SENSITIVE AND RAPID HOMOGENEOUS ENZYME IMMUNOASSAY FOR KETAMINE

#B-305

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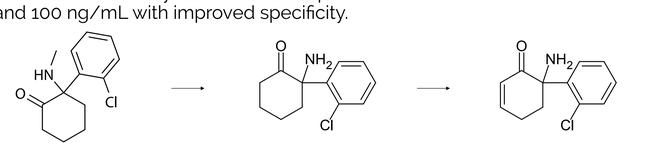
BACKGROUND

Ketamine is a synthetic, nonbarbiturate and rapid-acting dissociative anesthetic that is indicated for use in both human and veterinary surgical procedures. Ketamine is a Schedule III substance under the United States Controlled Substances Act for its potential for abuse and risk of dependence. Ketamine is structurally and pharmacologically similar to phencyclidine (PCP), but is less potent, has a faster onset and shorter duration of action relative to PCP. Ketamine has abuse/dependence potential and is occasionally used as a recreational drug. Ketamine is prescribed off-label for various pain management and for treating depression. The ARK Ketamine II Assay has been developed to detect ketamine in human urine at cutoffs of 50 and 100 ng/mL with improved specificity.



Ketamine Norketamine Dehydronorketamine

METHODS

The ARK Ketamine II Assay is a liquid-stable homogenous enzyme immunoassay consisting of two reagents. The assay has 50 ng/mL and 100 ng/mL cutoffs. The performance characteristics of this assay, including precision, spiked recovery, specificity, and method comparison to LC-MS/MS, were evaluated on the Beckman Coulter AU680 automated clinical analyzer.

RESULTS

PRECISION

Drug-free, negative human urine was supplemented with Ketamine (0 to 100 ng/mL for 50 ng/mL Cutoff, and 0.0 to 200 ng/mL for 100 ng/mL Cutoff). Each level was assayed in quadruplicate twice a day for 20 days (N=160) and evaluated qualitatively and semi-quantitatively. Results are summarized in the tables below.

Qualitative Precision

50 ng/mL Cutoff

Ketamine (ng/mL)	Relative % Cutoff	# of Results	Results
0.0	-100	160	160 Negative
12.5	-75	160	160 Negative
25.0	-50	160	160 Negative
37.5	-25	160	160 Negative
50.0	Cutoff	160	30 Negative / 130 Positive
62.5	+25	160	160 Positive
75.0	+50	160	160 Positive
87.5	+75	160	160 Positive
100.0	+100	160	160 Positive

100 ng/mL Cutoff

Ketamine (ng/mL)	Relative % Cutoff	# of Results	Results
0.0	-100	160	160 Negative
25.0	-75	160	160 Negative
50.0	-50	160	160 Negative
75.0	-25	160	160 Negative
100.0	Cutoff	160	69 Negative / 91 Positive
125.0	+25	160	160 Positive
150.0	+50	160	160 Positive
175.0	+75	160	160 Positive
200.0	+100	160	160 Positive

Semi-quantitative Precision

50 ng/mL Cutoff

Ketamine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
0.0	-100	160	1.04	160 Negative
12.5	-75	160	13.05	160 Negative
25.0	-50	160	26.09	160 Negative
37.5	-25	160	38.87	160 Negative
50.0	Cutoff	160	52.02	39 Negative / 121 Positive
62.5	+25	160	64.53	160 Positive
75.0	+50	160	76.96	160 Positive
87.5	+75	160	89.14	160 Positive
100.0	+100	160	103.06	160 Positive

100 ng/mL Cutoff

Ketamine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
0.0	-100	160	1.04	160 Negative
25.0	-75	160	26.09	160 Negative
50.0	-50	160	52.02	160 Negative
75.0	-25	160	76.96	160 Negative
100.0	Cutoff	160	103.06	52 Negative / 108 Positive
125.0	+25	160	128.59	160 Positive
150.0	+50	160	152.84	160 Positive
175.0	+75	160	178.96	160 Positive
200.0	+100	160	202.30	160 Positive

HISTOGRAM OVERLAP ANALYSIS (QUALITATIVE ANALYSIS)

Twenty replicates each of Negative Control, Cutoff Calibrator, and Positive Control were assayed together in a single run. Frequencies of distribution of ketamine values for each sample were shown by histogram analysis (Figure 1 and 2). The distributions of measurements did not overlap for both 50 ng/mL and 100 ng/mL cutoffs.

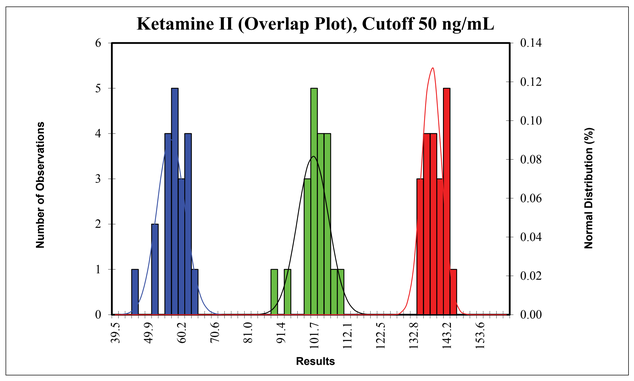


Figure 1. Histogram analysis, Blue (Low 25.0 ng/mL), Green (Cutoff 50.0 ng/mL), Red (High 75.0 ng/mL)

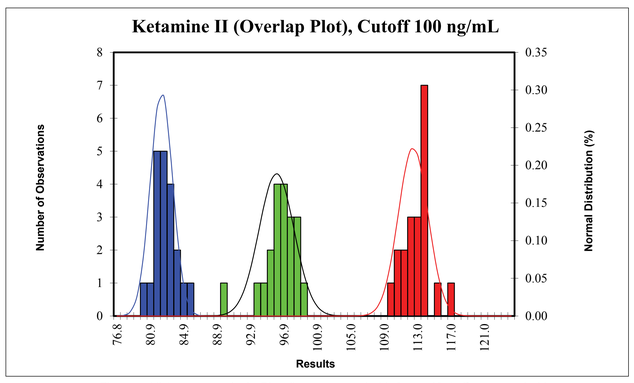


Figure 2. Histogram analysis, Blue (Low 75.0 ng/mL), Green (Cutoff 100.0 ng/mL), Red (High 125.0 ng/mL)

ANALYTICAL RECOVERY/LINEARITY

Recovery across the assay range was assessed using in-house prepared samples. Proportional dilutions of 625 ng/mL Ketamine were made with pooled negative human urine to give concentrations of 20 (LOQ), 50, 100, 200, 300, 400, and 500 ng/mL. Two separately calibrated runs with three replicates of each sample per run were assayed (N=6) in semi-quantitative mode. The percent recoveries ranged from 103.7% to 106.1% from 20.0 to 500.0 ng/mL.

Samples (ng/mL)	Mean (ng/mL)	SD	CV (%)	%Nominal	N
20.0	21.22	1.863	8.8	106.1	5
50.0	52.07	2.049	3.9	104.1	5
100.0	103.65	4.976	4.8	103.7	5
200.0	209.13	14.776	7.1	104.6	5
300.0	312.43	21.175	6.8	104.1	5
400.0	421.67	19.576	4.6	105.4	5
500.0	526.90	37.868	7.2	105.4	5

SPECIFICITY

Desirable Structurally Related Compounds

The cross-reactivity of ketamine metabolites, norketamine and dehydronorketamine, with ARK Ketamine II assay was tested in semi-quantitative mode to obtain the concentration of each compound equivalent to the 50 ng/mL and 100 ng/mL cutoffs.

Compound	Concentration approximately equivalent to 50 ng/mL Cutoff (ng/mL)	Concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)	% Crossreactivity	
			50 ng/mL Cutoff	100 ng/mL Cutoff
Nor-Ketamine	109.84	211.73	45.5	47.2
Dehydronorketamine	410.84	664.55	12.2	15.0

Undesirable Structurally Related Compounds

ARK Ketamine II Assay showed no cross reactivity to Tilidine, Nortilidine, and Venlafaxine. The cross reactivity of methoxetamine and its metabolites are shown in table below.

Compound	Concentration approximately equivalent to 50 ng/mL Cutoff (ng/mL)	Concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)	% Crossreactivity	
			50 ng/mL Cutoff	100 ng/mL Cutoff
Methoxetamine	50,000	100,000	0.1	0.1
Normethoxetamine	>100,000	>100,000	0.0	0.0
desmethylmethoxetamine (hydroxetamine)	17,000	32,500	0.3	0.3
Deoxymethoxetamine	50,000	100,000	0.1	0.1
Tilidine	>100,000	>100,000	0.0	0.0
Nortilidine	>100,000	>100,000	0.0	0.0
Venlafaxine	>100,000	>100,000	0.0	0.0

Structurally Unrelated Compounds

No interference was observed by testing the following structurally unrelated compounds at tested concentrations.

Up to 500,000 ng/mL:
11-nor-9-carboxy-THC, Acetaminophen, Butalbital, Caffeine, Diphenhydramine, Ibuprofen, S-(+)-Amphetamine, S(+)-Methamphetamine.

Up to 100,000 ng/mL:
(+)-MDA, 11-hydroxy-delta-9-THC, 1R,2S (-)-Ephedrine, 1S,2R (+)-Ephedrine, 4-Bromo-2,5-Dimethoxyphenethylamine, 6-Acetylcodeine, 6-Acetylmorphine, 6-Naltrexol, 7-Aminoclonazepam, 7-Aminoflurnitrazepam, 7-Aminonitrazepam , Acetylsalicylic Acid, Albuterol or Salbutamol (Ventolin), Alprazolam, Amitriptyline, Amobarbital, Aripiprazole (Abilify), Atenolol (Tenormin), Atorvastatin (Lipitor), Benzoylecgonine, Benzylpiperazine, Bromazepam, Budesonide (Pulmicort) , Buprenorphine, Bupropion, Buspirone (Buspar), Butabarbital, Cannabidiol, Cannabinol, Carbamazepine, Carbamazepine-10,11-epoxide, Carisoprodol, Chlordiazepoxide, Chlorpromazine, Ciprofloxacin, cis-Tramadol, Clobazam, Clomipramine, Clonazepam, Cocaine, Codeine, Cotinine, Cyanocobalamin (Vitamin B12), Cyclobenzaprine, Delta-9-THC, Demoxepam, Desalkylflurazepam, Desipramine, Desmethyl Ofloxacin, Dextromethorphan, Diazepam, Diclofenac (Voltaren), Digoxin, Dihydrocodeine, Doxepin, Doxylamine, Duloxetine (Cymbalta), Ecgonine, Ecgonine Methyl Ester, EDDP, Ethylmorphine, Ethyl-Dglucuronide, Famotidine (Pepcid), (-)-Fenfluramine, (+)-Fenfluramine, Fentanyl, Flunitrazepam, Fluoxetine, Flurazepam, Formoterol (Foradil), Gabapentin (Neurontin), Haloperidol, Heroin, Hexobarbital, Hydrocodone, Hydromorphone, Imipramine, Ipratropium (Atrovent), Lamotrigine, Levorphanol, Lidocaine, Loratadine (Claritin), Lorazepam, Lorazepam Glucuronide, Lormetazepam, Losartan (Cozaar), LSD, L-Thyroxine (Synthroid), Lurasidone (Latuda), Maprotiline, MDEA, MDMA, Meperidine, Meprobamate, Metformin (Glucophage), Methadone, Methaqualone, Methoxisopropamine, Methylphenidate, Methylphenidate Metabolite (Ritalinic Acid), Midazolam, Mirtazepine (Remeron), Montelukast (Singulair), Morphine, Morphine-3Dglucuronide, Morphine-6Dglucuronide, Nalorphine, Naloxone, Naltrexone, Naproxen, N-desmethyltapentadol, Nicotine, Nitrazepam, Norbuprenorphine, Norcodeine, Nordiazepam, Normorphine, Norpropoxyphene, Norpseudoephedrine, Norsertraline, Nortriptyline, Ofloxacin, Olodaterol (Striverdi Respimat), Omeprazole (Prilosec and Losec), Oxazepam, Oxcarbazepine (Trileptal), Oxycodone, Oxymorphone, Paliperidone (Invega), Paraxanthine, PCP, Pentazocine, Pentobarbital, Phenobarbital, Phentermine, Phenylephedrine, Phenylpropanolamine, Phenytoin, PMA, Prazepam, Prazosin (Minipress), Propoxyphene, Propranolol, Protriptyline, Quetiapine (Seroquel), R-(-) MHD (10-monohydroxy carbamazepine), R,R (-)-Pseudoephedrine, trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine, Ranitidine, S-(+) MHD (10-monohydroxy carbamazepine), S,S (+) Pseudoephedrine, Salicylic Acid, Secobarbital, Sertraline, Sufentanil Citrate, Temazepam, Testosterone, Theophylline, Thioridazine, Tianeptine (Stablon, Tatinol, and Coaxil), Tiotropium (Spiriva), Trazodone, Triazolam, Trifluoromethylphenylpiperazine, Trimipramine, Valacyclovir (Valtrex), Verapamil, Xylazine, Zolpidem Tartrate.

Up to 90,000 ng/mL: Budesonide (Pulmicort) (for 50 ng/mL cutoff)

Up to 50,000 ng/mL: Fluticasone Furoate (Trelegy Ellipta) (for 100 ng/mL cutoff)

Up to 17,000 ng/mL: Fluticasone Furoate (Trelegy Ellipta) (for 50 ng/mL cutoff)

METHOD COMPARISON

A total of two hundred seventy-three (273) unaltered clinical human urine specimens that are not individually identifiable were analyzed for ketamine with the ARK Ketamine II Assay. Based on LC-MS/MS results, there were five (5) samples with concentrations between the 50 ng/mL and the 100 ng/mL cutoffs. The data obtained with the ARK Ketamine II Assay were compared to LC-MS/MS. Results are summarized in the table below.

50 ng/mL Cutoff
Method comparison with LC-MS/MS as reference method

ARK Ketamine II Assay Results	<50% of cutoff concentration by LC-MS/MS (<25 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (25-49 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (50-75 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>75 ng/mL)
Positive	0	0	4	46
Negative	223	0	0	0

100ng/mL Cutoff
Method comparison with LC-MS/MS as reference method

ARK Ketamine II Assay Results	<50% of cutoff concentration by LC-MS/MS (<50 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (50-99 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (100-150 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>150 ng/mL)
Positive	0	0	5	40
Negative	223	5	0	0

CONCLUSIONS

The assay detects ketamine and its main metabolite, norketamine without any significant cross-reactivity to other undesirable structurally related and structurally unrelated compounds. Method comparison with LC-MS/MS showed 100% sensitivity and 100% specificity for both 50 ng/mL and 100 ng/mL cutoffs. The ARK Ketamine II Assay is applicable to a wide range of clinical chemistry analyzers and provides a sensitive, rapid, and reliable measurement of ketamine in human urine.

REFERENCES

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2. Drug Enforcement Administration, Office of Diversion Control, Drug & Chemical Evaluation Section. 2013. KETAMINE.
3. Goktas, E.F. and Arioz, F. 2017. A review of chromatographic methods for ketamine and its metabolites norketamine and dehydronorketamine. Biomedical Chromatography 32:e4014.

REGULATORY STATUS

Product under development. Not FDA cleared for sale in the U.S.