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### **K71 A Crazy Mini Heroin Epidemic in Richmond, Virginia**

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After attending this presentation, attendees will recognize the signs and symptoms of clenbuterol adulterated heroin and will be aware of the method development and validation of a method for the determination of clenbuterol in biological specimens.

This presentation will impact the forensic science community by presenting the clinical and potentially antemortem signs and symptoms of clenbuterol exposure.

Adulteration of illicit drugs like heroin is not an uncommon occurrence. This occurs to increase profit margin for the dealer, but users are then unaware of these products' purity or composition. Some adulterants may be relatively innocuous while others can result in moderate to severe toxicological reactions. Clenbuterol is a  $\beta_2$ -adrenergic agonist, but it acts like an anabolic steroid and causes muscle building. This provides clenbuterol with veterinary uses, but it is not United States Food and Drug Administration approved for human use; it is also a substance banned by the World Anti-Doping Agency and the International Olympic Committee. Clenbuterol has occasionally been reported as a heroin adulterant. This study describes a recent cluster of hospitalized patients with confirmed clenbuterol exposure resulting in serious clinical effects. Ten patients presented to emergency departments in the Richmond area over a ten-day period in the spring with unexpected symptoms shortly after heroin use. Heroin exposure was delivered by the following routes: five patients reported insufflation, three reported intravenous injection, and two patients were unaware.

The objective of this project was to develop a method for the qualification and quantification of clenbuterol in biological specimens.

Specimens were extracted using ISOLUTE<sup>®</sup> Supported Liquid Extraction (SLE) HXC columns. In brief, serum specimens were analyzed using a seven-point calibration curve ranging from 5ng/ml to 500ng/ml and quality control samples (5ng/ml, 15ng/ml, and 400ng/ml). Urine specimens were analyzed using a nine-point calibration curve ranging from 5ng/ml to 2,500ng/ml and quality control samples (5ng/ml, 15ng/ml, and 2,000 ng/ml). Clenbuterol and clenbuterol-d9 were extracted from the specimen using 0.5M ammonium hydroxide and ethyl acetate. The ethyl acetate was evaporated to dryness using a Biotage<sup>®</sup> TurboVap<sup>®</sup> 96 with nitrogen gas at 37°C. Analysis was performed using a Waters<sup>®</sup> ACQUITY<sup>®</sup> UPLC<sup>®</sup> with a TQD mass spectrometer, with positive Electrospray Ionization (ESI). The column was an ultra biphenyl 3 $\mu$ M, 2.1mm x 50 mm. The mobile phase was 10mM ammonium formate in water (A) and methanol (B) with a 95:5 to 5:95 gradient over three minutes.

Seven patients presented to the emergency department with their findings summarized. All patients were male with a median age of 40 years (range 28 years-46 years). Presenting symptoms included chest pain (6/7), dyspnea (5/7), palpitations (5/7), and nausea/vomiting (4/7). Troponin was positive in six patients at some point during their hospitalization. Three patients underwent cardiac catheterization; all revealed no significant coronary artery disease. Qualitative and quantitative clenbuterol concentrations were detected in the serum and urine of all seven patients. The results are as follows: serum median concentration 15ng/ml (range 6-38), urine median concentration 1,367ng/ml (range 13-3,389). The observed  $r^2$  values for the calibration curves were 0.99 or better. The limit of quantitation was administratively set at 5ng/ml for both serum and urine specimens. Validation criteria for calibrators and quality control specimens as well as carryover, matrix effect, precision, process efficiency, recovery, and specificity were acceptable.

The presence of drug adulterants in illicit drugs may result in atypical presentations of intoxication. Presentation of adrenergic symptoms and/or chest pain with hypokalemia, lactic acidosis, and hyperglycemia in heroin use suggests heroin adulteration with a beta agonist drug like clenbuterol. Clenbuterol adulteration of heroin can result in serious signs and symptoms and often requires hospitalization. A method is presented for the qualification and quantification of the clenbuterol in biological specimens.

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#### **Clenbuterol, Heroin, LC/MS/MS**