Development of Thermo Scientific™ DRI™ Zolpidem Homogeneous Enzyme Immunoassay for the Detection of Zolpidem and Its Major Metabolite Zolpidem Phenyl-4-COOH in Human Urine

Neha Betawar, Pong Kian Chua, Liang Xu, Chandrasekaran Raman, Tony Prestigiacomo, Lakshmi Anne Clinical Diagnostics Division, Thermo Fisher Scientific, 46500 Kato Road, Fremont CA 94538 USA

INTRODUCTION

Zolpidem, sold under trade names such as Ambien™ and Stilnoct™, is a schedule IV drug used to treat sleep disorders. It is an ideal insomnia drug because it has a quick onset with minimal residual daytime effects. Zolpidem is metabolized rapidly into Zolpidem Phenyl-4-COOH and Zolpidem 6-COOH, with only 1% of parent drug excreted in the urine. Zolpidem Phenyl-4-COOH accounts for >50% of all metabolites excreted in the urine, while Zolpidem 6-COOH comprises 11% of all metabolites. Commercially available immunoassays detect only zolpidem, but not its metabolites, which reduces the window of detection.

OBJECTIVE

The objective of this study was to develop a liquid ready-to-use homogeneous enzyme immunoassay that can detect Zolpidem and its major metabolite(s) in urine using the Thermo Scientific DRI immunoassay technology. Further, the antibody will have minimal cross-reactivity to structurally similar drugs that have imidazopyridine base structure.

MATERIALS AND METHODS

DRI technology is based on competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH), and free drug from the urine sample, for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH and causes a decrease in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring the conversion of nicotinamide adenine dinucleotide (NAD) to NADH. The performance of the assay was evaluated on the Beckman Coulter™ AU680™ analyzer.

The DRI Zolpidem Assay is a screening test. Confirmation of positive results were performed by LC-MS/MS.

RESULTS

Precision

Precision was carried out using 9 levels of spiked samples, at 25% increments or decrements from the cutoff. The samples were run in a random order, twice-a-day, over 5 days for a total of 20 replicates.

Table 1. Qualitative results

Spiked Concentration (ng//mL)	# of Determinants	# Neg / # Pos	Within-run CV (%)	Total-run CV (%)
0	20	20 / 0	0.61	0.50
5	20	20 / 0	0.38	0.47
10	20	20 / 0	0.39	0.57
15	20	20 / 0	0.49	0.47
20	20	15 / 5	0.33	0.41
25	20	0 / 20	0.45	0.55
30	20	0 / 20	0.41	0.70
35	20	0 / 20	0.38	0.60
40	20	0 / 20	0.50	0.58

Table 2. Semi-quantitative results

Spiked Concentration (ng//mL)	# of Determinants	# Neg / # Pos	Within-run CV (%)	Total-run CV (%)
0	20	20 / 0	N/A	N/A
5	20	20 / 0	19.10	22.51
10	20	20 / 0	15.04	14.56
15	20	20 / 0	4.51	6.48
20	20	14 / 6	3.01	6.05
25	20	0 / 20	2.59	2.97
30	20	0 / 20	2.78	3.82
35	20	0 / 20	0.91	1.30
40	20	0 / 20	2.69	2.80

Cross-Reactivity

The cross-reactivity of the assay against Zolpidem and its metabolites, Z-drugs, structurally un-related compounds were tested.

Table 3. Critical cross-reactivity results

Compound	Tested Concentration (ng/mL)	Result	Cross-Reactivity (%)
Zolpidem	20	Positive	100
Zolpidem Phenyl-4-COOH	20	Positive	100
Zolpidem 6-COOH	100,000	Negative	< 0.02
Zopiclone	100,000	Negative	< 0.02
Zaleplon	100,000	Negative	< 0.02

Table 4. Non-critical cross-reactivity results

Compound	Tested Concentration (ng/mL)	Result
Alprazolam	100,000	Negative
Clonazepam	100,000	Negative
Diazepam	100,000	Negative
Lorazepam	100,000	Negative
Nordiazepam	100,000	Negative
Oxazepam	100,000	Negative
Temazepam	100,000	Negative
Triazolam	100,000	Negative
11-nor-9 carboxy-delta-THC	100,000	Negative
6-Acetyl morphine	100,000	Negative
AB-PINACA pentanoic acid	100,000	Negative
Acetaminophen	100,000	Negative
Acetylsalicylic acid	100,000	Negative
Amphetamine	100,000	Negative

Table 4. Non-critical cross-reactivity results (continued)

Compound	Tested Concentration (ng/mL)	Result
Benzoylecgonine	100,000	Negative
Caffeine	100,000	Negative
Codeine	100,000	Negative
Dextromethorphan	100,000	Negative
Diphenhydramine	100,000	Negative
EDDP	100,000	Negative
Fentanyl	100,000	Negative
Hydrocodone	100,000	Negative
Hydromorphone	100,000	Negative
Hydromorphone-glucuronide	100,000	Negative
Ibuprofen	100,000	Negative
Imipramine	100,000	Negative
JWH-018 N-5 hydroxypentanyl metabolite	100,000	Negative
Lamotrigine	100,000	Negative
Methadone	100,000	Negative
Mitragynine	100,000	Negative
Morphine	100,000	Negative
Morphine-3β-D-glucuronide	100,000	Negative
Morphine-6β-D-glucuronide	100,000	Negative
Nalorphine	100,000	Negative
Naloxone	100,000	Negative
Omeprazole	50,000	Negative
Oxycodone	100,000	Negative
Oxymorphone	100,000	Negative
Oxymorphone-β-D-glucuronide	100,000	Negative
Phenobarbital	100,000	Negative
Tapentadol	100,000	Negative
Tramadol	100,000	Negative
UR-144 pentanoic acid	100,000	Negative

Interference

Endogenous substances, pH and specific gravity were tested in the DRI Zolpidem Assay.

Table 5. Interfering substances results

Compound	Tested Concentration (mg/dL)	Result
Acetone	500	Negative
Ascorbic acid	150	Negative
Creatinine	400	Negative
Ethanol	1000	Negative
Galactose	5	Negative
Glucose	1000	Negative
Hemoglobin	150	Negative
Human serum albumin	200	Negative
Oxalic acid	50	Negative
Riboflavin	3	Negative
Sodium chloride	1000	Negative
Urea	1000	Negative
pH	3 - 11	Negative
Specific Gravity	1.004 – 1.029	Negative

Accuracy

One hundred (100) patient samples were tested and immunoassay results compared to LC-MS/MS.

Table 6. Qualitative and Semi-Quantitative results

DRI Zolpidem Assay	< 50%of cutoff concentration by LC-MS/MS (< 10 ng/mL)	Between 50-100% of cutoff concentration by LC-MS/MS (10-19.9 ng/mL)	Between 100-150% of cutoff concentration by LC-MS/MS (20-30 ng/mL)	> 150% of cutoff concentration by LC-MS/MS (> 30 ng/mL)
Positive	0	5 *	5	45
Negative	45	0	0	0

* 5 samples are discrepant due to the presence of Zolpidem Phenyl-4-COOH

CONCLUSIONS

The proof-of-concept data on the DRI Zolpidem Assay demonstrates excellent specificity and sensitivity to Zolpidem and its major metabolite Zolpidem Phenyl-4-COOH, without any significant cross-reactivity to other commonly abused drugs.

TRADEMARKS/LICENSING

NOTE

The assay is currently in development and not available for sale.
The assay is not FDA 510(k) cleared.
The assay is not registered nor is it approved for sale in Europe.

ThermoFisher SCIENTIFIC