Investigating the Degradation of Benzodiazepines

Cailin Herl, BS*; J. Tyler Davidson, PhD; James T. Miller, MA; Alaina Anderson, MS; Mona Colca, BA; Geraldine Monjardez, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

ABSTRACT

The present study investigates the effect of pH on the stability of eleven benzodiazepine compounds and the identification of degradation products observed using UV-Vis spectrophotometry and gas chromatography-mass spectrometry (GC-MS). This research also aims to provide a more comprehensive understanding of the UV-Vis data obtained after exposure of these compounds to ultraviolet light under different pH conditions. This evaluation will provide a better understanding of the behavior of designer benzodiazepines when identified in casework.

INTRODUCTION

Benzodiazepines are a commonly prescribed central nervous system (CNS) depressant used for a variety of medical purposes including anxiety, seizures, sleep disorders, and alcohol withdrawal.

In recent years, there has been a significant increase in the number of novel psychoactive substances (NPS) encountered by crime laboratories, including designer benzodiazepines (examples shown in **Figure 1**), as a method to avoid legal sanctions associated with their scheduled drug class [1,2].

In 2022, the Houston Forensic Science Center (HFSC) detected eleven different benzodiazepines in seized drug evidence, eight of which were designer NPS. These drugs are frequently used in drug-facilitated crimes, such as robberies and sexual assaults. It is therefore essential to develop tools that can rapidly and reliably screen for these compounds, as well as their traditional benzodiazepine counterparts. While several studies have been conducted on traditional benzodiazepines, there is a gap in knowledge about designer benzodiazepines that needs to be addressed [3].

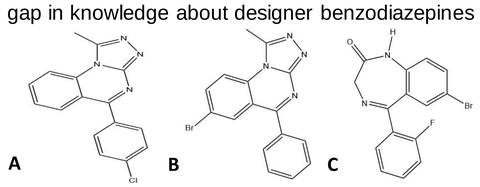

Figure 1. Chemical structures of select designer benzodiazepines studied in this research (A) 4'-chloro deschloroalprazolam, (B), bromazolam, and (C) flubromazepam

MATERIALS & METHODS

Linearity – For each of the 11 benzodiazepine standards, a 20 ppm stock solution was made in 2/3 N H_2SO_4 (pH 0.50). Serial dilutions were performed to create 16, 12, 8, 4, 2, 1.6, 1.2, 0.8, 0.4, and 0.2 ppm solutions. These were analyzed using the Thermo Scientific Evolution 60S UV-Visible Spectrophotometer (Waltham, Maryland) with the Thermo Scientific™ VISIONlite™ software to obtain the λ_{max} of each compound.

Alprazolam Degradation Study – Using the 20 ppm stock solution of alprazolam, aliquots were measured each week over 4 weeks to examine the possible degradation and shifts observed in the UV-Vis spectra.

- IN PROGRESS -

pH Effects – UV-Vis spectra were collected for each compound in methanol (pH 6). Two traditional benzodiazepines (alprazolam and diazepam) and two designer benzodiazepines (4'-chloro deschloroalprazolam and flubromazepam) were selected to be analyzed in sodium hydroxide (pH 13) and phosphate buffer (pH 7.4) as the basic and neutral solutions at a concentration of 20 ppm. Their UV-Vis spectra will be compared.

Extraction for GC-MS analysis – A liquid-liquid extraction will be performed on the selected benzodiazepines. 2 mL of ethyl acetate will be added to 2 mL of the 2/3 N H_2SO_4 solution, extracting the ethyl acetate layer and repeating for a total of three extractions. The fractions will be combined, dried down, and reconstituted in methanol for GC-MS analysis. To the remaining 2/3 N H_2SO_4 portion, NaOH will be added until pH 10 is reached before repeating the same ethyl acetate extraction procedure to collect the basic compounds. The extractions will then be analyzed with a GC-MS (Agilent Technologies 7890A GC-5975C MS) following the optimization of parameters.

RESULTS & DISCUSSION

- Linearity for seven of the eleven benzodiazepines in 2/3 N H_2SO_4 produced acceptable responses, with coefficient of determination values (R^2) above 0.99 (**Table 1**).
- The highest R^2 value was observed with 4'-chloro deschloroalprazolam and flubromazepam (**Table 1 and Figure 5**)
- Clonazepam, flunitrazepam, phenazolam and phenazepam showed the lowest R^2 values, ranging from 0.9951 to 0.9749 (**Table 1**).
- Benzodiazepines with lower R^2 values correlated with weak absorption in the UV region compared to the benzodiazepines with higher R^2 values.
- Unacceptable R^2 values could also be due to difficulty weighing out accurate amounts of certain benzodiazepines, as well as a change in pipette tips used.

Benzodiazepine	R^2 Value
4'-chloro deschloroalprazolam	1
Alprazolam	0.9999
Bromazolam	0.999
Bromazepam	0.9982 and 0.9995
Clonazepam	0.9760
Diazepam	0.9999 and 0.9997
Flubromazepam	1 and 0.9996
Flunitrazepam	0.9819
Lorazepam	0.9984
Phenazolam	0.9749
Phenazepam	0.9951 and 0.9822

Table 1. Coefficient of determination (R^2) values for each benzodiazepine analyzed in the study.

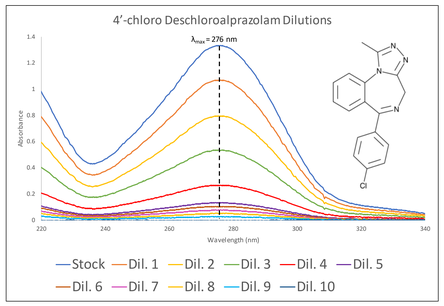

Figure 2. UV-Vis spectra for 4'-chloro deschloroalprazolam at each of the dilutions used for linearity with a λ_{max} at 276 nm.

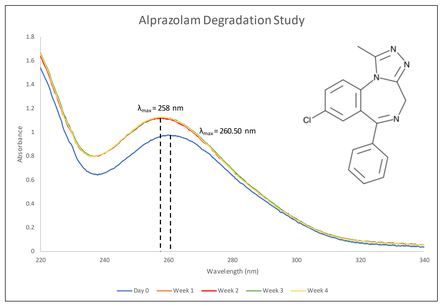

Figure 3. UV-Vis spectra for alprazolam indicating degradation as the λ_{max} shifts from about 260.50 nm to about 258 nm.

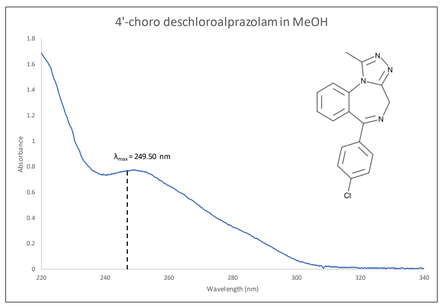

Figure 4. UV-Vis spectra for 4'-chloro deschloroalprazolam in methanol (20 ppm) with a λ_{max} at 249.50 nm.

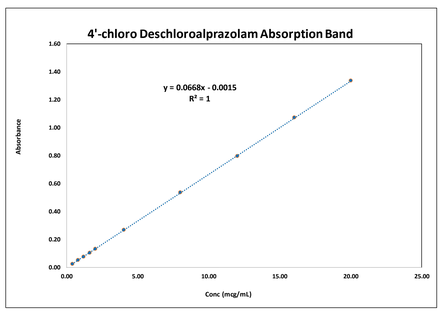

Figure 5. Linearity for the dilutions for 4'-chloro deschloroalprazolam with the equation y = 0.0668x – 0.0015.

- The initial lambda max (day 0) for alprazolam in 2/3 N H_2SO_4 was located at 260.50 nm (Blue spectrum in **Figure 3**).
- By Week 1, the lambda max shifted to 257.50 nm (orange spectrum in **Figure 3**).
- 257.50 nm lambda max was maintained (+/- 0.50 nm) for final three remaining weeks (red, green and orange spectra in **Figure 3**).
- In addition to the shift in wavelength, the absorbance of the lambda max was observed to be higher in weeks 1-4 (above 1) than for day 0 (between 0.8 and 1).
- In 2/3 N H_2SO_4 and methanol, a reproducible lambda max was identified for each benzodiazepine.
- In methanol, the UV-Vis spectra showed an absorption band with a different shape and a shifted lambda max than that observed for 2/3 N H_2SO_4.
- As shown in **Figures 2 and 4**, respectively, the lambda max for 4'-chloro deschloroalprazolam was located at 276 nm in 2/3 N H_2SO_4 while it was located at 249.50 nm in methanol.

CONCLUSIONS

- Reproducible UV-Vis spectra were obtained for all eleven benzodiazepines in 2/3 N H_2SO_4 and methanol.
- Benzodiazepines that showed lower absorbance in their UV-Vis spectrum exhibited lower R^2 values than those with stronger absorbance.
- Shift in band position and absorbance of alprazolam in 2/3 N H_2SO_4 was observed between day 0 and week 1 but remained stable at weeks 1-4.
- Effects of pH were determined to contribute to shifting of UV-Vis spectra more than UV radiation.

FUTURE WORK

- UV-Vis spectra will be collected for alprazolam, diazepam, 4'-chloro deschloroalprazolam, and flubromazepam in phosphate buffer and sodium hydroxide.
- Extractions from the sulfuric acid solution will be performed to isolate possible degradation products from the 2/3 N H_2SO_4 solution.
- GC-MS will be utilized to help in the structural elucidation of these degradation products.
- Further studies should be performed on the 7 remaining benzodiazepines to relate the pH to certain degradation products and evaluate how their stability is affected by their chemical structures.

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