitica*, *S. nemorosa*, *S. splendens*, and *S. officinalis*. Dried leaves from each species were extracted in triplicate for 16 hours, using a rotary agitation procedure. For GC/MS, the extraction solvent was dichloromethane while, for LC/MS, acetonitrile was used. All extracts were analyzed in triplicate using the appropriate chromatographic technique and, following analysis, two separate data sets were formed: one containing total ion chromatograms (TICs) obtained from GC/MS and the second containing TICs from LC/MS. Compounds present in extracts of each species were identified through mass spectral interpretation.

Prior to data analysis, TICs in each data set were subjected to various preprocessing procedures to remove instrumental sources of variance. This is necessary to ensure that subsequent data analysis procedures do not identify these non-chemical sources of variance as differences within the data set. Various preprocessing procedures were investigated for each data set, including background correction, smoothing, retention time alignment, and normalization. Background correction was used to minimize differences in background signal, while smoothing was used to minimize random variations in noise. Chromatograms were then retention time aligned, to account for retention time drift during analyses, and normalized, to account for variation in injection volume.

The pretreated data sets were then separately subjected to principal components analysis (PCA) to investigate differentiation of *S. divinorum* from the other four Salvia species based on the chemical profiles. Principal components analysis is an unsupervised statistical procedure that identifies groupings of samples within a data set. The procedure is termed "unsupervised" since no prior knowledge of groupings within the data set is required. The two outputs from PCA are a scores plot and loadings plots. The former is a scatter plot in which samples that are chemically similar are clustered closely, while chemically different samples are distinct. For both GC/MS and LC/MS analysis, *S. divinorum* was differentiated from the other four Salvia species in the corresponding scores plots. Student's t-tests were then used to statistically assess the positioning of the samples in the scores plots. Additionally, the PCA loadings plots were used to identify those compounds contributing most to the variance among the Salvia species. These compounds have potential use as chemical markers for the discrimination of Salvia species.

Using a combination of statistical procedures, discrimination of *S. divinorum* from four related Salvia species was demonstrated based on profiles of both the volatile compounds, obtained by GC/MS, and the non-volatile compounds, obtained by LC/MS.

***Salvia Divinorum*, Chromatographic Techniques, Multivariate Statistical Procedures**

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