K32 Detection of Ketamine in Urine of Nonhuman Primates After a Single Dose Using Microplate Enzyme-Linked ImmunoSorbent Assay (ELISA)

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After attending this presentation, attendees will understand the analysis of urine using newly developed ELISA screening technique for ketamine and its metabolites.

The general anesthetic ketamine (Ketalar®, Ketaject, Vetalar) (KET) is used in human and veterinary medicine for induction of anesthesia for short surgical procedures and routine veterinary examination. Its illicit use by teenagers in rave parties has been reported. It has recently been identified as a substance associated with sexual assault, socalled "date-rape" drug. Sexual predators use it for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. The objective of this study was to apply and evaluate a newly developed ELISA screening methodology for detection of KET and its metabolites in urine samples collected from five nonhuman primates which received a single dose of KET, and to study how long after drug administration, KET and its metabolites can be detected. The test kits and a microplate reader were kindly provided by the Neogen Corporation, Lexington, KY. The data are of great importance to law enforcement agencies and the forensic toxicology community in order to determine how long after sexual assault the urine samples can be collected from the victim to successfully prosecute the perpetrator. This study was reviewed and approved by the University of Illinois at Chicago Animal Care Committee. The aim of this study was: 1) to apply ELISA screening for detection of KET and its major metabolites directly in 20 µl of urine, 2) to increase a detectibility by extracting urine samples prior to ELISA screening, 3) to compare results from ELISA screening with previously obtained results of NCI-GC-MS analysis of urine samples for KET and its major metabolite norketamine (NKET).

Method: Urine was collected from five stumptail macaques (*Macaca arctoides*), four females (8-19 kg) and one male (17 kg) caged individually. All animals received a wash-out period of six months prior to the experiment. One urine sample was collected from each animal before KET administration. All monkeys received a single dose (5 mg/kg, I.M.) of KET. This dose represents an average I.M. dose in humans (3-8 mg/kg). Urine samples were collected from each animal for 18 hours every day (excluding weekends) up to 24 days then once every four days up to 35 days.

Urine screening procedure: The kit was first tested using urine spiked with KET or NKET at the following concentrations: 0, 5, 10, 25, 50, 75, 100, 500, 1000, 1500, and 2000 ng/ml. They were tested using the following procedure: 20µl of sample or control (positive or negative) was added to the appropriate well. 180 µl of a 1 to 180 dilution of enzyme conjugate in Neogen's EIA buffer was then added to each well. The plate was then covered with parafilm and incubated at room temperature for 45 minutes. Each well was then washed three times with 300 ?l of Neogen's wash buffer. 150 µl of Neogen's K-Blue Substrate was then added to each well and then incubated at room temperature for 30 minutes. The plate was gently shaken periodically throughout the incubation. To insure uniform color development. 50 µl of Neogen's Red Stop Solution was added to each well in order to stop the reaction. The plate was read at 650 nm. After the limitations of the kit and cross-reactivity with NKET were established, urine collected from monkeys dosed with ketamine was tested for the presence of KET.

Extraction: All urine samples (2 ml) were extracted from urine using HCX solid phase extraction (SPE) columns. To all control and study samples, 0.1 M acetate buffer (pH 4.5, 1 ml) and crude β-glucuronidase solution (50 µl) were added, and samples were incubated for 1.5 hours at 37^0C. After incubation 1.93 M acetic acid (1 ml) and deionized water (10 ml) were added. Each SPE column was conditioned with methanol (3 ml) deionized water (3 ml) and 1.93 M acetic acid (1 ml), the sample was added and the column was washed with deionized water (3 ml), 0.1 N HCl (1 ml) and methanol (3 ml). The final elution from the extraction column was achieved using methylene chloride:isopropanol:ammonia (78:20:2:, v/v/v, 3 ml). All extracts were evaporated to dryness in the stream of nitrogen, dissolved in 20 µl of the Neogen's buffer and transferred to microplates. They were treated as described above.

Results: KET and NKET were determined to be easily detectable at 25 ng/ ml. In one monkeys KET and its metabolites were detected in urine up to four days after drug administration, in two up to seven days, in one up to eleven days, and in one animal sixteen days after KET injection. Urine extraction followed by screening using ELISA methodology allowed for significant extension of the detection period in all animals from the study.

Date-Rape Drugs, Ketamine, ELISA

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