Detection of Cocaine and Its Major Metabolites in Rodent Bone Following Outdoor Decomposition after Chronic Administration with 2D LC/MS/MS Technology

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INTRODUCTION

In the field of forensic toxicology, several challenges exist with quantification analysis of cocaine in post mortem samples, including its rapid half-life due to hydrolysis within hours of death and postmortem redistribution. Cocaine can prove difficult to quantify in blood, urine, and soft tissues and correlate findings with drug dosage before death. Alternative matrices, such as hair, nails, and bone, could prove useful in detecting chronic drug use in postmortem toxicity cases. If a human body has undergone decomposition and putrefaction, toxicology screening of soft tissue samples is difficult to accomplish as well.

Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes, and 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 detection of drugs following decomposition of remains, specifically outdoor decomposition. A robust extraction and cleanup method, in which a homogenization step proceeds, is required to efficiently extract drugs from complex matrices, reach a target limit of detection (LOD) and to maintain instrument performance. The use of advanced hyphenated instrument platforms, such as UPLC/MS/MS, has allowed analysts to detect trace levels of analytes. However, there is a delay in analysis due to the extensive and time-consuming sample preparation protocols required to reach sub ng/mL levels. Traditional solid phase extraction techniques used in most laboratories require a lengthy evaporation step, which can take hours. A micro extraction protocol combined with a multi-dimension chromatography can decrease sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques.

METHOD

All rat specimens used for this study underwent 10-12 week 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 being euthanized. Average daily dosages for each rat fell within a range of 13-19 mg/kg. Fourteen cocaine positive rats were placed outside and above ground in a gated facility for a period of 12 months. All recoverable pelts and skeletal samples were collected for testing. A second group consisting of 16 cocaine-positive rats were placed outside and above ground in a gated facility for 1 week. A group of 4 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 rats were also acquired by placing drug-free rats above ground, until full decomposition occurred.

The homogenization process started by measuring 0.5 g of bone sample followed by an addition of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact homogenator to a suspension with the use of 4 mL of acetonitrile in a 15 mL plastic tube.

RESULTS

CONCLUSIONS

1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
%
0
100
1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00
ppb
Innominate 3 – T2 years 0.052 ppb
Femur 19 – T2 years 0.137 ppb
Humerus + ulna 16 – T2 years 0.183 ppb

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