K65 Determining Zolpidem Compliance: Urinary Metabolite Detection and Prevalence in Chronic Pain Patients

David M. Schwope, PhD, Aegis Sciences Corporation, 515 Great Circle Rd, Nashville, TN 37228; Anne DePriest, PharmD, 515 Great Circle Rd, Nashville, TN 37228; David L. Black, PhD, Aegis Sciences Corporation, 515 Great Circle Rd, Nashville, TN 37228; Edward J. Cone, PhD, Cone Chem Research, LLC, 441 Fairtree Dr, Severna Park, MD 21146; Yale H. Caplan, PhD, National Scientific Services, 3411 Philips Dr, Baltimore, MD 21208-1827; and Rebecca Heltsley, PhD, Aegis Sciences Corporation, 515 Great Circle Rd, Nashville, TN 37228*

After attending this presentation, attendees will be able to describe a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method for the simultaneous identification and quantification of zolpidem (Ambien®) and its primary urinary metabolite, zolpidem 4-phenyl carboxylic acid (ZCA), in human urine.

This presentation will impact the forensic science community by offering a novel analytical method for sensitive and specific simultaneous quantification of zolpidem parent and metabolite in a single urine extract, as well as providing useful data detailing zolpidem metabolite prevalence in a chronic pain patient population.

Introduction: Zolpidem is the most prescribed insomnia treatment in the United States; however, little is known about zolpidem metabolite excretion in chronic pain patients. As zolpidem is extensively metabolized *in vivo*, metabolite detection may provide improved accuracy for compliance determinations, thereby improving clinical decisions and treatment courses. It is believed that no reported method simultaneously quantifies both parent zolpidem and ZCA in urine.

Method: This study was IRB-approved. Zolpidem and ZCA were extracted from 1mL human urine by mixed-mode solid-phase extraction following buffering with 0.1M acetic acid. Samples were eluted, evaporated to dryness, and reconstituted in 200µL aqueous mobile phase. Samples were injected onto an LC/MS/MS instrument comprised of a Shimadzu Prominence HPLC and ABSciex™ API 3200 tandem mass spectrometer. Ionization was by electrospray (positive mode) with Multiple Reaction Monitoring (MRM) mode employed for detection and quantification. Gradient chromatographic separation starting at 20% B (0.1% formic acid in acetonitrile) was achieved using a C_{18} column (100 x 2.1mm, 3µm particle). Flow rate was 0.7mL/min with an overall run time of 1.8 min.

Results: Conservative Limits Of Quantification (LOQ) were 4ng/mL for both analytes. The assay was validated for linearity from 4 – 1,000ng/mL for zolpidem and 4 – 5,000ng/mL for ZCA (r^2>0.990 and concentrations within ±15% of target). Inter-day recovery (bias) and imprecision (n=20) were 100% – 107% of target and 2.4% – 3.7% relative standard deviation, respectively. Extraction efficiencies were 78% – 90%. Freeze-thaw, processed sample, and autosampler stability were examined (n=6 each), with concentration changes <6.0% observed in all cases. No quantifiable carryover was observed at the method Upper Limit Of Quantification (ULOQ).

A total of 3,264 urine samples were obtained from chronic pain patients over five months and analyzed, with 3,142 (96.3%) meeting qualitative acceptance criteria. Results were de-identified and examined for zolpidem and ZCA prevalence, with concentrations normalized to urine-specific gravity. Zolpidem was detected > LOQ in 720 specimens (22.9%) while ZCA was detected in 1,579 specimens (50.3%). Two specimens (0.06%) contained zolpidem > ULOQ and 45 specimens (1.43%) contained ZCA > ULOQ. Of specimens within the dynamic linear range, median (range) zolpidem and ZCA concentrations were 28.3 (4.08 – 805) ng/mL and 2,038 (4.53 – 23,000) ng/mL, respectively. Only five specimens (0.16%) contained zolpidem alone (median concentration 488ng/mL). As ZCA was observed without parent zolpidem in 864 samples, addition of this metabolite to the assay increased detection rates by 27.5% in this cohort.

Conclusions: An LC/MS/MS method for simultaneous detection and quantification of zolpidem and ZCA in human urine is presented. Addition of zolpidem metabolite to compliance determinations resulted in substantially more positive samples compared to zolpidem alone at the same LOQ. This method is rapid and conducive to a high-throughput environment. Improved detection windows for zolpidem intake should prove useful in both clinical and forensic settings.

Zolpidem Metabolite, Compliance, LC/MS/MS

 ** Presenting Author*