HOMOGENEOUS ENZYME IMMUNOASSAY FOR XYLAZINE AND ITS METABOLITES

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BACKGROUND

Xylazine belongs to a class of compounds known as alpha-2 adrenergic agonists. It is a non-opiate sedative, analgesic, and muscle relaxant only authorized in the United States for veterinary use. However, xylazine has increasingly been identified in various illicit drug mixtures, associated with a rising incidence of drug overdose fatalities. It is most commonly found in combination with fentanyl known as,'tranq' or 'tranq dope' in the USA, but has also been detected in mixtures containing cocaine, heroin, and a variety of other drugs. The chronic use of xylazine can lead to severe side effects such as necrotic skin ulcerations, abscesses, and infections. Currently, there are no commercially available urine drug screening immunoassays for the detection of xylazine on automated chemistry analyzers. ARK Diagnostics has developed the first sensitive homogeneous enzyme immunoassay to detect xylazine in human urine at a cutoff concentration of 10 ng/mL.

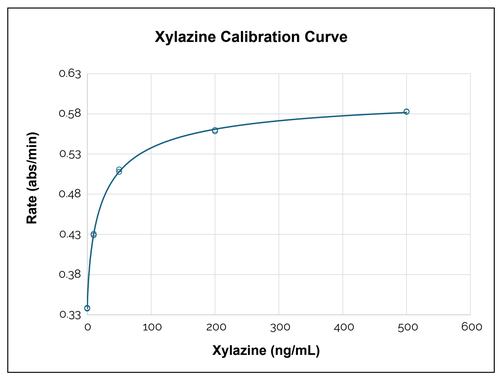
METHODS

The ARK™ Xylazine Assay is a liquid-stable homogenous enzyme immunoassay consisting of two reagents. The performance characteristics of this assay, including precision, spiked recovery, specificity, and method comparison to LC-MS/MS, were evaluated on the Beckman Coulter AU480 automated clinical analyzer.

RESULTS

SEMI-QUANTITATIVE CURVE

A semi-quantitative curve was established using xylazine calibrators at 0, 10, 50, 200, and 500 ng/mL concentrations on the Beckman AU480.



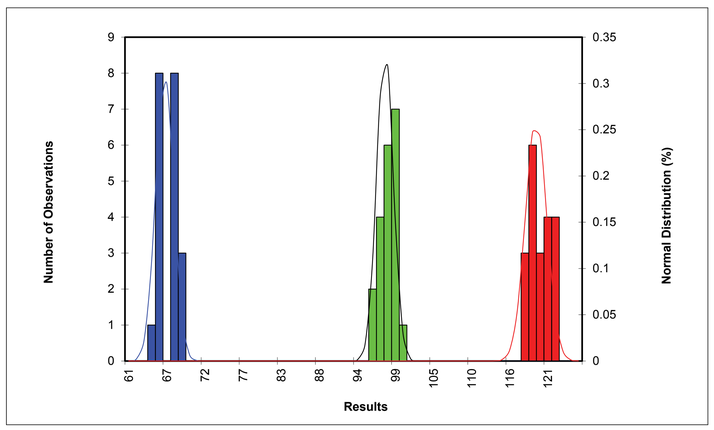
PRELIMINARY PRECISION

Pooled human urine was spiked with xylazine to achieve concentrations at 50% increments from the cutoff calibrator (10 ng/mL). Twenty (20) replicates of each sample were assayed in semi-quantitative mode.

Xylazine (ng/mL)	Cutoff (%)	Mean (ng/mL)	SD	CV (%)
5	-50	4.4	0.151	3.4
10	Cutoff	9.5	0.237	2.5
15	+50	14.7	0.444	3.0

HISTOGRAM OVERLAP ANALYSIS (QUALITATIVE ANALYSIS)

Frequency of distribution of xylazine values for each sample is shown by histogram analysis. Twenty replicates each of Negative Control (5 ng/mL), Cutoff Calibrator (10 ng/mL), and Positive Control (15 ng/mL) were assayed together in a single run. The distributions of measurements did not overlap.

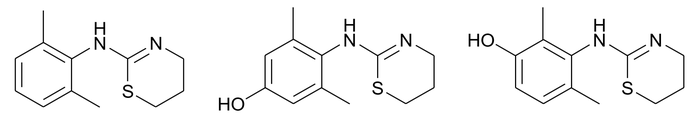


ANALYTICAL RECOVERY

Spike recovery was evaluated using in-house prepared samples. Seven (7) samples were tested in semi-quantitative mode using the AU480 analyzer. One calibration curve was generated, and five (5) replicates of each sample were assayed. Mean, SD, %Nominal and %CV were calculated for each level. Percent nominal ranged from 91.74 to 107.4%.

Samples (ng/mL)	Mean (ng/mL)	SD	CV (%)	Nominal (%)	N
6	5.5	0.14	2.6	91.7	5
14	14.4	0.32	2.2	102.9	5
40	42.9	0.47	1.1	107.4	5
100	102.8	3.71	3.6	102.8	5
150	154.4	9.03	5.9	102.9	5
300	292.9	18.90	6.5	97.6	5
450	428.3	42.26	9.9	95.2	5

SPECIFICITY - XYLAZINE METABOLITES



Xylazine | 4-Hydroxy Xylazine | 3-Hydroxy Xylazine

The following metabolites of xylazine were prepared in drug-free negative human urine. Their corresponding concentration approximately equivalent to the 10 ng/mL xylazine cutoff was investigated using a dose-response curve.

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
4-Hydroxy xylazine	25.0	40.0
4-Hydroxy xylazine glucuronide	38.2	26.2
3-Hydroxy xylazine	9.1	109.8

SPECIFICITY - α-2 AGONIST AND α-2 ANTAGONIST COMPOUNDS

The cross-reactivity to α-2 Agonist and α-2 Antagonist compounds with the ARK Xylazine Assay was tested in semi-quantitative mode to obtain the concentration of each compound equivalent to the 10 ng/mL cutoff.

Compound	Concentration zzzApproximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
Atipamezole	>100,000	<0.01
Brimonidine	20,400	0.05
Clonidine	1,500	0.67
Detomidine	>100,000	<0.01
Etomidate	>100,000	<0.01
Eutylone	>100,000	<0.01
Medetomidine	>100,000	<0.01
Metamizole	>100,000	<0.01
Romifidine	2,200	0.45
Tizanidine	8,000	0.13
Tolazoline	>100,000	<0.01
Yohimbine	>100,000	<0.01

SPECIFICITY –STRUCTURALLY UNRELATED COMPOUNDS

No interference was observed by testing the following forty (40) structurally unrelated compounds at a minimum concentration of 75,000 ng/mL.

Compound	Concentration Tested (ng/mL)	Compound	Concentration Tested (ng/mL)
Acetaminophen	1,000,000	Lidocaine	100,000
Alprazolam	100,000	Lorazepam	100,000
Amphetamine	100,000	MDMA	100,000
Bupivacaine	100,000	Meperidine	100,000
Buprenorphine	250,000	Methadone	100,000
Caffeine	100,000	Methamphetamine	100,000
Chlorpromazine	100,000	Morphine	100,000
Cimetidine	100,000	Naloxone	100,000
Ciprofloxacin	100,000	Norfentanyl	100,000
Cocaine	100,000	Ofloxacin	100,000
Desmethyl Ofloxacin	75,000	Oxycodone	100,000
Diphenhydramine	500,000	Procaine	100,000
(+)-(1S, 2R)-Ephedrin	100,000	Quetiapine	100,000
(-)-(1R, 2S)-Ephedrin	100,000	Quinine	100,000
Fentanyl	100,000	Risperidone	100,000
Hydrocodone	100,000	Salicylic Acid	100,000
Ibuprofen	1,000,000	Thioridazine	100,000
Imipramine	100,000	Tramadol	100,000
Ketamine	100,000	Trazodone	100,000
Levofloxacin	100,000	Δ9-Tetrahydrocannabinol	100,000

METHOD COMPARISON

Forty-seven (47) positive samples and one hundred (100) negative samples were analyzed qualitatively and semi-quantitatively by ARK Xylazine Assay and by LC-MS/MS. The ARK Xylazine Assay used a 10 ng/mL xylazine as the cutoff.

ARK Xylazine Assay Results	True Negatives	Low Negative <50% of cutoff concentration by LC-MS/MS (< 5.0 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (5.0 - 9.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (10.0 – 14.9 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (≥ 15.0 ng/mL)
Positive	0	0	3*	2	30
Negative	100	9	3	0	0

*Three samples with LC-MS/MS values of 7.4, 9.7, and 9.6 ng/mL tested positive in the ARK Xylazine Assay.

The 47 xylazine positive urine samples contained LC-MS/MS values ranging from 1.5 to 3377 ng/mL xylazine. Fifteen of these samples had concentrations below the ARK Xylazine Assay cutoff level of 10 ng/mL. All 32 samples with confirmed xylazine LC-MS/MS concentrations greater than 10 ng/mL were detected as positive with the ARK Xylazine Assay.

CONCLUSIONS

The ARK™ Xylazine Assay enables a sensitive, rapid, and reliable measurement of xylazine and its metabolites in human urine, applicable to a wide range of clinical chemistry analyzers.

PROPOSED INTENDED USE

For Criminal Justice and Forensic Use Only

The ARK Xylazine Assay is a homogeneous enzyme immunoassay intended for the qualitative detection and/or semi-quantitative estimation of xylazine and its metabolites in human urine at a cutoff concentration of 10 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers.

The semi-quantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures.

The ARK Xylazine Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed positive analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

REGULATORY STATUS

In Development. For Criminal Justice and Forensic Use Only