Phenazepam and its Effects on Driving

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Phenazepam use in the state of Georgia has increasingly become a trend for a drug market looking at new and different recreational drugs. This paper examines the psychomotor effects of phenazepam on individuals and their ability to operate a motor vehicle. This study reviewed phenazepam cases of impaired drivers that were submitted to the Georgia Bureau of Investigation’s Division of Forensic Sciences between March, 2010, and August, 2011. A total of 11 cases were reviewed, of which five had only phenazepam detected and six had multiple drugs detected in addition to phenazepam. Concentrations ranged from 0.04 to 3.2 mg/L, with a median of 0.17 mg/L and a mean of 0.50 mg/L (0.23 mg/L excluding the 3.2 mg/L blood concentration). The observed effects where symptomatic of central nervous system depression with slurred speech, lack of balance, slow reactions, drowsiness and confusion. This review indicates that the use of phenazepam at concentrations similar to other low-dose benzodiazepines such as clonazepam can have a significant impact on an individual’s ability to drive.

Introduction

The internet has made the recreational drug market more global, suggesting that more novel and foreign drugs will find their way into the case work of toxicologists (1–2). Phenazepam [7-bromo-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one] was first synthesized in 1975, applied to clinical use in 1978 as a sedative hypnotic drug in the USSR, and is still being prescribed in Russia and other Commonwealth of Independent State (CIS) countries (3–5) (Figure 1). Reports of phenazepam abuse have been documented in Finland (5–6). A reported fatal case of phenazepam in combination with poppy seed tea was submitted to the Georgia Bureau of Investigation’s Division of Forensic Sciences between March, 2010, and August, 2011. With central nervous system (CNS) depression and side effects in common with other benzodiazepines, such as drowsiness, impaired balance, confusion, ataxia and memory loss, phenazepam has the potential to significantly affect an individual’s driving ability (12, 13). The effects of phenazepam and its combined actions with other drugs were examined to assist in the evaluation of cases.

Materials and Methods

Chemicals and reagents

Phenazepam was purchased from Toronto Research Chemicals (North York, ON, Canada). Diazepam-d₅ was purchased from Cerilliant (Round Rock, TX). Acetone (HPLC grade), methanol (HPLC grade), water (HPLC grade), pH 7 buffer (0.05 M potassium phosphate monobasic–sodium hydroxide) and formic acid (88%, w/v) were purchased from Fisher Scientific (Pittsburgh, PA). Ammonium formate (99%) was purchased from Sigma Aldrich (St. Louis, MO). Negative blood was screened for drugs of abuse and therapeutic drugs prior to use. Mobile phase A was 0.1% formic acid and ~15 mM ammonium formate in Fisher Optima grade water and mobile phase B was 0.1% formic acid and ~15 mM ammonium formate in Fisher Optima grade methanol. The cloned enzyme donor immunoassay (CEDIA) reagents were purchased from Thermo Scientific (Fremont, CA).

Sample preparation and analysis

Sample pretreatment was performed for a combined CEDIA and liquid chromatography tandem mass spectrometry (LC–MS-MS) analysis. This process involves an acetone precipitation procedure that requires a 1.0 mL aliquot of blood sample for analysis with an addition of 2.5 mL of acetone (vortexing samples during acetone addition). The samples were allowed to stand for 10 min, then vortexed for approximately 15 s and centrifuged for 10 min. The supernatant was decanted through filtering reservoirs into test tubes containing a glass boiling head, reservoirs were rinsed with 0.5 mL of acetone and removed. Samples were dried at 75°C for 20 min, reconstituted with 0.5 mL 1:1 methanol–pH 7 buffer solution, vortexted until the residue was suspended, centrifuged for 10 min and then transferred to sample cups for analysis (14).

The precipitate was then divided into two aliquots for screening. One aliquot received a six-panel CEDIA screen for benzodiazepines, barbiturates, opiates, amphetamines, cocaine and tetrahydrocannabinol (THC). The second aliquot received a 73 drug panel screen using LC–MS-MS for qualitative identification of phenazepam (15). A separate quantitative analysis was performed, utilizing the same acetone precipitation procedure on LC–MS-MS using a 200 μL aliquot of blood sample, and with the addition of diazepam-d₅ as the internal standard and final reconstitution before analysis in a 1:1 mobile phase A–mobile phase B.
**Instrumentation**

Phenazepam testing was performed on an Applied Biosystems QTRAP 3200 (Carlsbad, CA), with electrospray ionization (ESI) in positive mode. Chromatography was achieved by injecting 10 \( \mu \)L of the specimen on a MetaSil Basic RP (3 micron, 50 × 2.0 mm) column at a temperature of 30°C. Separation was achieved by gradient elution using mobile phase A and mobile phase B. The gradient conditions were initially 95% A and 5% B for 1 min, decreasing to 5% A and 95% B at 17 min and resetting to the initial conditions for a final 2 min, producing a total run time of 20 min. The retention time for phenazepam using these settings was 13.6 min. Instrumental parameters for quantitative analysis were set for multiple reaction monitoring (MRM), and the parameters for qualitative analysis were set to perform an enhanced product ion (EPI) scan for full mass spectral identification to a library match internally generated by the Georgia Bureau of Investigation (Figure 2).

**Validation**

A method currently implemented by the laboratory and reported elsewhere was validated for use in the analysis of phenazepam (15). Phenazepam was infused on the instrument to determine the optimal parameters (Table I). A limit of detection (LOD) was established at 12 \( \mu \)g/L, with the criteria that the method had to produce a qualitative full mass spectrum identification when compared to an internal library reference, while producing a signal-to-noise ratio of greater than 3:1. The limit of quantitation (LOQ) was determined at 0.028 mg/L, the concentration at which the signal-to-noise ratio proved to be greater than 10:1. The variability was determined to be 18% based upon a 95% confidence interval, with a percent coefficient of variation (CV) of 9%, using a series of fortified control sample analyses (N = 30). A seven-point calibration curve with concentrations ranging from 0.10 to 1.6 mg/L was incorporated, and the correlation coefficient \( r \) and coefficient of determination \( r^2 \) were 0.997 and 0.994, respectively. Analyzed positive control samples were to have an anticipated concentration of 0.80 mg/L.

**Case Histories**

A total of 11 suspected impaired drivers were found to have phenazepam as one of the major drug findings in their toxicological analysis. The police officers' reports were summarized...
showing their circumstances of arrest, officers’ observations, field sobriety tests (if conducted), and lab analyzed drug concentrations (Table II). Field sobriety test results are reported with the number of clues (indicators of impairment) the subject exhibits during the evaluation, which include a total of six clues for horizontal gaze nystagmus (HGN), eight clues for the walk and turn and four clues for the one-leg stand. Concentrations below 0.1 mg/L were approximated because they were below the lowest calibrator of 0.1 mg/L.

**Subject 1**
An 18-year-old white male was involved in an accident after driving past a school crossing guard who was signaling him to stop. The officer noted that the individual seemed lethargic, with slurred speech during questioning, and was unable to stand without assistance. It was also noted that the individual had redness in his eyes and was not able to focus on the officer. At the hospital for the blood draw, the subject was observed to have an abnormal heart rate. No field sobriety tests were performed. Toxicology analysis revealed blood results of phenazepam at 0.18 mg/L and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA) at 28 ng/mL.

**Subject 2**
A 27-year-old white male was pulled over after failure to maintain lane. The officer observed slow reactions, slurred speech, disorientation, drowsiness and lack of balance. During conversations with the subject, the officer observed that his pupils were slow to react to light. When blood was drawn at the hospital, the subject’s heart rate was noted at 111 beats per minute. No field sobriety tests were conducted due to the subject’s refusal. Toxicology analysis revealed blood results of phenazepam at 0.50 mg/L and cyclobenzaprine at 0.0061 mg/L.

**Subject 3**
A 22-year-old white male was pulled over for leaving the scene of a single car accident, where the subject hit a mailbox. The officer noted that the subject had extremely slurred speech and could not maintain balance. No field sobriety tests were conducted due to the subject’s refusal. Toxicology analysis revealed blood results of phenazepam at 0.75 mg/L and trazodone (reported qualitatively).

**Subject 4**
A 29-year-old white female was pulled over for failure to maintain lane after the officer observed that the subject almost collided with another car. The officer observed constricted pupils, slow and slurred speech, bloodshot eyes and lack of balance. Field sobriety tests noted one clue on the walk and turn, and two clues during the one leg stand, and the driver estimated 37 s to be 30 s during the Romberg evaluation (in which the subject tilts their head back and estimates 30 s). HGN was not conducted due to an eye injury. Toxicology analysis revealed blood results of phenazepam at 0.31 mg/L, amphetamine at 0.17 mg/L and urine results positive for THCA (due to institutional testing protocols, the blood was not tested for THCA).

**Subject 5**
A 39-year-old white male was found pulled over in a parking lot with automobile damage, after a concerned citizen called to report the subject holding a baby, which he dropped multiple times. The officer noted that the subject had slow and mumbled speech, acted confused and lacked balance. Field sobriety tests noted six clues for the HGN evaluation. The subject’s heart rate was noted to be 104 beats per minute. Toxicology analysis revealed blood results of phenazepam at 0.17 mg/L and urine results positive for THCA (due to institutional testing protocols, the blood was not tested for THCA).

**Subject 6**
A 23-year-old white male was involved in a multi-car accident after failing to stop at a stop sign. The officer noted that the subject had slurred speech, acted disoriented and seemed sedated. Field sobriety tests noted six clues for the HGN evaluation, two clues during the walk and turn and two clues during the one leg stand. Toxicology analysis revealed blood results of phenazepam at 0.14 mg/L and gabapentin (reported qualitatively).

**Subject 7**
A 22-year-old white male was involved in a single car accident, having driven off the road and collided with a tree. The officer noted that the subject did not remember the accident he was just involved in, or the fact that a female passenger was riding with him at the time of the accident. The officer observed the subject acting disoriented, with dilated pupils and slow and slurred speech. No field sobriety tests were conducted. Toxicology analysis revealed blood results of phenazepam at 3.2 mg/L, which was obtained by sampling a reduced volume that fell within the calibration range.

**Subject 8**
A 40-year-old white male was pulled over for failure to maintain lane and traveling with a flat tire. The subject showed a lack of awareness about where he was going, and about the fact that he was traveling with a flat tire. The officer observed confusion, constricted pupils and slurred speech. Field sobriety tests noted one clue on the walk and turn, two clues during the one leg stand and two clues on the HGN analysis. Toxicology analysis revealed blood results of phenazepam at an approximate concentration of 0.04 mg/L.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Phenazepam (mg/L)</th>
<th>Additional drugs</th>
<th>Circumstance</th>
<th>Observations/impairment</th>
<th>Field sobriety Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>Male</td>
<td>0.18</td>
<td>THCA (28 ng/mL)</td>
<td>Involved in an accident after driving past a school crossing guard who was signaling him to stop</td>
<td>Redness of the conjunctiva, lethargic, slurred speech, lack of balance and an abnormal heart rate</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>Male</td>
<td>0.5</td>
<td>Cyclobenzaprine (0.0061 mg/L)</td>
<td>Pulled over after failure to maintain lane</td>
<td>Slow reactions, slurred speech, drowsiness, lack of balance, pupils slow to react to light and a heart rate of 111 bpm</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>Male</td>
<td>0.75</td>
<td>Trazodone</td>
<td>Pulled over for leaving the scene of a single car accident where the subject hit a mailbox and failure to maintain lane</td>
<td>Slurred speech and lack of balance</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>Female</td>
<td>0.31</td>
<td>Amphetamine (0.19 mg/L), quetiapine</td>
<td>Pulled over for failure to maintain lane after the officer saw the subject almost hit another car</td>
<td>Constricted pupils, slow and slurred speech, redness of the conjunctiva, and lack of balance</td>
<td>Walk and turn: missed heel to toe, one leg stand: body swayed and put foot down, romberg: estimated 37 s to be 30 s</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>Male</td>
<td>0.17</td>
<td>THCA (urine)</td>
<td>Found already pulled over in a parking lot with automobile damage</td>
<td>Slow mumbled speech, confusion, lack of balance and a heart rate of 104 bpm</td>
<td>HGN: lack of smooth pursuit, nystagmus was detected prior to 450 and at maximum deviation in both eyes</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>Male</td>
<td>0.14</td>
<td>Gabapentin</td>
<td>Involved in a multi-car accident after failing to stop at a stop sign</td>
<td>Slurred speech, disoriented and sedated</td>
<td>HGN: lack of smooth pursuit, nystagmus was detected prior to 450 and at maximum deviation in both eyes, walk and turn: missed heel to toe, used arms for balance, one leg stand: body sway, put foot down</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>Male</td>
<td>3.2</td>
<td>—</td>
<td>Involved in a single car accident, driving off the road and hitting a tree</td>
<td>Memory loss, disoriented, dilated pupils and slow slurred speech</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>Male</td>
<td>0.04* (estimated)</td>
<td>—</td>
<td>Pulled over for failure to maintain lane, and driving with a flat tire</td>
<td>Slurred speech, confusion, lack awareness and constricted pupils</td>
<td>HGN: lack of smooth pursuit with the eyes horizontally and vertically, walk and turn: missed heel to toe, one leg stand: raised both arms for balance, put foot down</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>Female</td>
<td>0.05* (estimated)</td>
<td>—</td>
<td>Involved in an accident in which the subject ran into the back of another car at a stop light</td>
<td>Lethargic, heart rate of 150 and appearance of being intoxicated</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>Male</td>
<td>0.12</td>
<td>—</td>
<td>Officer found the subject’s car half way in the road out of gas</td>
<td>Lack of balance</td>
<td>Romberg evaluation: swayed and almost fell, walk and turn: failed to touch heel to toe, took the wrong number of steps and stepped off the line, one leg stand: put his foot down and swayed</td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>Male</td>
<td>0.08* (estimated)</td>
<td>—</td>
<td>Subject ran off the road</td>
<td>Lethargic, appearance of being intoxicated</td>
<td>HGN: lack of smooth pursuit, nystagmus was detected prior to 450 and at maximum deviation in both eyes, walk and turn: missed heel to toe, stepped off the line, and took to many steps, one leg stand: put his foot down, used arms for balance, and swayed</td>
</tr>
</tbody>
</table>

*Lower than the lowest calibrator of 0.1 mg/L.
Subject 9
A 24 year-old white female was involved in an accident, having run into the back of another car at a stop light. The officer observed that the individual appeared lethargic and intoxicated. When the blood was drawn, a heart rate of 150 beats per minute was noted. Toxicology analysis revealed blood results of phenazepam at an approximate concentration of 0.05 mg/L.

Subject 10
A 29 year-old white male ran out of gas in the middle of the road. The subject was witnessed stumbling around the road trying to flag down cars, when the officer arrived and observed a lack of balance. Field sobriety tests noted body sway and a near fall during the Romberg evaluation, three clues during the walk and turn and two clues during the one leg stand. Toxicology analysis revealed blood results of phenazepam at 0.12 mg/L.

Subject 11
A 21 year-old white male was involved in a single car accident in which he drove his vehicle off the road. The officer observed lethargy and the appearance of intoxication. Field sobriety tests noted six clues during the HGN evaluation, three clues during the walk and turn, and three clues during the one leg stand. Toxicology analysis revealed blood results of phenazepam at an approximate concentration of 0.08 mg/L.

Conclusions
Phenazepam concentrations from case histories ranged from 0.04 to 3.2 mg/L, with a median of 0.17 mg/L and a mean of 0.50 mg/L (0.23 mg/L, excluding the 3.2 mg/L blood concentration). Concentrations at which phenazepam was the only drug detected ranged from 0.04–3.2 mg/L, with a median of 0.08 mg/L and a mean of 0.69 mg/L (0.096 mg/L, excluding the 3.2 mg/L blood concentration). All calculated means and medians are approximate because some of the concentrations fell below the lowest calibrator of 0.1 mg/L. The concentrations of phenazepam observed in Georgia drivers are above concentrations that would typically be observed in prescribed doses, which is most likely due to the self dosing of the individuals. Since the first case appearance in March, 2010, the concentrations detected in phenazepam cases have been steadily decreasing. The most recent cases are typically below 0.1 mg/L, indicating that the users may have discovered that they can obtain the desired effects of phenazepam at lower concentrations. This information shows to be consistent with reports of phenazepam’s potency even at low concentrations (12, 16).

Phenazepam symptoms observed in these cases were those of CNS depression, with the most commonly observed symptoms of slurred speech, lack of balance, slow reactions, drowsiness and confusion, which is consistent with the effects of benzodiazepines in general (13). Substantial memory loss was also observed in the one case in which phenazepam was reported at a concentration almost fourteen times the average concentration of the other subjects. Impairment was noted in all of the case histories, including the lowest concentrations at which phenazepam was the only drug detected. Benzodiazepines in combination with other CNS depressants typically have an additive effect (10). Based upon the observations of this study, phenazepam has the potential to elicit a similar response, but it is difficult to determine whether increases in impairment are due to higher phenazepam concentrations or the addition of other drugs. This is, in part, because of the wide range of concentrations detected. It is reasonable to conclude that phenazepam causes impairing CNS depressant effects similar to those of other benzodiazepines, and in concentrations similar to those of low-dose benzodiazepines such as clonazepam.

References