K35 Sensitive Method For Detection of Cocaine and Metabolites by Liquid Chromatography Tandem Mass Spectroscopy (LC - MS/MS) in Urine

Loralie J. Langman, PhD, Hilton 761-B, 200 First Street, SW, Rochester, MN 55905; Matthew Bjergum, BSc, Mayo Clinic, Gu CL-052, Rochester, MN 55905; and Frank W. Crow, PhD, Mayo Clinic, 5-25 Hilton Building, 200 1st Street, Southwest, Rochester, MN 55905*

After attending this presentation, attendees will be able to understand the uses of an assay for cocaine and metabolites with a lower limit of detection.

This presentation will impact the forensic science community by describing a method with a low limit of detection for cocaine and metabolites that will improve the ability to investigate drug use patterns.

Cocaine (COC) is an alkaloid found in *Erythroxylon coca*, and is a potent CNS stimulant that results in a state of increased alertness and euphoria. Cocaine can block the reuptake of neurotransmitters norepinephrine, dopamine, and serotonin. COC is rapidly metabolized to major metabolites like benzoylecgonine (BE) which is further metabolized to minor metabolites like m-hydroxybenzoyleconine (HOBE) and Norcocaine (NC). Cocaethylene (CE) is formed by trans- esterification of cocaine with ethyl alcohol when used simultaneously. Anhydroecgonine methyl ester (AEME) is a unique metabolite of smoked cocaine, and anhydroecgonine ethyl ester (AEEE) has been identified in cocaine smokers who also use ethyl alcohol. A method has been developed for the detection of and quantiation of COC, HOBE, AEME, AEEE, NC, CE, and BE in urine at low concentrations.

Sixty-eight randomly selected previously confirmed BE positive by GC-Ms were collected from May 19th to 22nd and June 20th to 27th. Samples were extracted using SPE and 30uL of the reconstituted extract was injected. Chromatographic separation was achieved using a gradient consisting of Mobile phase A [20mM ammonium formate (pH = 2.7)] and Mobile phase B [50%/50% methanol/acetonitrile], and an XDB-C8, 1.8micron, 2.1x50mm column, with a flow rate of 270 ul/min. Concentrations were calculated by comparing the drug peak area with the internal standard peak-area. The ratio was plotted against a standard curve.

The assay displayed linearity from 1.0m- 1000 ng/mL for all analytes. Between-run CVs were <10% throughout the linear range. Of the 68 BE positive samples, 56 were positive for cocaine, 32 for AEME, 7 for AEEE, 64 for HOBE, 29 for NC, and 32 for CE at > 1.0 ng/mL.

Standard surveys to determine methods of cocaine use rely on individual responses to survey questions, and assumes that users responses are honest. We report here on a sensitive method to identify cocaine metabolites at concentrations as low as 1.0 ng/mL. Because of the unique derivation of some of the metabolites, the pattern of the metabolites makes it possible to determine the user's method of cocaine ingestion. This assay could be used to validate or challenge current survey techniques.

Cocaine, Cocaine Metabolites, LC-MS/MS

 ** Presenting Author*