

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., Fremont, Ca 94538, USA.

Background: Zolpidem, a schedule IV controlled substance, commonly known as Ambien and Ambien CR, is a prescription only treatment for insomnia and is one of the most commonly prescribed sleep aids in the U.S. 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 urinalysis plays an important role in cases of misuse, diversion, and forensics. The sole commercially available homogeneous immunoassay detects zolpidem at a relatively high cutoff of 20 ng/mL and poor cross-reactivity (0.02%) to major metabolite zolpidem 4-carboxylic acid. The ARK™ Zolpidem Assay detects 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 $\pm 50\%$ controls and the cutoff, and method comparison with LC-MS/MS were evaluated.

Results: In semi-quantitative mode, total precision ranged from 2.6 to 3.1% CV. Spiked recovery of zolpidem ranged from 88.6% (2.5 ng/mL) to 96.8% (10 ng/mL). The major metabolite, zolpidem phenyl-4-carboxylic acid, showed an approximate equivalence to the 10 ng/mL zolpidem cutoff at 30 ng/mL (33.3% cross-reactivity). Histogram overlap analysis showed no overlap between cutoff and control levels. Method correlation with LC-MS/MS using authentic urine samples showed an excellent agreement with a specificity of 100% and sensitivity of 100% (15 positives and 100 negatives).

Conclusion: 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.

Keywords: Zolpidem, Homogeneous Enzyme Immunoassay, Zolpidem phenyl 4-carboxylic acid