DEVELOPMENT OF A HIGH SENSITIVITY ELISA FOR THE MEASUREMENT OF NORKETAMINE.

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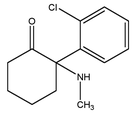
INTRODUCTION

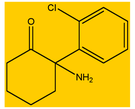
Ketamine is a dissociative anaesthetic agent used in human and veterinary medicine.[1,2] It became a drug of abuse shortly after it was introduced to clinical use and is becoming increasingly popular.[3,4] Ketamine is metabolized by the hepatic microsomal cytochrome P450 enzymes to two major metabolites, norketamine and dehydronorketamine.[5,6]

The use of immunoassays for the specific and sensitive detection of the major metabolite norketamine is advantageous for assessment of intake with applications in clinical, therapeutic and regulatory fields. To our knowledge this is the first report of the development of a high sensitivity ELISA for the measurement of norketamine, the major metabolite of ketamine. This represents an effective screening assay for monitoring ketamine abuse.

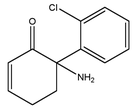
Chemical structures:



KETAMINE



NORKETAMINE



DEHYDRONORKETAMINE

METHODOLOGY

The immunoassay developed is a competitive ELISA. The highly specific capture monoclonal antibody (KT3459, Randox Laboratories, Crumlin, Northern Ireland) was immobilised and stabilised on the microtitre plate. After addition of 50 µl of calibrator/sample and 75 µl of horseradish peroxidase labelled conjugate, the microtitreplate was incubated for 1 hour at 25°C, followed by a washing step. Substrate was then added and after 20 minutes incubation at 25°C, the enzymatic reaction was stopped. Measurement was carried out by reading the absorbance at 450 nm, which was inversely proportional to the concentration of the analyte present in the calibrator/sample.

Assay evaluation parameters:

- The IC50 for each analyte was calculated by taking 50% of the optical density (OD) from the zero calibrator and reading this OD value from the x-axis (concentration in ng/ml) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.
- Specificity: the specificity, expressed as %cross-reactivity (%CR) was calculated as follows: %CR=[IC50(norketamine)/IC50 (cross-reactant)]x100
- Intra-assay precision was determined from the results of 12 replicates at different concentration levels within the same run. Results were expressed as %CV.

Ketamine detection in equine urine post-administration:

Ketamine was administered intravenously to a thoroughbred gelding (age: 17 years, weight: 535 kg) at a dosage of 0.22 mg/kg. 27 post-administration urine samples were collected at regular intervals over a period of 82 hours. Determination of ketamine and/or its metabolites was performed with this developed ELISA assay and other commercially available systems for comparison.

RESULTS

Results corresponding to the initial evaluation of the assay are presented.

IC50 and specificity

Analyte	Calibration range (ng/ml)	IC50 (ng/ml)	Specificity (% cross-reactivity)
Norketamine	0-300	3.3	100
Ketamine	0-2000	155.2	2.1
Dehydronorketamine	0-2000	90.5	3.6

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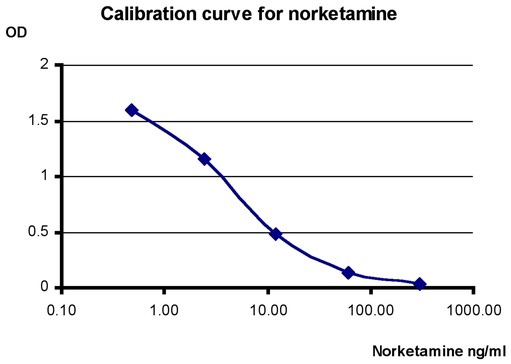
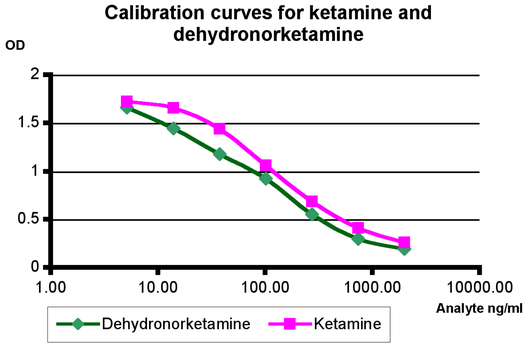
Calibration curves





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Intra-assay precision

Norketamine ELISA	Intra-assay precision (n=6x12)					
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
%CV	2.8	3.1	4.0	5.2	7.9	7.8

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Detection of ketamine in equine urine post-administration

This table summarizes the results obtained from the analysis of 27 urine samples collected at regular intervals from a thoroughbred gelding over 82 hours post-administration.

Detection of ketamine in equine urine post-administration

Assay	Sample Preparation	Number of post-administration urine samples in which analyte was detected	Detection post-administration (hours)
Reported norketamine ELISA	Dilution	13	45.58
Reported norketamine ELISA	One-step extraction	15	53.25
Commercial system A	Neat	7	23.00
Commercial system B	Dilution	3	11.42

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CONCLUSION

- To our knowledge this is the first report of the development of a **high sensitivity ELISA** for the measurement of **norketamine**, the major metabolite of ketamine (IC50=3.3 ng/ml).
- It can also be used for the detection of **ketamine and dehydronorketamine** by expanding the calibration ranges of the assay.
- Evaluation in equine urine after administration of ketamine showed a longer detection window (45.58 hours sample dilution method, 53.25 hours after one step extraction) when compared with other commercial systems.
- This ELISA represents a valuable tool for the effective screening of samples for clinical, therapeutic and regulatory purposes.

REFERENCES

1. Bergman, S.A. Ketamine: review of its pharmacology and its use in pediatric anesthesia. *Anesth. Prog.* 1999, **46:** 10-20.
2. Wright, M. Pharmacologic effects of ketamine and its use in veterinary medicine. *J. Am. Vet. Med. Assoc.* 1982, **180:** 1462-1471.
3. Smith, K.M., *et al.* Club drugs: Methylenedioxymethamphetamine, flunitrazepam, ketamine hydrochloride and gamma-hydroxybutyrate. *Am. J. Health Syst. Pharm.* 2002, **59:** 1067-1076.
4. Lua, A.C., *et al.* Profiles of urine samples from participants at rave party in Taiwan: Prevalence of ketamine and MDMA abuse. *Forensic Sci. Int.* 2003, **136:** 47-51.
5. Adams, J.D., *et al.* Studies on the biotransformation of ketamine.1. Identification of metabolites produced in vitro from rat liver microsomal preparations. *Biomed. Mass Spectrom.* 1981, **8:** 527-538.
6. Hijazi, Y., *et al.* Stability of ketamine and its metabolites norketamine and dehydronorketamine in human biological samples. *Clin. Chem.* 2001, **47:** 1713-1715.

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