K50 Detection and Quantification of Low Levels of Benzoylecgonine in Equine Urine

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After attending this presentation, attendees will understand some principles of testing race horses for cocaine and other drugs.

This presentation will impact the forensic community and/or humanity by providing understanding of a problem of so-called low level cocaine concentrations in horse urine.

Cocaine (COC) is a local anesthetic and psychostimulant plant alkaloid widely abused by humans by IV injections, snorting or smoking. After administration, COC is quickly and completely metabolized and excreted in urine. Benzoylecgonine (BE) is a primary COC metabolite detected in human and equine urine. COC has no accepted therapeutic applications in equine veterinary practice. Many horse racing toxicology laboratories in the United States and in other countries occasionally detect low concentrations of BE (<150 ng/mL) in urine samples collected from winning animals. It is known that these very low BE concentrations in horse urine are a result of an accidental transfer of COC from humans or environmental contamination rather than premeditated administration to increase a horse's performance during the race. In response to the controversy of very low BE concentrations in urine, in February 2005 the Illinois Racing Board issued new rules establishing the threshold level of 150 ng/mL for BE in urine. According to the new rule, the first three positive BE laboratory reports below 150 ng/mL are accompanied by increasing fines ($250, $500, and $1000, respectively). The presence of BE in urine at a concentration equal to or higher than the threshold level is treated as a Class 1 drug as defined in the Association of Racing Commissioners International Uniform Classification Guidelines for Foreign Substances. Methods: A solid phase extraction method for extraction of BE from 2 mL of equine urine followed by EI-GC-MS analysis after derivatization with BSTFA with 1% TMCS was developed and validated. D_3-BE was used as an internal standard for quantitation of BE in equine urine samples. The following ions were monitored: for BE *m/z* 240 (used for quantitation), 256, 361, and D_3-BE *m/z* 243, 259, 364. The standard curve for BE in urine ranged from 5 – 300 ng/mL. In order to validate the method, two levels of controls prepared in naive horse urine were analyzed on different days (15 and 75 ng/mL).

Results: In this paper the results from analysis of horse urine samples collected at four race tracks in the Greater Chicago Area between July 1, 2004 and June 30, 2005 are presented. During that period of time a total of 15 samples (0.16%) were reported positive for BE, five collected from thoroughbred and ten from harness horses. Out of 15 samples, three were reported positive without BE quantification (July 2004 to February 2005) and none of the estimated concentrations exceeded 25 ng/mL. The concentrations of BE in the remaining 12 samples ranged from 5 – 57 ng/mL. The limit of quantitation for BE was 5 ng/mL and the limit of detection was 1 ng/mL. The intra-day accuracy and precision for the low control was 2.8% and 20.6%, respectively, and for the high control urine preparations 2.2% and –2.3%, respectively. The inter-day accuracy and precision for the low controls was 10.4% and 9.5%, respectively, and for the high controls was 6.8% and 2.9%, respectively.

Conclusions: None of the BE concentrations reached or exceeded the threshold level of 150 ng/mL. The authors then postulate that these low concentrations found in urine are most likely a result of the external contamination and not premeditated cocaine administrations to horses.

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