SENSITIVE AND RAPID HOMOGENEOUS IMMUNOASSAY FOR THE DETECTION OF ZOLPIDEM AND ITS MAJOR METABOLITE IN URINE

Hennessey V., Chung K., Singh R., Houts T., and O'Malley R.
ARK Diagnostics Inc., 48089 Fremont Boulevard, Fremont, Ca 94538, USA.

BACKGROUND

Zolpidem, a schedule IV controlled substance under the Controlled Substances Act, is commonly known as Ambien and Ambien CR, a prescription only treatment for insomnia. Zolpidem is one of the most common prescribed sleep aids in the U.S. due to its rapid sedative effects. The recommended duration of treatment is between two days and four weeks, as long-term use of zolpidem increases the risk of habit formation. Addiction to zolpidem can cause severe side effects, including memory loss, delusion, and in extreme cases, fatal overdose. From 2005-2010, Zolpidem-related emergency room visits doubled, underscoring the need for accurate testing as zolpidem testing plays an important role in cases of misuse, diversion, and forensics. The sole commercially available homogeneous immunoassay for the detection of zolpidem in urine has a relatively high cutoff at 20 ng/mL and poor cross-reactivity (0.02%) to major metabolite zolpidem 4-carboxylic acid. ARK Diagnostics has developed the ARK™ Zolpidem Assay to detect zolpidem at a cutoff concentration of 10 ng/mL with high cross-reactivity to its metabolite, Zolpidem phenyl 4-carboxylic acid and no cross-reactivity to zaleplon and zopiclone at concentrations below 100,000 ng/mL.

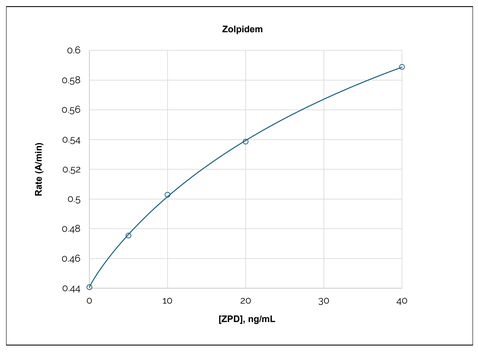
METHODS

The ARK™ Zolpidem Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, with a cutoff concentration of 10 ng/mL and semi-quantitative range up to 40 ng/mL. Preliminary performance characterization for this assay was evaluated on the Beckman Coulter AU680 Automated Clinical Chemistry Analyzer. Precision, analytical recovery, specificity, Histogram Overlap Analysis of ±50% controls and the cutoff, and method comparison with LC-MS/MS were evaluated.

RESULTS

SEMI-QUANTITATIVE CURVE

A semi-quantitative curve was established using zolpidem calibrators at 0, 5, 10, 20, and 40 ng/mL concentrations on the Beckman AU680.



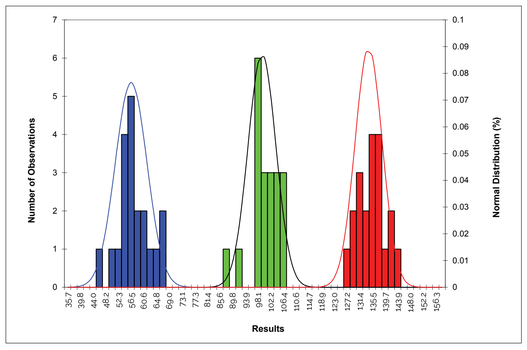
PRELIMINARY PRECISION

Pooled human urine was spiked with zolpidem 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.

Samples (ng/mL)	Cutoff (%)	Mean (ng/mL)	SD	CV (%)
5.0	-50	4.9	0.13	7.4
10.0	Cutoff	10.2	0.38	6.1
15.0	+50	15.5	0.20	2.9

HISTOGRAM OVERLAP ANALYSIS (QUALITATIVE ANALYSIS)

Frequency of distribution of Zolpidem values for each sample is shown by histogram analysis. Twenty (20) replicates each of Negative Control (5.0 ng/mL), Cutoff Calibrator (10.0 ng/mL), and Positive Control (15.0 ng/mL) were assayed together in a single run. The distributions of measurements did not overlap.



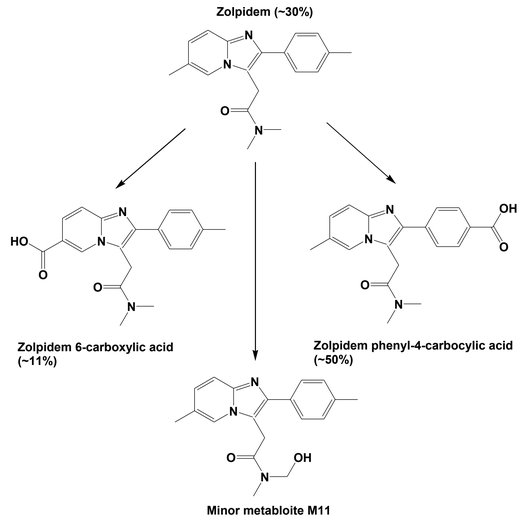
ANALYTICAL RECOVERY

Spike recovery was evaluated using in-house prepared samples. Ten (10) samples were tested in semi-quantitative mode using the AU680 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 88.6 to 96.8%.

Samples (ng/mL)	Mean (ng/mL)	SD	CV (%)	Nominal (%)	N
2.5	2.2	0.20	8.9	88.6	5
5.0	4.8	0.52	10.8	96.7	5
7.5	6.8	0.42	6.1	91.2	5
10.0	9.7	0.67	7.0	96.8	5
15.0	14.1	0.60	4.2	93.7	5
30.0	27.9	1.13	4.1	92.9	5

SPECIFICITY - ZOLPIDEM METABOLITES

Zolpidem is rapidly absorbed through the gastrointestinal tract with a half-life of 2-3 hours. Zolpidem is metabolized via hepatic methylation and hydroxylation mediated by the following cytochrome P450 isoenzymes: CYP3A4 (~60%), CYP2C9 (~22%), CYP1A2 (~14%), CYP2D6 (~3%), and CYP2C19 to 3 inactive metabolites. The major metabolites found in urine are zolpidem phenyl-4-carboxylic acid and zolpidem 6-carboxylic acid.



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

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Cross-reactivity (%)
Zolpidem phenyl-4-carboxylic Acid	30	33.3
Zolpidem 6-carboxylic Acid	197	5.1

SPECIFICITY – BENZODIAZEPINE AND Z-DRUGS

The following benzodiazepines and Z-drugs were tested in drug-free negative human urine

Compound	Concentration Tested (ng/mL)	Result (POS/NEG)	Cross-reactivity (%)
Alprazolam	100,000	NEG	<0.01
Chlordiazepoxide	100,000	NEG	<0.01
Clonazepam	100,000	NEG	<0.01
Flurazepam	100,000	NEG	<0.01
Lorazepam	100,000	NEG	<0.01
Nordiazepam	100,000	NEG	<0.01
Oxazepam	100,000	NEG	<0.01
Temazepam	100,000	NEG	<0.01
Triazolam	100,000	NEG	<0.01
Zaleplon	100,000	NEG	<0.01
Zopiclone	100,000	NEG	<0.01

SPECIFICITY –STRUCTURALLY UNRELATED COMPOUNDS

No interference was observed by testing the following thirty-one (31) structurally unrelated compounds at a minimum concentration of 75,000 ng/mL.

Compound	Concentration Tested (ng/mL)	Compound	Concentration Tested (ng/mL)
+/- amphetamine	100,000	Lidocaine	100,000
Acetaminophen	500,000	Meperidine	100,000
Acetylsalicylic Acid	1,000,000	Methadone	100,000
Caffeine	100,000	Methamphetamine	100,000
Ciprofloxacin	100,000	Morphine	100,000
Cocaine	100,000	Naloxone	100,000
Diphenhydramine	500,000	N-Desmethyl ofloxacin	75,000
Ecgonine	100,000	Pentazocine	100,000
EDDP	100,000	Protriptyline	100,000
Fentanyl	100,000	Quinine	100,000
Hydrocodone	100,000	Risperidone	100,000
Hydroxychloroquine	100,000	Thioridazine	100,000
Ibuprofen	500,000	Tramadol	100,000
Ketamine	100,000	Trazodone	100,000
Levofloxacin	100,000	11-OH Δ9-Tetrahydrocannabinol (THC)	100,000
Δ9-Tetrahydrocannabinol (THC)	100,000		

METHOD COMPARISON

Fifteen (15) positive samples and one hundred (100) negative samples were analyzed qualitatively and semi-quantitatively by ARK™ Zolpidem Assay and by LC-MS/MS. The ARK Zolpidem assay used a cutoff concentration of 10 ng/mL and LC-MS/MS had an LLOD of 10 ng/mL. Results showed an overall agreement of 100.0%, 100.0% clinical specificity, and 100.0% clinical sensitivity.

		LC-MS/MS 10 ng/mL LLOD Zolpidem	
		(+)	(-)
ARK Zolpidem Assay 10 ng/mL Zolpidem Cutoff	(+)	15	0
	(-)	0	100

CONCLUSIONS

The ARK™ Zolpidem Assay measures zolpidem and its major metabolite zolpidem phenyl-4-carboxylic acid in human urine with acceptable performance. The assay is sensitive, rapid, and applicable to a wide range of clinical chemistry analyzers. The assay demonstrates superior specificity and simplicity compared to commercially available zolpidem immunoassays and GC-MS or LC-MS/MS methods that require multiple pre-analytical steps or hydrolysis.

PROPOSED INTENDED USE

The ARK™ Zolpidem Assay is intended for the qualitative detection and/or semi-quantitative estimation of zolpidem 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™ Zolpidem Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed 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 positive test result is positive.

REGULATORY STATUS

In Development. This product is not FDA cleared for sale in the US.