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 post-mortem toxicology cases. If a human body has undergone decomposition and putrefaction, toxicology screens of soft tissue samples are difficult to accomplish as well.

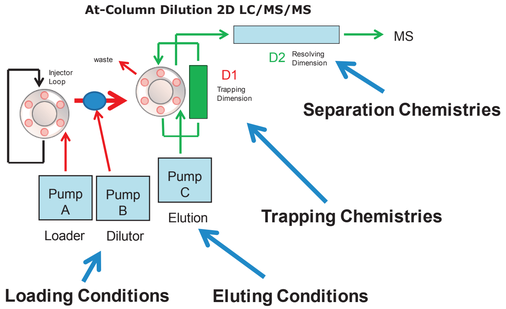
Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes, and inability to detect an analyte at trace levels. Further, analysis of drugs in hard tissues, such as hair and bone, has only been attempted in recent years. Even fewer studies have investigated detection of drugs following decomposition of remains, specifically outdoor decomposition. A robust extraction and clean up methodology, in which a homogenization step precedes, 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 instrumentation 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

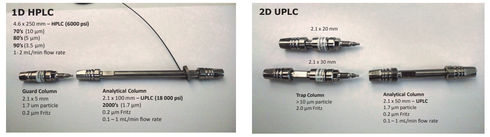
All rat specimens used for this study underwent 10-12 week chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before being euthanized. Average daily dosages for each rat fell within 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 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 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 rat bones were also acquired by placing drug-free rats outdoors, above ground, until full decomposition occurred.

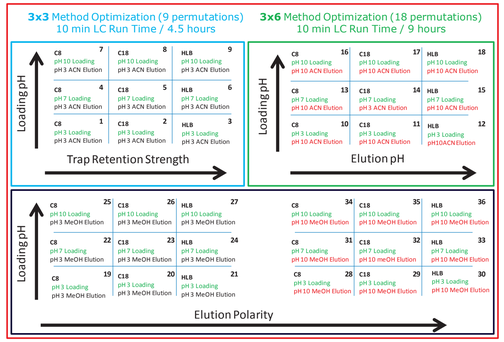
The homogenization process started by measuring 0.5 g of bone sample followed by an addition of 4 mL of acetonitrile in a 15 mL plastic tube. The bone sample was homogenized using a high speed impact process with ceramic ball bearings. The ACN homogenate was centrifuged at 4000 rpm for 5 min and the supernatant collected for further extraction. The solid homogenate was re-extracted with 4 mL of water and the aqueous supernatant was pooled with the previous method extract for the next step. With the ACN and aqueous supernatant pooled together was diluted in 100 ml of MilliQ water. The extraction process was performed on pre-conditioned mixed mode reversed-phase/ion exchange sorbent (6 cc Oasis™ MCX SPE barrel).

INSTRUMENTATION

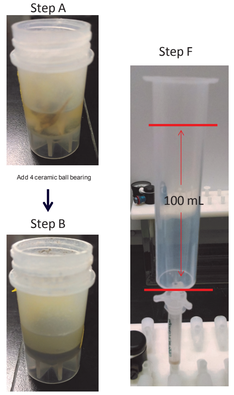


2D LC Column Hardware





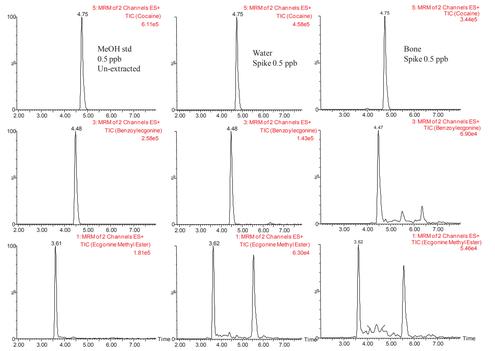
RESULTS

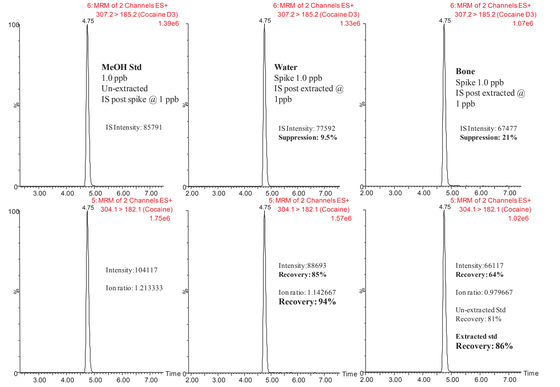


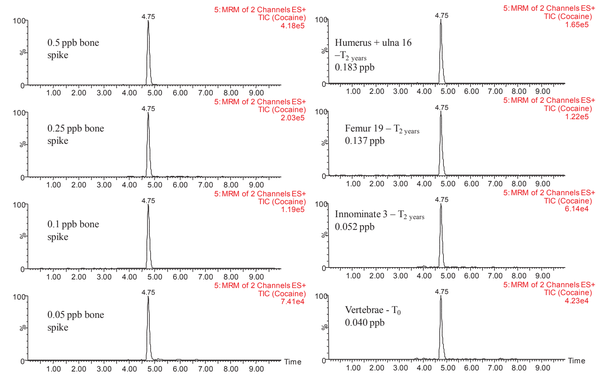
- **A** • Weight 1 g Bones • Add 4 mL ACN
- **B** • Homogenize 2x 90 sec • Centrifuge at 4000 RPM (5 min) • Collect Supernatant
- **C** • Filter Supernatant 4000 RPM (5 min)
- **D** • Repeat extraction with H_2O
- **E** • Pool ACN + Water supernatant
- **F** • Dilute with 100 mL MilliQ water • Load onto MCX 150 mg cartridge • Wash A: 5 mL 100 % Water + 0.1 N HCl • Wash B: 5 mL 100 % MeOH + 2 % FA
- **G** • Elute 2 mL MeOH 5% NH_4OH • add 20 ul IS deuterated Mix

Caution: Do Not Evaporate to Dryness

- **H** • Inject 100 µL







	Coc. ng/g	Benz. ng/g	Ecgo. ng/g
A	0.052	0.050	ND
B	0.137	0.050	ND
C	0.183	0.029	ND
D	0.016	TLD	ND
Ref	0.040	0.165	0.201

A: Innominate 3
B: Femur 19
C: Humerus + Ulna 16
D: Tibio-Fibula 10
Ref: Vertebrae T_0

CONCLUSIONS

- **0.998 R^2 Linearity from 10 to 0.05 ppb**
- **LOQ set at 50 ppt in bones**
- **LOD set at 100 ppt**
- **40 min extraction - No evaporation**
- **100 uL injection—clean MeOH extract**
- **90 sec homogenization process**
- **10 min LC/MS/MS analytical run**

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