K50 The Detection of Cocaine and Its Major Metabolites in Rodent Bone Following Outdoor Decomposition After Chronic Cocaine Administration Using 2D-Liquid Chromatography/Tandem Mass Spectrometry (2D-LC/MS/MS)

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After attending this presentation, attendees will better understand how to perform an extraction of a drug of abuse from bone in approximately one hour. Additionally, attendees will understand the background and utility of multidimensional chromatography.

This presentation will impact the forensic science community by showcasing a procedure offering quick sample preparation and short extraction time of a very complex matrix (bone), which can be applied to any laboratory using LC/MS/MS to analyze and quantify drugs of abuse in any matrix.

Objective: In the field of forensic toxicology, several challenges exist with quantification of cocaine and metabolites in postmortem samples. Cocaine can prove difficult to detect and quantify in blood, urine, and soft tissues following extensive decomposition. Alternative matrices, such as hair, nails, and bone, could prove useful in detecting chronic drug use in postmortem toxicology cases. Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes and the inability to detect an analyte at trace levels. Further, analysis of drugs in hard tissues, such as hair and bone, has only been attempted in recent years. Even fewer studies have investigated the detection of drugs following the decomposition of remains, specifically outdoor decomposition. The objective of this study was to develop a robust extraction and clean-up methodology with preceding homogenization to efficiently extract drugs from complex matrices, to reach a target Limit Of Detection (LOD), and to maintain instrumental performance.

Method: In this study, a method analyzing cocaine and its major metabolites benzoylecgonine and ecgonine methyl ester was developed. All rat specimens used for this study underwent 10-12 weeks of chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before euthanization. Average daily dosages for each rat fell within a range of 13mg/kg-19mg/kg. Fourteen cocaine-positive rats were placed outside and above ground in a gated facility for a period of 12 months. All recoverable pelt and skeletal samples were collected for testing. A second group consisting of 16 cocaine-positive rats was placed outside and above ground in a gated facility for one week. A group of four cocaine-positive rats were removed for testing on the second week and every week following. All recoverable skeletal samples were collected for testing. Drug-free control rat bones were also acquired by placing drug-free rats outdoors, above ground, until full decomposition occurred.

After homogenization of whole bones, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent, which yields two eluting fractions — one with neutral and acidic entities and a second one with basic analytes. The use of a 2D-LC/MS/MS technology eliminates the need for a lengthy evaporation step in the extraction method. The chosen 2D-LC/MS/MS used in this application was identified using a 6x6 automated

method development protocol. The manual extraction of the bone samples was completed in less than one hour. The analysis was performed using 100µL of the final organic solvent (MeOH) extracts.

Results: The Limit Of Quantitation (LOQ) for cocaine and its metabolites was measured at 100ng/g sample material. The response factor of analytes was high enough that the LOD was estimated at 10ng/g (10ppt).

Conclusion: The micro extraction protocol combined with multidimensional chromatography decreased sample preparation time without sacrificing the quality seen with current single-dimension chromatography techniques. The procedure developed in this study can be utilized on bone and completed in less than one hour before injection of 100µl final extract into the 2D/LC/MS/MS system.

LC/MS/MS, Multidimensional Chromatograph, Bone