Analysis of Zolpidem in Postmortem Fluids and Tissues Using Ultra-Performance Liquid Chromatography-Mass Spectrometry

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February 2014

Final Report
Zolpidem is a nonbenzodiazepine sedative hypnotic drug used for the short-term treatment of insomnia. Its use is common and wide-spread. While quite effective in producing sedation, zolpidem has potentially hazardous side effects when put in the context of complex tasks. Side effects include drowsiness, dizziness, amnesia, nausea, double vision, diminished reflexes, and a lack of coordination.

Due to its potentially deleterious effects on aviation safety, it is important for our laboratory to more fully understand its postmortem concentrations and distribution. Therefore, our laboratory has developed a sensitive method to identify and quantitate zolpidem in biological specimens. Furthermore, we have evaluated the distribution of this compound in various postmortem tissues and fluids from 10 fatal aviation accident cases. Each of these cases had a majority of the desired biological tissues and fluids available for analysis (blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, heart, and brain). This method incorporated a modified acetonitrile “crash and shoot” extraction and a Waters Xevo TQ-S (MS/MS) with an Acquity UPLC. The linear dynamic range was 0.39 – 800 ng/mL. The extraction efficiencies ranged from 78 – 87%, depending on concentration. Postmortem blood zolpidem concentrations in these 10 cases ranged from 8 – 77 ng/mL.

The highest concentrations of zolpidem present in each victim were found in the liver, spleen, lung, and kidney tissues. Distribution coefficients for zolpidem were determined for each of the specimen types analyzed. These coefficients are expressed relative to the blood concentration in each case. This method proved to be simple, accurate, and robust for the identification and quantitation of zolpidem in postmortem fluids and tissues.
An Analysis of Zolpidem in Postmortem Fluids and Tissues Using Ultra-Performance Liquid Chromatography-Mass Spectrometry

INTRODUCTION

Zolpidem is a nonbenzodiazepine sedative hypnotic drug used to treat insomnia by slowing the activity in the brain. It is prescribed as a short-term treatment for insomnia and has been available in the United States since 1993 (1). Zolpidem is an imidazopyridine derivative with the chemical name \( [N,N-6\text{-trimethyl-2-(4-methylphenyl)}\text{imidazo}[1,2-\alpha] \text{pyridine-3-acetamide}] \) (Figure 1). It is a Schedule IV controlled substance, sold under the brand name Ambien in the United States.

The recommended dosage for zolpidem is 10 mg just prior to bedtime (2,3). The dosage for women, the elderly, and patients with decreased liver function is generally 5 mg (2,4). An oral dose of 10-20 mg can have effects lasting up to 4-5 hr and may last as long as 8-16 hr if used with other central nervous system depressants (4). Zolpidem is readily absorbed from the gastrointestinal tract. It is quickly eliminated with a half-life of approximately 2.5 hr (5,6). Research indicates the half-life is less in children and greater in the elderly (4). Peak plasma concentrations occur at approximately 1.6 hr (5). When zolpidem is taken with food, blood concentrations are less. In blood, the therapeutic range for zolpidem is 0.08-0.15 mg/L (7). Blood concentrations average 0.06 mg/L at 1.6 hr for a 5 mg dose and 0.12 mg/L at 1.6 hr for a 10 mg dose (3,4). Toxic effects are seen at 0.5 mg/L, and the lethal effects begin at 2-4 mg/L (7).

There are many possible side effects from zolpidem. The psychological effects range from drowsiness and dizziness to amnesia and memory impairment. Physiological effects range from nausea to slow and slurred speech, including diminished reflexes and a lack of coordination. Other side effects include, but are not limited to, nightmares, hallucinations, leg cramps, double vision, and dry mouth (3,8).

Scientific information concerning the postmortem distribution of zolpidem is mostly limited to drug overdose cases or accidental consumption (6,8,9). Scant information concerning postmortem concentrations and distribution of zolpidem at therapeutic levels has been reported. At therapeutic concentrations, scientific information concerning the distribution of zolpidem is very limited. Consequently, our laboratory set out to determine its distribution in various postmortem tissues and fluids. A search of the Civil Aerospace Medical Institute (CAMI) toxicology database identified 10 fatalities from separate aviation accidents that were reported positive for zolpidem and also had most of the desired biological tissues and fluids available for analysis. These specimen types included blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, heart and brain. This report describes the analysis and distribution of zolpidem in postmortem specimens.

MATERIALS AND METHODS

Chemicals and Reagents

Zolpidem and zolpidem d7 were purchased from Cerilliant (Cerilliant Corp.; Round Rock, TX) at concentrations of 1.00 mg/mL in methanol and 100 µg/mL in methanol, respectively. Potassium oxalate A.C.S. and sodium fluoride (NaF) A.C.S. were purchased from Sigma-Aldrich (Sigma-Aldrich; St. Louis, MO). LCMS grade water, LCMS grade acetonitrile, and Formic Acid LC/MS were purchased from Fisher Scientific (Fisher Scientific, Inc.; Pittsburgh, PA). Double deionized water (DDW) was generated using an ELGA PURELAB Ultra water system (ELGA, Inc.; Lowell, MA). Bovine blood was obtained from Country Home Meat Co. (Country Home Meat Co.; Edmond, OK).

Immediately upon collection, sodium fluoride and potassium oxalate were added to the blood and mixed to produce a final sodium fluoride/potassium oxalate concentration of 1.0% (w/vol). LCMS grade acetonitrile was mixed with formic acid in a 999:1 (v:v) ratio, respectively, for mobile phase B. Mobile phase A was prepared with LCMS grade H2O and formic acid in a 999:1 (v:v) ratio, respectively. A wash solution was prepared at an 80:20 (v:v) ratio with LCMS grade water and LCMS grade acetonitrile.

Sample Selection and Storage

A search of the CAMI toxicology database (ToxFLO, DiscoverSoft Development, LLC; Oklahoma City, OK) identified 10 zolpidem-positive fatalities from separate accidents that occurred during a period of 4 years (2007-2010). Each of these cases had a majority of the desired biological tissues and fluids.
available for analysis (blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, heart and brain). The blood from the cases was stored at -20°C in tubes containing 1.00% (w/v) sodium fluoride/ potassium oxalate prior to analysis. All other specimen types were stored without preservation -20°C prior to analysis.

**Ultra Performance Liquid Chromatographic/Mass Spectroscopic Conditions**

All analyses were performed utilizing a WatersXevoTQ-S Acquity UPLC system. The mass spectrometer portion of the LCMS was optimized for zolpidem using the Waters Intellistart technology. Intellistart calibrates the mass, sets resolution, provides optimum tuning for samples, optimizes the ionization source conditions, and optimizes instrument parameters. The source temperature was set at 150°C, desolvation temperature at 500°C, and the desolvation gas flow at 1,000 L/hr. Initially, we evaluated the applicability of 4 modes of compound ionization (APCI ± and ESI ±) and determined that +ES mode provided maximum ionization. Precursor (parent) and product (daughter) ions, cone voltage, collision energy, and ion mode are listed in Table 1. The precursor ion for zolpidem was 308.2; product ions were 263.1 and 235.3; the quantitative ion was 263.1. For zolpidem d7, the precursor ion was 315.1; product ions were 270.2 and 242.0; and the quantitative ion was 270.2.

Chromatographic separation was achieved using an ACQUITY UPLC BEH C18 column (2.1 x 100-mm, 1.7-µm). The UPLC system and column were obtained from Waters (Waters Corporation; Milford, MA). The UPLC was operated with a gradient of 70:30 A:B mobile phase (A - water with 0.1% formic acid:B - acetonitrile with 0.1% formic acid) to 35:65 A:B at 2.8 min, 5:95 A:B at 2.85 min, and back to 70:30 A:B at 3.88 min, holding until 4.5 min. The total run time was 4.5 min with a flow rate of 0.400 mL/min. The sample injection volume was 2 µL. The UPLC was equilibrated for approximately 30 min prior to use. Typical UPLC pressures observed for these conditions were around 12,000 psi. Following use, the column was washed and stored in a 20:80 mixture of methanol:H₂O.

**Calibrator and Control Preparation**

A calibration curve for zolpidem was prepared by serial dilution, using bovine whole blood as the diluent. Calibrators and controls were prepared using separate 1 mg/mL methanolic zolpidem drug standards. A 100 ng/mL working internal standard was prepared from a 100 µg/mL methanolic zolpidem d7 drug standard. Serial dilution produced a calibration curve at concentrations ranging from 0.2 to 800 ng/mL. A minimum of 7 calibrators were used to construct each calibration curve. A negative control was prepared using negative bovine whole blood. Controls were prepared at concentrations of 1, 10, 100, and 500 ng/mL and analyzed with the batch of unknowns to verify the accuracy of the established calibration curve. The controls were also used to determine the accuracy and precision of the method, both intra-day (within day) and inter-day (between day).

**Sample Preparation and Extraction Procedure**

Calibrators, controls, and specimens were prepared using the following procedure. Tissue samples were prepared for homogenization by adding 1.00% NaF solution to the tissue sample in a 2:1 w:w (1% NaF solution:tissue) addition. Tissue samples were homogenized using an OMNI post-mounted mixer homogenizer (Omni International; Kennesaw, GA). To individual 16 x 100mm screw-top tubes, 100 µL aliquots of each calibrator, control, postmortem fluid, and 0.3 g aliquots of each tissue homogenate (0.1 g wet tissue) were transferred. To each tube, 100 µL of 100 ng/mL internal standard was added (10 ng total). The tubes were vortexed while 1.00 mL of cold acetonitrile was added to each tube, capped, and thoroughly mixed. The tubes were then centrifuged at 1500xg for 10 min in a Thermo Jouan C4i Centrifuge (Thermo Electron Corp.; Chateau-Gontier, France). Centrifugation removed the proteins and cellular debris from the samples. Once centrifuged, 200 µL of the supernatant from each tube was filtered through 16 x 100-mm round bottom tubes and placed in the TurboVap LV nitrogen evaporator (Caliper Life Sciences; Hopkinton, MA), set at 40°C and evaporated to dryness under a stream of

<p>| Table 1. MS parameters for Zolpidem and D7-Zolpidem. |
|---------------------------------|----------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Precursor Ions (m/z)</th>
<th>Cone Voltage (V)</th>
<th>Product Ions (m/z)</th>
<th>Collision Energy (CE)</th>
<th>Ion Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zolpidem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>308.2</td>
<td>42</td>
<td>263.1</td>
<td>26</td>
<td>ES+</td>
</tr>
<tr>
<td>308.2</td>
<td>42</td>
<td>235.3</td>
<td>36</td>
<td>ES+</td>
</tr>
<tr>
<td>D7-Zolpidem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>315.1</td>
<td>42</td>
<td>270.2</td>
<td>26</td>
<td>ES+</td>
</tr>
<tr>
<td>315.1</td>
<td>42</td>
<td>242.0</td>
<td>38</td>
<td>ES+</td>
</tr>
</tbody>
</table>
nitrogen at 15 psi. Once dried, the residue was reconstituted in 1.0 mL of 70:30% LCMS grade water:acetonitrile, vortexed, and 100 µL of the reconstituted sample was transferred to 2 mL LC vials with inserts. The vials were capped (pre-cut septa) and transferred to the auto sampler. All specimens were analyzed at one time to avoid inter-assay variations.

**RESULTS AND DISCUSSION**

**Analysis of Zolpidem**

The extraction method described herein is a simple and rapid modified “crash and shoot,” using a minimal amount (100 µL) of specimen. The LC/MS/MS provided superior chromatography, sensitivity, and dynamic range. The small column particle size associated with UPLC generates narrow peaks and enhanced chromatographic resolution, reducing the chances of co-eluting interferences (10, 11). A chromatogram of a typical calibrator is illustrated in Figure 2. Minimal, if any, matrix effect was observed.

LC/MS/MS ion suppression, or enhancement, was determined by analyzing multiple zolpidem-spiked solvent samples and spiked post-extraction blood samples, and then comparing their response. If the post-extraction blood sample had a lower signal than the solvent control sample, it is ion suppression. If it has a higher signal, then it is ion enhancement. Any ion suppression or enhancement observed for zolpidem or zolpidem D7 was less than 3% and is statistically insignificant.

The Federal Aviation Administration’s (FAA’s) Bio-aeronautical Sciences Research Laboratory has 3 criteria that must be met before a drug can be reported as positive by LC-MS/MS. An analyte must have a minimum signal-to-noise ratio of 10, a retention time ± 5% of the average calibrator retention time, and product ion ratio ±20% of the average calibrator product ion ratio. The retention times for the samples did not exceed ±2% of the average calibrator retention time. It must be noted that while the laboratory accepts as much as ±20% of the ion ratio, the specimen ion ratios never varied more than ±10%. We observed that the LC/MS ion ratio variation in this instance appeared to have much smaller variation than do ion ratios in current laboratory GC/MS methods.
Figure 2. Zolpidem Chromatogram.
The linear dynamic range (LDR) is the concentration range between which the calibration curve remains linear. The LDR for zolpidem in bovine whole blood was 0.39 – 800 ng/mL. Thirteen points were used for the calibration curve and is shown in Figure 3. The correlation coefficient for this curve exceeded 0.9999, with a weighting factor of 1/x. The limit of detection (LOD) is the lowest concentration of the drug that meets the identification criterion. The LOD was found to be 0.20 ng/mL. The limit of quantitation (LOQ) is the lowest concentration that meets the same criteria as the LOD, plus has an experimentally determined value within ±20% of its prepared concentration (see Table 2). The LOQ was found to be 0.39 ng/mL.

Accuracy, expressed as relative error (%E), was determined by calculating the difference between the target value and the measured value. Precision was expressed as the coefficient of variation (CV) of multiple analyses at a given control level. Control values for Days 1, 2, 3, and 7 were processed from the curve injected on Day 1. Controls were prepared at 1, 10, 100, and 500 ng/mL, in large lots, to ensure a sufficient amount of each control was available for the entire accuracy, precision, and stability study. The maximum %E on Day 1 (intra-day) was 9%, with a maximum CV of 4%. The inter-day accuracy and precision was reproducible and compared closely to Day 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LDR (ng/mL)</th>
<th>LOD (ng/mL)</th>
<th>LOQ (ng/mL)</th>
<th>Extraction Efficiency (%) ± SD* 1 ng/mL</th>
<th>100 ng/mL</th>
<th>500 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zolpidem</td>
<td>0.39 - 800</td>
<td>0.20</td>
<td>0.39</td>
<td>78 ± 2</td>
<td>83 ± 2</td>
<td>87 ± 2</td>
</tr>
</tbody>
</table>

* n=5 for all measurements
The maximum %E and CV for Days 2, 3, and 7 were 7% E and 4% CV. Accuracy and precision values are listed in Table 3. Intra-day and inter-day controls processed against the curve from Day 1 further exemplified the robustness of this method. Carryover from one sample to the next was found to be non-existent. It was initially investigated and subsequently monitored by the use of solvent injections. Acetonitrile blanks were injected following the 800 ng/mL calibrator, as well as after each set of controls. There was no sample-to-sample contamination. Additionally, blanks were used randomly throughout the sample sequence to verify that no carryover occurred.

The extraction efficiency of zolpidem at various concentrations was surprisingly high, considering the simplicity of this modified crash and shoot (protein precipitation) method. The procedure for determining the extraction efficiency was described in detail by Johnson et al (12). Zolpidem extraction efficiency was 77.5 ± 1.8% at 1 ng/mL, 83.1 ± 2.1% at 100 ng/mL, and 87.3 ± 2.0% at 500 ng/mL (see Table 2). Since the LOQ was significantly below the therapeutic range, no attempts were made to further optimize the extraction efficiency.

The short-term and intermediate stability of zolpidem in bovine whole blood stored at 4°C was determined by evaluating controls at 1, 10, 100, and 500 ng/mL on Days 7 and 43 of the experiment. The maximum deviation from the target (% error) found for Days 7 and 43 was 6%, with the exception of the 1 ng/mL on Day 43, which was 34%. This 34% error was accompanied by a large CV (13%). Since the other 3 controls provided accurate values on Day 43, we believe that the 34% error found for the 1 ng/mL was an operator or extraction error. Long-term stability of zolpidem stored at -20°C was evaluated by reviewing the Bioaeronautical Sciences Research Laboratory QC data. Over a 3-year period, a zolpidem QC blood control was analyzed 8 separate times, with a mean of 0.090 µg/mL ± 0.006. This QC material was analyzed 5 additional times during this study, yielding a mean of 0.086 µg/mL ± 0.001. Hence, zolpidem is, indeed, a stable compound in blood.

Postmortem Concentrations of Zolpidem

Due to the violent nature of aviation accidents, accident victims may be badly dismembered, fragmented, and/or burned. When an aviation accident occurs, an investigation is necessary to determine its cause. A part of this investigation includes a toxicological evaluation of the pilot and other crew members to determine if drugs (prescription or otherwise) or alcohol were a contributing factor in the accident. Following an aviation accident, specimens are collected at autopsy and forwarded to the FAA’s Bioaeronautical Sciences Research Laboratory for toxicological analysis. The laboratory receives blood in approximately 70% of cases received from an aircraft accident; thus, it relies solely on tissues for approximately 30% of the cases (13). Therefore, a general understanding of the postmortem distribution of commonly encountered drugs is very important.

Therapeutic blood concentrations for zolpidem range from 0.080 to 0.150 µg/mL (7). Toxic levels of zolpidem have been reported at approximately 0.500 µg/mL (7). Lethal levels of zolpidem concentrations begin in the 2.00 to 4.00 µg/mL range (7). Blood concentrations observed in the current study ranged from 0.008 – 0.077 µg/mL, which appear to be in the sub-therapeutic to low therapeutic concentration range. With the short half-life of zolpidem(5,6), low concentrations would be expected in toxicological samples of postmortem specimens from aviation accidents if the zolpidem ingestion were from the “night before.” Additionally, since the site from which the blood was collected at autopsy is unknown for many of these cases and due to possible postmortem redistribution or other factors, these blood concentrations may not be representative of the actual levels prior to death.
Table 3. Intra and Inter-Day Accuracy and Precision.

<table>
<thead>
<tr>
<th>Target (ng/mL)</th>
<th>Mean (ng/mL)</th>
<th>CV%</th>
<th>%E</th>
<th>Mean (ng/mL)</th>
<th>CV%</th>
<th>%E</th>
<th>Mean (ng/mL)</th>
<th>CV%</th>
<th>%E</th>
<th>Mean (ng/mL)</th>
<th>CV%</th>
<th>%E</th>
<th>Mean (ng/mL)</th>
<th>CV%</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.09 ± 0.04</td>
<td>4</td>
<td>9</td>
<td>1.07 ± 0.04</td>
<td>4</td>
<td>7</td>
<td>1.07 ± 0.03</td>
<td>3</td>
<td>7</td>
<td>1.06 ± 0.03</td>
<td>3</td>
<td>6</td>
<td>1.34 ± 0.17</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>10</td>
<td>10.3 ± 0.08</td>
<td>1</td>
<td>3</td>
<td>10.5 ± 0.16</td>
<td>2</td>
<td>5</td>
<td>10.6 ± 0.20</td>
<td>2</td>
<td>6</td>
<td>10.6 ± 0.22</td>
<td>2</td>
<td>6</td>
<td>10.3 ± 0.14</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>104 ± 1.10</td>
<td>1</td>
<td>4</td>
<td>105 ± 1.16</td>
<td>1</td>
<td>5</td>
<td>106 ± 1.86</td>
<td>2</td>
<td>6</td>
<td>106 ± 1.36</td>
<td>1</td>
<td>6</td>
<td>104 ± 0.64</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>500</td>
<td>511 ± 3.70</td>
<td>1</td>
<td>2</td>
<td>526 ± 9.42</td>
<td>2</td>
<td>5</td>
<td>518 ± 6.40</td>
<td>1</td>
<td>4</td>
<td>515 ± 5.98</td>
<td>1</td>
<td>3</td>
<td>513 ± 5.21</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Note. n=5 for all measurements. Accuracy measured as relative error (%E) from target concentration. Precision measured as CV in replicate measurements.
The concentration of zolpidem in each postmortem specimen analyzed from these 10 cases are presented in Table 4. The following mean concentration (µg/mL, µg/g) of zolpidem was detected in each specimen type: blood 0.027 (range 0.008 – 0.077, n=10), urine 0.021 (range 0.012 – 0.036, n=3), liver 0.063 (range 0.015 – 0.236, n=10), spleen 0.042 (range 0.014 – 0.123, n=9), brain 0.014 (range 0.003 – 0.041, n=9), kidney 0.038 (range 0.013 – 0.122, n=9), muscle 0.011 (range 0.003 – 0.020, n=8), heart 0.027 (range 0.003 – 0.091, n=9), vitreous humor 0.015 (range 0.002 – 0.028, n=2), and lung 0.039 (range 0.013 – 0.099, n=9).

On average, the highest concentrations of zolpidem present in each victim were found in the liver, spleen, lung, and kidney tissues. The general trend for highest concentration to lowest concentration of zolpidem was: liver, spleen, lung, kidney, blood, heart, urine, vitreous humor, brain, and muscle. The concentrations of zolpidem found in the tissues were expected considering the low volume of distribution (Vd), which ranged from 0.54 to 0.68 L/kg (14).

<table>
<thead>
<tr>
<th>Case</th>
<th>Blood</th>
<th>Urine</th>
<th>Liver</th>
<th>Spleen</th>
<th>Brain</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Heart</th>
<th>VH *</th>
<th>Lung</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.077</td>
<td>-</td>
<td>0.236</td>
<td>0.123</td>
<td>0.041</td>
<td>0.122</td>
<td>0.016</td>
<td>0.091</td>
<td>0.028</td>
<td>0.099</td>
</tr>
<tr>
<td>2</td>
<td>0.028</td>
<td>-</td>
<td>0.081</td>
<td>0.048</td>
<td>0.003</td>
<td>0.030</td>
<td>0.016</td>
<td>0.035</td>
<td>-</td>
<td>0.040</td>
</tr>
<tr>
<td>3</td>
<td>0.008</td>
<td>-</td>
<td>0.014</td>
<td>0.016</td>
<td>0.003</td>
<td>0.014</td>
<td>0.003</td>
<td>0.003</td>
<td>-</td>
<td>0.013</td>
</tr>
<tr>
<td>4</td>
<td>0.015</td>
<td>0.014</td>
<td>0.040</td>
<td>0.014</td>
<td>0.008</td>
<td>0.012</td>
<td>0.005</td>
<td>0.007</td>
<td>-</td>
<td>0.024</td>
</tr>
<tr>
<td>5</td>
<td>0.052</td>
<td>-</td>
<td>0.084</td>
<td>0.070</td>
<td>0.029</td>
<td>0.064</td>
<td>0.020</td>
<td>0.039</td>
<td>-</td>
<td>0.069</td>
</tr>
<tr>
<td>6</td>
<td>0.025</td>
<td>-</td>
<td>0.032</td>
<td>0.021</td>
<td>0.007</td>
<td>-</td>
<td>0.007</td>
<td>0.016</td>
<td>-</td>
<td>0.028</td>
</tr>
<tr>
<td>7</td>
<td>0.018</td>
<td>0.036</td>
<td>0.031</td>
<td>0.023</td>
<td>0.011</td>
<td>0.027</td>
<td>0.008</td>
<td>0.032</td>
<td>-</td>
<td>0.029</td>
</tr>
<tr>
<td>8</td>
<td>0.021</td>
<td>-</td>
<td>0.035</td>
<td>0.032</td>
<td>0.013</td>
<td>0.028</td>
<td>-</td>
<td>0.004</td>
<td>-</td>
<td>0.021</td>
</tr>
<tr>
<td>9</td>
<td>0.018</td>
<td>0.012</td>
<td>0.045</td>
<td>0.030</td>
<td>0.012</td>
<td>0.028</td>
<td>0.010</td>
<td>0.020</td>
<td>-</td>
<td>0.033</td>
</tr>
<tr>
<td>10</td>
<td>0.008</td>
<td>-</td>
<td>0.027</td>
<td>-</td>
<td>-</td>
<td>0.017</td>
<td>-</td>
<td>-</td>
<td>0.002</td>
<td>-</td>
</tr>
</tbody>
</table>

Note. - Specimen type not available for analysis. All concentrations are shown in units of µg/mL or µg/g.
* Vitreous Humor
The distribution coefficients for zolpidem, expressed as specimen concentration/blood concentration, are listed in Table 5. The summary of distribution coefficients are: urine 1.2 ± 0.56, liver 2.2 ± 0.73, spleen 1.4 ± 0.36, brain 0.50 ± 0.16, kidney 1.4 ± 0.37, muscle 0.39 ± 0.12, heart 0.86 ± 0.47, vitreous humor 0.29 ± 0.08, and lung 1.4 ± 0.27. The %CV values ranged from 18 – 55%. Basic drugs with large Vd can readily undergo postmortem redistribution (15). However, zolpidem has a low Vd, which may partially explain the relatively low CVs observed for a few of the postmortem distribution coefficients (lung, kidney, spleen), suggesting postmortem redistribution may not have been significant in all tissues.

The large CVs associated with the distribution coefficients for many of the tissues and fluids were not unexpected, as many unknown variables exist in these cases. These variables include such things as inconsistent blood collection sites at autopsy, postmortem interval, postmortem redistribution, contamination, time from drug administration to death, and dosage, just to name a few (16). In the majority of the cases where the blood collection site is noted, typically collection site is chest cavity. If more information relating to each accident victim were available, the conclusions drawn from these data may be more substantial. However, unknown variables are common in the postmortem toxicology world.

### CONCLUSION

A rapid, reliable, and sensitive method for identification, confirmation and quantitation of zolpidem was developed using a Waters XEVO TQ-S MS/MS and an Acquity UPLC. A modified “crash-and-shoot” method was incorporated to minimize preparation time and sample and solvent volumes. The LOD was determined as 0.2 ng/mL, and the LDR was 0.39 – 800 ng/mL. Seventy-eight tissue and fluid samples, from a total of 10 aviation accident fatalities, were analyzed to determine the zolpidem concentrations and postmortem distribution. The blood concentrations were all in the sub-therapeutic to low therapeutic range. This methodology was demonstrated to be highly effective for the identification and quantitation of zolpidem in various postmortem fluid and tissue specimens.
REFERENCES


