K31 Analysis and Stability Determination of Salvinorin A and B in Human Blood, Plasma, and Urine by Liquid Chromatography Tandem Mass Spectrometry

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After attending this presentation, attendees will be able to describe the origins of and effects associated with abuse of *Salvia divinorum*, optimum methods for its analysis by liquid chromatography/tandem mass spectrometry, and limitations on its analysis based on analyte stability.

This presentation will impact the forensic science community by identifying a novel analytical approach to detection of an emerging hallucinogenic drug of abuse.

Salvinorin-A is an hallucinogenic compound that has no approved medical use in the United States. It is a naturally occurring, non- nitrogenous kappa opioid receptor agonist, and is the active component of the plant, *Salvia divinorum*, belonging to the mint family. The leaves of the plant are typically dried, crushed, and smoked for their dissociative hallucinogenic effect. Plant concentrate or extract is also commercially available. Salvia is a potent hallucinogen with effects distinct from LSD, mescaline, and other hallucinogens. An effective dose in humans is reportedly in the 200 to 1,000 microgram range when smoked. Salvinorin A and Salvinorin B have both been identified in the leaf and leaf extract; however, Salvinorin B is present in much smaller amounts. The Salvinorin A and Salvinorin B contents have been determined to be in the range of 3.2–5.0/0.10–0.17 mcg/mg in the dried leaf products, and 4.1–38.9/0.26–2.42 in the "concentrated extract" products.

After smoking Salvia, subjects experience rapid onset of an intense hallucinatory dissociative effect, during which they cannot speak or recognize their surroundings, lose psychomotor coordination and are highly impaired. Acute symptoms resolve within 8 to 12 minutes; however, longer term and residual effects have not been studied.

A validated a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed for the identification and quantitation of Salvinorin A and B in human blood, plasma, and urine.

Salvinorin A and B were extracted from biological matrices treated and preserved with sodium fluoride by a single step liquid/liquid extraction. Salvinorin A was analyzed under positive mode ESI- LC/MS/MS and Salvinorin B was analyzed under negative mode ESI LC/MS/MS (ABI 5000 Tandem Mass Spectrometer, Shimadzu SIL 20A, HPLC). Ions monitored for Salvinorin A and its internal standard Salvinorin A-d3 are: m/z 433/373; 436/373. Ions monitored for Salvinorin B and its internal standard are: m/z 389/313; 391/359. HPLC conditions included 2% methanol in water gradient, vs water, at 1mL/min, on a Phenomenex Luna C8(2) 150cm column.

The linear range for this assay was established as 1–40 ng/mL for whole blood, plasma and urine. Response was linear, and the LLOQ was established at 1 ng/mL for both analytes. LLOD was approximately 0.25ng/mL. Within-run precision at the LLOQ was 3.2 % for Salvinorin A and 2.5% for Salvinorin B. The within-run accuracy was determined as 100±5% for both Salvinorin A and B.

Following development, the assay was validated according to laboratory procedure including assessment of inter- and intra- batch precision and accuracy, storage, extraction and autosampler stability, freeze thaw stability, dilution integrity, and recovery.

The stability experiments indicated that Salvinorin A and B in unpreserved urine were stable for 28 days refrigerated and frozen, Salvinorin A was stable for less than nine days at room temperature. Both compounds were unstable in sodium fluoride/potassium oxalate preserved whole blood at room temperature and refrigerated, being undetectable after one day. Samples that were preserved with sodium fluoride and EDTA and frozen, were stable for at least 28 days.

Challenges resulting from limited stability and likely low concentrations in human subjects make this a challenging assay for medico-legal applications and require the use of LC/MS/MS techniques. **Salvia Divinorum, Salvinorin A, LC/MS/MS**

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