ELISA ASSAY FOR APOMORPHINE

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Apomorphine ((R)-5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo[de,g]quinolone-10,11-diol) is a dopamine agonist used as an emetic in dogs. In horses, the drug has a stimulant affect on locomotor activity. As such, apomorphine is classified as a Class 1 drug in the RCI Uniform Classification Guidelines for Foreign Substances (2001). The current methods for detection of apomorphine employ instrumental methods, but due to complexity and cost, these methods are unsuitable for high volume screening of samples. Methods employing thin layer chromatography have limited sensitivity and shorter duration of detections. Therefore, a user-friendly ELISA should prove a valuable screening method for the racing industry.

For this assay, an apomorphine-specific antisera was produced in rabbits by injecting a protein:hapten immunogen. The drug hapten was also conjugated to horseradish peroxidase to form an enzyme:drug conjugate. Antisera coated onto microwells were optimized with the enzyme conjugate using a TMB substrate system. Standard curves were generated by competing the conjugate with known levels of apomorphine in buffer. The I-50 (a measure of assay sensitivity) in buffer was optimized to 40 ng/mL.

Background studies using cleared equine and canine track samples revealed no interference with the assay.

Standard curves were generated using four common sample matrices (equine plasma, equine serum, equine urine and canine urine). The I-50 is 100 ng/mL in equine plasma, and the I-50 in equine serum is 136 ng/mL. The I-50 in equine urine is 35 ng/mL. The I-50 in canine urine is 40 ng/mL.

Cross-reactivates for several illegal drugs, therapeutic drugs, masking agents, vitamins, and drug vehicles were determined. Significant cross-reactivity was seen with apocodeine. Other morphine analogs demonstrated little cross reactivity.

Equine administration samples were collected from a Standardbred mare after a single 30 mg IV dose of apomorphine. The urine was collected up to 24 hours post administration. Apomorphine equivalents were detectable in the urine at a peak concentration by 1 hour post-administration and were still detectable at 8 hours post-administration. Urine samples had to be diluted 100 fold to allow for approximate concentration calculations, suggesting that the immunoassay is highly sensitive for apomorphine equivalents in equine urine.

A sensitive, specific ELISA assay has been developed for apomorphine. Little interference is seen from sample matrices.