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### **A Method for the Estimation of Drug Concentrations in Dried Bloodstains**

William A. Dunn<sup>1</sup>, Laura M. Labay<sup>2</sup> and Jonathan J. Lipman<sup>3</sup>

<sup>1</sup>Regional Medical Examiner Office, Newark, NJ, U.S.A.; <sup>2</sup>NMS Labs, Willow Grove, PA, U.S.A.; <sup>3</sup>Neuroscience Consulting Inc., Unicoi, TN, U.S.A. and East Tennessee State University, Psychiatry Dept, Johnson City, TN, USA

## **Abstract**

Dried blood is a commonly encountered specimen type in both the clinical and forensic laboratory. In the clinical environment dried blood is an ideal specimen choice due to the ease of its collection, transport and storage. It is used for several types of tests including screening newborns for the detection of certain inherited genetic disorders such as Hepatorenal tyrosinemia and Niemann-Pick, evaluating lipid levels (e.g., Gaucher disease), in drug discovery testing and assessing lead exposure. In the forensic environment, dried blood is most commonly used to determine serological markers such as blood grouping (ABO) and sub-grouping, enzyme polymorphisms and for DNA testing purposes. Less frequently, dried blood can be used for the detection of drugs and their metabolites, metals and anticoagulants. Most of these analyses are either qualitative or the final quantitative result is based upon an assumed volume of blood. Here we report an alternative method for the estimation of drug quantity by normalizing the concentration of drug and drug metabolite(s) to the amount of iron (Fe) found in the dried blood. We present a case to illustrate the use and application of this method. In our analytical protocol, two near-equivalent sections of material, one containing dried blood and the other appearing to be negative for blood, underwent a one-step extraction process with methanol. The methanolic extracts were screened by Enzyme Multiplied Immunoassay Technique (EMIT) and any confirmation work performed by Gas Chromatography-Mass Spectrometry (GC/MS). The iron concentrations in the sample and control areas were determined by performing an acid digestion on the material followed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Some of the assumptions that were made include the following: 1) The blood is from a single individual, 2) The drug and metabolites come only from the blood and not from local surface contamination or sweat, 3) The blood is uniformly distributed over the stained area, 4) Any background iron is uniformly distributed throughout the entire material, 5) The individual is of normal hematological status (i.e. neither anemic nor polycythemic), 6) The compounds of interest are stable in the dried blood matrix and will not appreciably change in identity (i.e. cocaine will not degrade to benzoylecgonine).

The amount of iron in the blood stained area, normalized to a gram of cloth, when corrected for background iron was 1177  $\mu\text{g}$  iron/g of cloth. Given that in healthy adult males the amount of iron in whole blood is in the range of 440 to 625 mg/L, then a blood stained cloth weighing 697 mg would have a blood volume between 1.31 to 1.87 mL.

The results of our analysis in this case were that the drugs in the blood shed in vivo onto the defendant's jeans contained (with the estimated concentration range): cocaine 364 ng (195 to 277 ng/mL of blood), cocaethylene 25 ng (13 to 19 ng/mL of blood) and benzoylecgonine 623 ng (334 to 474 ng/mL of blood).

## Case History

On the date of the homicide the defendant, RG, was a 30 year old white male with a lengthy history of alcohol and substance abuse. The offense occurred while RG was in a hotel room with a female who was offering to sell him crack. Believing that he was being cheated an altercation ensued (RG had barricaded the room, pushing the bed and the chest of drawers in front of the door) and the female – whom he said he thought was stunned or unconscious from the struggle - died of positional asphyxia. RG described his state as one of terror, shock and fear. The pounding on the hotel door caused him to call '911' for help but in a mounting state of panic he broke a window and escaped. He did not take the decedent's money, but fled into the night, cutting himself on the window glass during his exit. He eventually found security inside a large cardboard box. He was arrested, charged with murder, and under police pressure confessed to strangling the woman with his hands, although the autopsy later found she had not in fact been strangled. The decedent had not shed any blood at the scene. At the time of his arrest a lavender top tube was drawn, presumably for serological purposes.

In preparing for his criminal defense, RG's attorneys required a better understanding of his state of mind at the time of this victim's demise and consulted JJJ to perform a forensic neuropharmacological evaluation.

At the time of his forensic neuropharmacological evaluation, months after his arrest, the only remaining evidence of RG's drug use at the time of the offense were the lavender top tube of blood, his blood stained clothing and blood on the broken glass of the hotel room window. Approximately one and one half years after being drawn and collected the lavender top blood tube and blood stained clothing were submitted to National Medical Services (NMS Labs) for toxicological examination.

He was found guilty of second degree murder and is currently serving a 20 year sentence in prison.

## Materials And Methods

Samples submitted to National Medical Services (NMS Labs) were one lavender top tube and one pair of blue jean pants. It was noted that the pants were unclean and had a significant amount of dirt on them as well as being heavily blood stained. In addition there were many areas that appeared to be stained with an off white colored paint. Blood from the lavender top tube was analyzed for volatiles cocaine and cocaine metabolites using Headspace Gas Chromatography and Gas Chromatography-Mass Spectrometry (GC/MS), respectively, using previously published methods.

Drug screening and confirmation as well as iron determination on the clothing samples were analyzed as follows: two near equivalent sections of material, one containing dried blood and the other appearing to be negative for blood underwent a one-step extraction process with methanol for screening and any conformational procedures. Briefly, the control and blood stained areas were immersed in approximately 7 mL of HPLC grade methanol. The methanol and sample were sonicated for 1.5 hours then allowed to stand at room temperature for 2 days. The methanol was then transferred into a properly labeled test tube and taken to dryness at 40 °C under a gentle stream of air.

The methanolic extracts were reconstituted with 1.0 mL of EMIT™ buffer and screened by Enzyme Multiplied Immunoassay Technique (EMIT™). To the confirmation extracts the appropriate amount of internal standards were then added and the extracts' analyzed by Gas Chromatography-Mass Spectrometry (GC/MS).

The iron concentrations in the sample and control areas were determined by performing an acid digestion on the material followed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

## Results

Blood from the lavender top tube was negative for alcohols, cocaine, cocaethylene. Benzoylecgonine was detected at a level below our administrative cut-off of 50 ng/mL (~29 ng/mL).

After correcting for background iron in the control cloth sample, the amount of iron in the blood stained area was 1177 µg iron/g of cloth, normalized to a gram of cloth. Since it is known that in healthy adult males the amount of iron in whole blood is in the range of 440 to 625 mg/L (µg/mL), it follows that the blood stained cloth sample weighing 697 mg would contain iron corresponding to an original blood volume of between 1.31 to 1.87 mL (see equations below)..

$$\text{Blood Volume} = (\text{weight of cloth}) \times \left( \frac{\frac{\mu\text{g Fe on stained area}}{\text{gram of blood stained cloth}}}{\left( \frac{\mu\text{g Fe}}{\text{mL of whole Blood}} \right)} \right)$$

$$\text{Blood Volume} = (0.697) \times \left( \frac{1177}{(440)} \right) = 1.86 \text{ mL}$$

$$\text{Blood Volume} = (0.697) \times \left( \frac{1177}{(625)} \right) = 1.31 \text{ mL}$$

EMIT™ screening on the blood stained area was presumptively positive for cannabinoids and benzoylecgonine. All other EMIT™ tests were either negative or were inconclusive because of an interfering compound(s). The control area was presumptively positive for cannabinoids. Because of the circumstances of this case and the fact that cannabinoids cross reactives' were detected in both the blood stained and control areas, conformation testing for cannabinoids was not performed.

Cocaine, cocaethylene and benzoylecgonine were determined by Gas Chromatography-Mass Spectrometry (GC/MS) using appropriate standards and controls in a serum matrix. D3-cocaine was used as the internal standard for cocaine and cocaethylene, D3-benzoylecgonine was the internal standard for benzoylecgonine.

The results of our analysis in this case were that the drugs in the blood shed in vivo onto the defendant's jeans contained (with the estimated concentration range): cocaine 364 ng (195 to 277 ng/mL of blood), cocaethylene 25 ng (13 to 19 ng/mL of blood) and benzoylecgonine 623 ng (334 to 474 ng/mL of blood).

## Conclusions

1. The cocaine found in the bloodstain indicates that cocaine was present in the blood of the defendant on the night of the homicide.
2. Cocaethylene was detected in spite of the prolonged exposure to pure methanol. The presence of cocaethylene is indicative of ethanol also being present as well on the night in question; however the actual blood ethanol concentration cannot be calculated by this method.
3. The benzoylecgonine measured in the blood from the lavender top tube was significantly less than the benzoylecgonine calculated to be in the bloodstain. We believe this is due to the following reasons:

- a. There was a significant period of time (approximately 18 hours from his exiting the hotel room and having his blood drawn) where the cocaine, cocaethylene and benzoylecgonine were undergoing *in vivo* hydrolysis and elimination
  - b. There was a significant period of time (approximately 18 months) between the time of blood collection and the analysis for cocaine and metabolites during which time *in vitro* hydrolysis of the cocaine, cocaethylene and benzoylecgonine was occurring. In addition the blood was stored in a lavender top tube which uses EDTA as an anticoagulant but not sodium fluoride.
  - c. Upon drying further hydrolysis cannot occur.
4. The cocaine concentration is consistent with impairment and therefore highly likely to have contributed to the defendants' behavior on the night in question.
  5. Because the amount of drug measured is normalized to the amount of iron present (and therefore the volume of blood) the clothing does not have to be saturated.
  6. Using the assumptions listed previously, this method is a valid way of calculating the concentration of drugs and metabolites that were present on the bloodstream of the blood donor at the time of bleeding.

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