K11 A Validated Method for the Quantitative Determination of Zolpidem, Zopiclone, and Zaleplon (ZZZ Drugs) in Blood, Stomach Contents, and Liver by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will better understand a validated method for the quantitation of ZZZ drugs in blood, stomach contents, and liver by basic Liquid-Liquid Extraction (LLE) and LC/MS/MS.

This presentation will impact the forensic science community by describing a method validation to rapidly and simultaneously confirm all three ZZZ drugs with matching deuterated internal standards (zolpidem-D6, zopiclone-D4, and zaleplon-D4).

Zolpidem, zopiclone, and zaleplon are sedative hypnotics.[1] Due to their rapid onset of action and short half-lives, ZZZ drugs have become the standard alternative to short-acting benzodiazepines for the treatment of onset and maintenance forms of insomnia.[2] ZZZ drugs are GABA agonists and their poly-use with benzodiazepines, ethanol, or other Central Nervous System (CNS) depressants can increase impairment and lead to toxicity or death.[3] If taken in compliance, the drugs should exist at or below therapeutic concentrations with little to no residual effects upon waking. ZZZ drugs are commonly detected both independently and in conjunction with benzodiazepines and ethanol in Driving Under the Influence of Drugs (DUID) and postmortem cases.[4] Current methods for the detection of ZZZ drugs in blood are generally performed with Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) either in tandem with benzodiazepines or are not quantitative for all three analytes.[1,3,5] The goal of this work was to validate a method for the quantification of zolpidem, zopiclone, and zaleplon by LC/MS/MS.

Both acidic and basic LLE methods were investigated during method development. The basic extraction yielded higher area counts, more uniform peak symmetry, and easier isolation of the organic layer. The basic extraction method utilized saturated sodium borate (pH 12), ethyl acetate, and matching deuterated internal standards. An Agilent® 1290 Infinity® II Stack and 6460 Triple quadrupole/Mass Spectrometry (QqQ/MS) system was employed in positive electrospray ionization mode with Multiple Reaction Monitoring (MRM) transitions selected by the Agilent® Optimizer program. Separation was achieved on an Agilent® InfinityLab Poroshell 120 EC-C18 column (3.0mm x 100mm, 2.7µm) with a 0.6mL/min flow rate of 0.1% formic acid in H_2O (A) and 0.1% formic acid in CH_3CN (B). The gradient was initialized at 20% B for 0.8min, increased to 45% B over 0.8min, and 95% B over 1min. An isocratic hold was placed at 95% B for 1.5min, followed by a decrease to 20% B over 0.3min, for a total run time of 4.7min. Method validation was conducted according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

Seven non-zero calibrators were used to establish a 10ng/mL-1,000ng/mL working range with a 1/x weighting factor. Standard residual plots indicated that zopiclone was best fit with a linear model while zolpidem and zaleplon required a quadratic model. Studies assessing the limit of detection are currently underway and the Limit Of Quantitation (LOQ) was set at the lowest non-zero calibrator (10ng/mL) as ZZZ drugs tend to be found, in blood, at concentrations exceeding the lowest calibrator.[2,6] Carryover following the 1,000ng/mL calibrator was determined to be 0.35%, 0.23%, and 1.28% of the LOQ for zolpidem, zopiclone, and zaleplon, respectively. Three concentration levels (25ng/mL, 400ng/mL, 750 ng/mL) in triplicate were used to determine the bias and precision. Bias and precision were calculated at ≤15% for all three analytes, concentrations, and matrices, with the exception of zopiclone in stomach contents at 17.3% CV. Ion suppression was assessed by a post-extraction spike of mobile phase and negative blood, stomach content, liver, and urine matrices at low and high Quality Controls (QCs). Although ion suppression was present at values exceeding SWGTOX guidelines, it had no impact on accuracy or precision of quantitation. No significant interferences were present in mobile phase samples spiked with common drugs of interest (fentanyl, opiates, cocaine, diphenhydramine, trazodone, buspirone, PCP, 3-MeO-PCP, dextromethorphan, ketamine, duloxetine, venlafaxine, tramadol/ nortramadol, amitriptyline/ nortriptyline, clozapine, doxepin/nordoxepin, fluoxetine/norfluoxetine, olanzapine, quetiapine/norquetiapine). The selected MRM transitions were not triggered by endogenous compounds in negative human blood, stomach contents, liver, or urine matrices. Bias and precision of dilution integrity fell ≤20% and was monitored by preparing a 1,500ng/mL ZZZ calibrator and diluting in triplicate (x25, x10, x4) in negative blood. The preceding validation method meets the requirements of SWGTOX guidelines.

Reference(s):

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