A Validated Method for the Quantitative Determination of Zolpidem, Zopiclone, and Zaleplon in Blood, Stomach Contents, and Liver by LC-MS/MS

ABSTRACT

After attending this poster session, attendees will have been introduced to a validated method for the quantitation of zolpidem, zopiclone, and zaleplon (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 addressing the lack of a simple validated method able to rapidly and simultaneously confirm all three ZZZ drugs with matching deuterated internal standards (zolpidem-D6, zopiclone-D4, zaleplon-D4) rather than characteristically similar benzodiazepine internal standards. The method described meets the requirements of SWGTOX guidelines.

INTRODUCTION

• Zolpidem, zopiclone, and zaleplon (ZZZ drugs) are sedative hypnotics prescribed to treat onset and maintenance forms of insomnia.1,2
• These GABA receptor agonists cause central nervous system (CNS) depressant activity with a faster onset of action and shorter half-life than benzodiazepines.3
• Residual effects include eating, drinking, driving, or having sex while sleeping2
• Often found in driving under the influence of drugs, post-mortem, and drug-facilitated sexual assault cases in combination with benzodiazepines, ethanol, or other CNS depressants.4

MATERIALS & METHODS

ZZZ drug analytes and deuterated internal standards were obtained as 1 mg/mL solutions from Cerilliant (Round Rock, TX). Negative sheep’s blood was obtained from Colorado Serum Company (Denver, CO). Human urine, stomach contents, and liver matrices from drug-free individuals were used for the preparation of quality controls (QCs).

Sample Preparation & Extraction

The ZZZ drugs were prepared in combined working solutions of 5 and 0.5 µg/mL. Seven calibrators (10, 25, 50, 100, 250, 500, and 1000 ng/mL) were prepared in blood as the representative matrix. Three quality control samples (25, 400, 750 ng/mL) were prepared in 500 µL blood. Methods were for blood, stomach contents (SC), and liver (LV). The deuterated internal standard was added to 10 µL aliquots from a combined working standard of 1.0 µg/mL. The basic liquid-liquid extraction method utilized 200 µL saturated sodium borate (pH 12) and 2 mL ethyl acetate. Samples were mixed for five minutes and centrifuged for ten minutes at 3500 rpm. The organic layer was isolated and dried under a nitrogen flow. Samples were reconstituted in 2 mL 80:20 0.1% formic acid (FA) in H2O/1% FA in CH3CN.

Validation

Method validation was carried out according to SWGTOX guidelines.7 The within-run and between-run bias/precision was calculated according to equations found in the SWGTOX Standard Practices for Method Validation. The calibration models were determined using standard calibrators (10, 25, 50, 100, 250, 500, and 1000 ng/mL) were prepared in blood as the representative matrix. Three quality control samples (25, 400, 750 ng/mL) were prepared in 500 µL blood. Methods were for blood, stomach contents (SC), and liver (LV). The deuterated internal standard was added to 10 µL aliquots from a combined working standard of 1.0 µg/mL. The basic liquid-liquid extraction method utilized 200 µL saturated sodium borate (pH 12) and 2 mL ethyl acetate. Samples were mixed for five minutes and centrifuged for ten minutes at 3500 rpm. The organic layer was isolated and dried under a nitrogen flow. Samples were reconstituted in 2 mL 80:20 0.1% formic acid (FA) in H2O/1% FA in CH3CN.

RESULTS

Ionization Suppression/Enhancement (%CV)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
<th>Collision Energy (eV)</th>
<th>SWGTOX Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zolpidem</td>
<td>1.11</td>
<td>41.89</td>
<td>25.4</td>
<td>24.3</td>
<td>≤20%</td>
</tr>
<tr>
<td>Zopiclone</td>
<td>1.12</td>
<td>41.89</td>
<td>25.4</td>
<td>24.3</td>
<td>≤20%</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>1.13</td>
<td>41.89</td>
<td>25.4</td>
<td>24.3</td>
<td>≤20%</td>
</tr>
</tbody>
</table>

DISCUSSION

• The method adheres to SWGTOX validation guidelines.
• No interferences were detected from the matrices (blood, SC, LV, urine), deuterated ISTDs, or other commonly encountered analytes.

CONCLUSION

• Advantages of the method include the rapid extraction, use of deuterated internal standards, and the short run time (4.7 min).
• The method is capable of detecting ZZZ drugs at forensically relevant concentrations (Table 1).
• Using matrix matched controls, quantitative analysis was performed using a blood calibration curve.
• The method adheres to SWGTOX validation guidelines.

REFERENCES