K32 Quantitative Analysis of Salvinorin A: (Salvia) in Blood

Lyndsi J. Ayers, MS, Sam Houston State University, 1003 Bowers Boulevard, Huntsville, TX 77341; and Sarah Kerrigan, PhD, Sam Houston State University Regional Crime Laboratory, 8301 New Trails Drive, Suite 125, The Woodlands, TX 77341*

After attending this presentation, attendees will be familiar with a technique for the extraction and quantification of Salvinorin A from blood specimens using solid phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS).

This presentation will impact the forensic science community by providing a new procedure for both qualitative and quantitative

determination of the potent hallucinogen, Salvinorin A, from whole blood samples using GC/MS.

Salvia divinorum is a naturally occurring herb found within the *Lamiaceae* (mint) family. Salvinorin A is the trans-neoclerodane diterpene contained within its leaves that produces the plant's psychotropic properties. The drug is a potent naturally occurring hallucinogen. The availability and psychotropic effects associated with Salvinorin A have led to an increase in its use within the past decade. Studies have shown a growing trend in the *Salvia divinorum*-related media and internet traffic, as well as the use of the drug in persons age
12 or older. Salvinorin A is listed on the United States Drug Enforcement Administration's Drugs and Chemicals of Concern List but is not currently scheduled under the Federal Controlled Substances Act. Many states and other countries have already scheduled the drug and some are currently in the process.

Despite growing concerns regarding the recreational use of *Salvia divinorum,* published scientific literature describing Salvinorin A identification in toxicological specimens is very limited. Liquid chromatography/mass spectrometry (LC/MS) and related techniques have been reported. The objective for this study was to develop and validate a method for qualitative and quantitative identification of Salvinorin A in whole blood using a technique that was universally available in forensic toxicology laboratories (i.e. GC/MS). The development of the procedure evaluated potential protein precipitants, wash solvents, and elution solvents. The assay involves protein precipitation with 0.2 M zinc sulfate/methanol (20/80, v/v), mixed-mode solid phase extraction, a double wash step using hexane followed by hexane/dichloromethane (90/10, (v/v) and dichloromethane/ethyl acetate (80/20, v/v) as the elution solvent. Testosterone-d3 was used as the internal standard, and quantification was performed in selective ion monitoring mode. The ions selected were m/z 432, 273, and 94 for Salvinorin A and m/z 291, 249, and 124 for testosterone-d3 (quantitation ions underlined). The total run time was 23 minutes and the retention times for Salvinorin A and testosterone-d3 were 10.655 and 5.479, respectively.

The optimized GC/MS assay was evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, analytical recovery, linearity, interference, and carryover. The LOD and LOQ for the assay were determined to be 2 ng/mL. Precision and accuracy were evaluated at 15 and 150 ng/mL in blood. Both intra- and inter-assay CVs were in the range 4.5 - 7.7%. The 95% confidence intervals (95% CI) at 50 and 1000 ng/mL were 55.8 ± 5.3 and 1059.2 ±
43.5 ng/mL, respectively. Accuracy determined over a range of concentrations was 87-104% and analytical recovery of Salvinorin A was 88%. Calibrations were linear at concentrations as high as 5,000 ng/mL (the highest concentration tested). Carryover was evident at 2,000 ng/mL but this greatly exceeds concentrations expected in blood samples of forensic interest, which are typically one hundred-fold lower. The interference study included 27 commonly encountered drugs of abuse in addition to other her structurally related Salvinorins and divinatorins. No interferences were present, either qualitatively or quantitatively. This presentation provides a reliable and effective method for the detection and analysis of salvinorin A in whole blood by GC/MS at low concentrations of forensic interest.

Salvinorin A, Gas Chromatography/Mass Spectrometry (GC/MS), Blood

 ** Presenting Author*