B130 Pepper Plants and Magic Mint—The Application of Ambient Mass Spectral Analysis for the Rapid Detection and Quantification of Psychoactive Compounds in the Complex Matrices of Plant-Based Legal-High Substances

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Learning Overview: After attending this presentation, attendees will better understand how mass spectrometric techniques can be applied to the identification of plant-based legal-high substances and the quantification of the psychoactive compounds in complex plant matrices.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a protocol that will enable law enforcement and forensic crime laboratories to rapidly detect and quantify plant-based legal-high substances that are otherwise indistinguishable from plants that are non-psychoactive.

Piper methysticum and *Salvia divinorum*, commonly known as kava and salvia, respectively, are psychoactive plants identified by the United Nations Office on Drugs and Crime (UNODC) as two of the "20 plants of concern" containing psychoactive compounds. The non-characteristic appearance of these plants of abuse and their derived products make it difficult for law enforcement and forensic crime laboratories to distinguish them from plants that are not psychoactive or mind-altering. Current methods of analysis often include time-consuming sample preparation, lengthy run-times, overlapping retention times, and are heavy on the use of solvents. Although neither of these plants are federally classified as controlled substances, in states where they are scheduled or restricted, cases are rarely prosecuted because of the difficulties and challenges associated with proving the plant material to be what it is. The investigation presented here focused on resolving these problems with a method that circumvents the challenges associated with other methods, rapidly screens for unique chemical biomarkers of these plants, and quantifies their psychoactive constituents. Because of its unique attributes and features, the use of Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART®-HRMS) as the instrumental approach assists in the accomplishment these goals. DART®-HRMS is an ambient ionization technique that allows for the direct analysis of samples, rapid screening and identification of compounds, and semi-automated quantification with the use of a linear rail system to produce consistent and reproducible results.

Representative kava and salvia samples were used to determine if DART®-HRMS could rapidly detect the relevant analytes of interest in these plants. The six major kavalactones and three flavokavains were detected in the kava products, and the compound salvinorin A was detected in the salvia products. DART®-HRMS was then used to confirm that all 28 of these products originated from either kava or salvia plant material. Because yangonin and salvinorin A are the major psychoactive components of kava and salvia plants respectively, standard curves developed using calibrators and internal standards of either yangonin-d_3 or cholesterol were used to quantify their presence in the various products. The ratio between the protonated yangonin and yangonin-d_3 isotope peaks was preserved in all calibrators, as was the ratio between the salvinorin A $[M+H]^+$ and cholesterol $[M+H-H_2O]^+$ peaks, and could therefore be used to develop standard curves to confirm their concentration in quality control samples. With the success of three runs for each of the psychoactive chemical standards, all validated according to the Food and Drug Administration (FDA) guidelines, the protocols were considered acceptable for application to commercial products with unknown yangonin or salvinorin A content.

Eighteen kava products were purchased from eight vendors and consisted of powders, tinctures, capsules, roots, and pastes. Although two samples fell just below the validated curve range, the remaining 16 kava products contained yangonin concentrations that fell within the linear range of the standard curve. Tincture and liquid samples had yangonin concentrations that were between 1.03 and 4.59mg/mL, while the solid samples exhibited a range from 2.71 to 8.99mg/g. Eight salvia products were purchased from two vendors and included raw salvia leaf and enhanced leaf extracts of varying potencies. The salvia leaves had an average salvinorin A concentration of 1.54mg/g, while the enhanced leaf extracts had concentrations ranging from 13.54 to 53.24mg/g depending on the reported strength of the extract (ranging from 5x to 60x). In addition, the concentration of salvinorin A in the leaves and extracts were relatively consistent with previously reported salvinorin A concentrations in *S. divinorum* products.

These results demonstrate the development of a protocol that can rapidly detect plant-based legal-high substances in a variety of complex plant matrices, in addition to enabling the quantification of the psychoactive components in the various sample types, and it circumvents many of the problems encountered during analysis of these plants by conventional methods.

Psychoactive Substances, Plant-Based Legal-High Substances, Mass Spectrometry

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