The Effect of Food on the Absorption of Rohypnol using the RIVM in vitro Digestion Model

Nicole McLeod¹, Jayden Mayville¹, Shashi K. Jasra¹*

Abstract: The goal of this study is to compare levels of flunitrazepam in an in vitro digestion system with and without the presence of food. Food and a spiked drink were combined with simulated intestinal juice mixture, designed by RIVM, for a set duration. A colourimetric reaction was then carried out to quantify the amount of flunitrazepam in the sample. The concentration of flunitrazepam in the control sample and experimental sample are 8.802 ppm and 1.900 ppm respectively; this shows that food decreases the amount of flunitrazepam available in the small intestine to be absorbed into the bloodstream.

Keywords: alcohol, digestion, flunitrazepam, forensic science, food, RIVM, rohypnol, sexual assault

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Introduction

Several studies have considered the use of drugs in sexual assault cases. Elsohly et al. found a strong association between drugs and alleged cases of sexual assault. Alcohol was often found to be the most common substance used in sexual assaults. However, it was suggested that at least 20 different substances can be associated with sexual assault, and they are often combined. For instance, alcohol can act as a delivery system and enhance the effects of other drugs, such as gamma-Hydroxybutyric acid (GHB) and flunitrazepam (rohypnol), two of the more commonly used “date rape” drugs. Both these drugs alone act rapidly, reducing inhibition and causing anterograde amnesia.

Flunitrazepam is a benzodiazepine, a tasteless, colourless, odorless drug that acts as a central nervous system suppressant, causing temporary cognitive impairment. It is frequently used in Europe to treat insomnia and anxiety. However, a dose as small as 1 milligram (mg) can cause impairment for 8-12 hours. When combined with depressants, such as alcohol, it can cause acute sedation and unconsciousness, which is why it has a tendency to be abused, such as in cases of sexual assault. The studies conducted by Eloshly, Slaughter and Schwartz all support the concept that drug-facilitated sexual assault is common and suggest that more measures need to be taken to test for drugs in alleged cases of sexual assault.

When a drug-facilitated sexual assault occurs, it is unrealistic to believe that all victims will file a report and go to the hospital for sexual assault testing promptly. The detection window for flunitrazepam depends on the dosage and the bodily fluid analyzed. It has been found that a 2 mg dose was detected in a urine sample in a 14-28 day window, and a 1 mg dose was detected in urine 3-5 days after consumption and less than 6 hours in oral fluids. The longer victims wait, the more the evidence becomes degraded, decreasing the available samples for collection as with drugs that are eliminated from the body through metabolic processes.

Studies have found that the presence of food in the stomach prior to alcohol consumption can slow the absorption rate of ethanol in the body. The decreased absorption rate means that the peak concentration of alcohol is lower but the alcohol remains in the body for a longer period, creating a larger detection window. With a larger detection window, victims of drug facilitated sexual assault that don’t immediately come forward would still be able to provide samples of their bodily fluid for drug testing and it could still be used as viable evidence. The aim of this research project is to determine if the consumption of food prior to drinking an alcoholic beverage laced with rohypnol will affect its absorption into the body. A slower absorption rate may lengthen the duration that the drugs can be detected from bodily fluids.
Materials

- SnCl$_2$ (5%)
- HCl (4%)
- 4-(Dimethylamino)cinnamaldehyde (DMAC)
- Ethanol (95%)
- Distilled Water
- Flunitrazepam solution (1.0 mg/mL)
- Spectronic 20D+ by Thermo Scientific
- Beefeater London Dry Gin (40.0% alcohol)
- President’s Choice® Club Soda - Low Sodium
- Tostitos® Scoops!® tortilla chips
- President’s Choice® Medium Salsa
- NaCl (175.3g/L)
- CaCl$_2$ • 2H$_2$O (22.2 g/L)
- HCl (37% g/g)
- NaHCO$_3$ (84.7 g/L)
- KH$_2$PO$_4$ (8 g/L)
- KCl (89.6 g/L)
- MgCl$_2$ (5 g/L)
- Urea (25g/L)
- Bovine serum albumin
- Pancreatin
- Lipase
- Bovine Bile
- Stirring hot plate
- Magnetic stir bars
- Scale
- 2 - 600mL beakers
- Test tubes

Methods

Beverage Preparation

The beverage utilized in this experiment was gin and tonic because it is colourless solution like the gin that was used in Friedman’s experiment allowing a similar colour to be expressed. The gin and tonic beverage was composed of 25 milliliters (mL) of gin and 75 mL of tonic water. The amount of flunitrazepam added is enough to create a 10 ppm flunitrazepam solution.
**Colorimetric Reaction**

A coloured product was required in order to measure the absorbance of solutions at a particular wavelength. The colour reaction chosen was designed by Arthur J. Friedman, where flunitrazepam was hydrolyzed by 4% hydrochloric acid and reduced by 5% tin (II) chloride\textsuperscript{12}. The colouring reagent Friedman utilized was 0.25% 4-dimethylaminocinnamaldehyde (DMAC) in 95% ethanol\textsuperscript{12,13}.

This colour reaction can be done at room temperature and requires 1 mL of the reducing/hydrolyzing agent to be mixed with 1 mL of sample for 5 minutes. Then 2.5 mL of the DMAC visualizing agent was added and the colour is allowed to produce for 10-15 minutes.

**Maximum Absorbance Determination**

The wavelength at which the colour reaction can display maximum absorbance must be determined in order to quantify the concentration of flunitrazepam in the test and standard solutions. The Spectronic 20D+ was used to analyze the 10 ppm sample, measuring the absorbance in the visible spectrum from 350 nm to 825 nm in 25 nm increments to narrow down the range of the maximum absorbance. The range was narrowed down within 50 nm of the maximum absorbance reading to determine the precise wavelength that would be used in the colorimetric determinations of flunitrazepam.

**Flunitrazepam Standard Solutions**

Standard solutions of flunitrazepam with known concentrations were used to create a Beer’s Law graph as a reference to compare the experimental and control absorbance readings and approximate their concentrations. The standards were created by diluting a 10 ppm solution with distilled water to concentrations of 9 ppm, 8 ppm, 7 ppm, 6 ppm, 5 ppm, 4 ppm, 3 ppm, 2 ppm and 1 ppm as well as a sample with a concentration of 0 ppm to create an absorbance curve for flunitrazepam at the determined peak wavelength.

**Mechanical Food Disruption and Intestinal Cavity**

The food used in the experiment was represented by 15 g of tortilla chips and 30 mL of salsa. Chewing was simulated by a mortar and pestle; the food was grinded down for one minute.

The chemical mixture for the intestinal cavity (Table 1) was the duodenal juice and bile mixtures developed by RIVM (Rijksinstituut voor Volksgezondheid en Milieu), the National Institute for Public Health and Environment of the Netherlands\textsuperscript{14}. For the experimental trial, the duodenal and bile mixtures were combined with 100 mL of 10 ppm flunitrazepam gin and tonic.
solution and the chip and salsa mixture. The control trial contains the duodenal and bile mixtures, combined with 100 mL of 10 ppm flunitrazepam and gin and tonic solution. Once the trial mixtures were created, they were mixed on a stirring hot plate, kept at approximately 37°C for two hours. After the trials ran, the contents were allowed to settle, separating the liquid and solid phase before a sample of the trials were taken for analysis. The samples were analyzed in the same manner as the flunitrazepam standards.

Table 1: Chemical Components of the duodenal juice and bile from Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants\textsuperscript{14}.

<table>
<thead>
<tr>
<th></th>
<th>Duodenal Juice</th>
<th>Bile</th>
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<tbody>
<tr>
<td><strong>Inorganic Solution</strong></td>
<td>40 ml NaCl 175.3 g/L</td>
<td>30 ml NaCl 175.3 g/L</td>
</tr>
<tr>
<td></td>
<td>40 ml NaHCO3 84.7 g/L</td>
<td>68.3 ml NaHCO3 84.7 g/L</td>
</tr>
<tr>
<td></td>
<td>10 ml KH2PO4 8 g/L</td>
<td>4.2 ml KCl 89.6 g/L</td>
</tr>
<tr>
<td></td>
<td>6.3 ml KCl 89.6 g/L</td>
<td>200 μl HCl 37% g/g</td>
</tr>
<tr>
<td></td>
<td>10 ml MgCl2 5 g/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>180 μl HCl 37% g/g</td>
<td></td>
</tr>
<tr>
<td><strong>Organic Solution</strong></td>
<td>4 ml urea 25 g/L</td>
<td>10 ml urea 25 g/L</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td>9 ml CaCl\textsubscript{2} 2H2O 22.2 g/L</td>
<td>10 ml CaCl\textsubscript{2} 2H2O 22.2 g/L</td>
</tr>
<tr>
<td></td>
<td>1 g BSA</td>
<td>1.8 g BSA</td>
</tr>
<tr>
<td></td>
<td>3 g pancreatin</td>
<td>6 g bile</td>
</tr>
<tr>
<td></td>
<td>0.5 g lipase</td>
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</table>

**Trial Concentration Determination**

The absorbance for three samples was tested for both the experimental and control solution, the average of each was used in conjunction with the linear regression of the Beer’s Law graph to calculate the concentration of the experimental and control solution.

**Results**

In the first set of wavelengths utilized in analyzing the 10 ppm flunitrazepam standard from wavelengths of 350nm to 825nm, peak absorption was observed at 400nm (Figure 1). Because of this peak, the refined wavelength range was based on a wavelengths ±25nm around 400nm, resulting in a range of 375nm to 425nm.
Figure 1: Absorption of 10 ppm flunitrazepam standard solution from wavelengths 350 nm to 825 nm; peaking at 400 nm.

Within the range of 375nm and 425nm, the wavelength with the highest absorption reading from the 10 ppm flunitrazepam sample was 413 nm (Figure 2). This is the wavelength that is used to analyze the control and experimental samples and the 11 standard flunitrazepam samples (Table 2).

Figure 2: Absorption of 10 ppm flunitrazepam standard solution from wavelengths 375 nm to 425 nm; peaking at 413 nm
The absorbance values measured from the 11 standard samples were used to create a Beer’s Law graph (Figure 3). While creating the linear regression the standard solutions of 1 ppm, 3ppm and 4 ppm were excluded to give a linear regression with a coefficient of determination ($R^2$) value closer to 1. The linear regression of the Beer’s Law graph with an equation of: $y=0.0126x + 1.692$, where $x$ represents the concentration in parts per million and $y$ represents the absorbance at 413 nm.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.700</td>
</tr>
<tr>
<td>1</td>
<td>1.740</td>
</tr>
<tr>
<td>2</td>
<td>1.720</td>
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<tr>
<td>3</td>
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<td>4</td>
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<td>6</td>
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<td>1.760</td>
</tr>
<tr>
<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>1.800</td>
</tr>
<tr>
<td>10</td>
<td>1.850</td>
</tr>
</tbody>
</table>

**Figure 3:** Beer’s Law Graph of Standard Flunitrazepam Samples at $\lambda = 413$ nm with calculate control and experimental data plotted. The linear regression has an equation of: $y=0.0126x + 1.692$; where $x$ represents the concentration in parts per million and $y$ represents the absorbance at 413 nm.
Using the linear regression equation and the average absorbance of the samples (Table 3), the concentrations of the experimental and control solutions were calculated to be 1.900 ppm and 8.802 ppm, respectively.

Discussion

The results show that the experimental sample, containing food, had a lower concentration than the control sample; this implies that the presence of food assists in absorbing the flunitrazepam present in the small intestine, decreasing the amount of flunitrazepam in the liquid portion of the intestinal contents to be absorbed into the bloodstream. Food consumption has a similar effect on alcohol metabolism in the human body\textsuperscript{15}, where the presence of food decreased the amount of alcohol detected in the bloodstream, resulting in a lower peak of blood-alcohol level compared to the consumption of alcohol without the addition of food. This addition of food has the potential to reduce the peak levels of flunitrazepam in the bloodstream that in turn would reduce the effect that flunitrazepam would have on the central nervous system, as it did in the study by Jones and Jönsson\textsuperscript{15}.

This research could be improved upon by the inclusion of the mouth and stomach cavity to observe how the inclusion of those cavities influences the level of flunitrazepam available to be absorbed into the bloodstream. As well, the large intestine and colon could also be included to determine how much of the flunitrazepam that was absorbed by the food would migrate to the bloodstream to give a better idea of the detection window of flunitrazepam.

Conclusion

This evidence supports the concept that the absorption of drugs into the system can be decreased by the consumption of food, although, more research is required in order to get a more detailed understanding of how food affects the absorption of flunitrazepam into the human body. With more knowledge about this topic, more support and assistance may be given to those who have fallen victim to drug facilitated sexual assault.

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References


