Under the Light: UV-Vis Spectrophotometry as a Tool for Characterizing Designer and Traditional Benzodiazepines in Varying Solvent Systems

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INTRODUCTION

Traditional benzodiazepines and designer benzodiazepines (DBZDs) have been increasingly encountered in seized drug casework over recent years. DBZDs are typically made to have higher abuse potentials in comparison to traditional benzodiazepines and evade legal repercussions, which heightens their risk to public health[1]. All benzodiazepines can be categorized into three families, and those included in this study can be classified either as 1,4-benzodiazepines or triazolobenzodiazepines (**Figure 1**)[2].

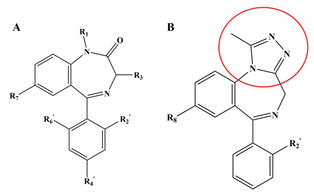

Figure 1: Chemical structures of the two families of benzodiazepines investigated in this study (**A**) 1,4-benzodiazepine and (**B**) triazolobenzodiazepine

UV-Vis spectrophotometry is a common presumptive technique in forensic laboratories and has been utilized for the analysis of benzodiazepines. However, differentiating emerging DBZDs encountered in casework remains challenging. Given their novelty, DBZDs require further characterization to aid seized drug analysts in their identification.

Since 2022, the Houston Forensic Science Center (HFSC) has identified twelve different benzodiazepines in their casework, eight of which are DBZDs. UV–Vis spectrophotometry provides a valuable method for the preliminary characterization of both traditional and novel benzodiazepines. In this study, the pH effect of various solvent systems on the appearance of characteristic UV–Vis absorption bands for 11 benzodiazepines was evaluated. Additionally, a linearity study was conducted to examine the relationship between benzodiazepine concentration in 0.67 N H_2SO_4 and the corresponding UV–Vis spectral response.

MATERIALS & METHODS

Solvents: Phosphate buffer (pH 7.4) was prepared from purchased phosphate buffered saline powder. ACS grade sulfuric acid 95-98% to prepare a 0.67 M solution (H_2SO_4, pH 0.5), methanol (MeOH, pH 6.0), and sodium hydroxide pellets to prepare a 0.45 M solution (NaOH, pH 13.0) were purchased.

Benzodiazepines: Six traditional benzodiazepines (Alprazolam, Bromazepam, Clonazepam, Diazepam, Flunitrazepam, and Lorazepam) and five DBZDs (4'-Chloro Deschloroalprazolam, Bromazolam, Flubromazepam, Phenazolam, and Phenazepam) were purchased.

Sample Preparation: 1.0 mg of each benzodiazepine was dissolved in 50 mL of 0.67 M H_2SO_4 to create a 20-ppm solution, which was diluted to 16, 12, 8, 4, 2, 1.6, 1.2, 0.8, 0.4, and 0.2-ppm. 1.0 mg of each benzodiazepine was dissolved in 50 mL of 0.45 M NaOH, and 0.5 mg in 25 mL of MeOH and phosphate buffer, respectively.

Data Collection: A Thermo Scientific Evolution 60S UV-Visible Spectrophotometer with Thermo Scientific™ VISIONlite™ software was used to collect all spectra. The spectra were collected in a quartz cuvette in absorbance mode with a wavelength range of 220 - 340 nm, fast scan speed, single scan mode, and a sampling interval of 0.5 nm.

Data Processing: All UV-Vis spectra were plotted in Microsoft Excel v. 16. All linearity values were determined by plotting concentration of solution versus absorbance of the λ_{max}.

RESULTS & DISCUSSION

Effect of Solvent System pH on Characterization

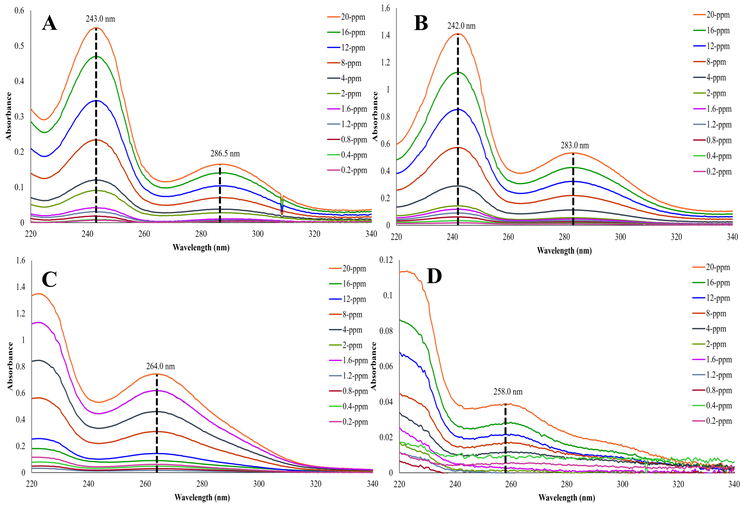

Figure 2: UV-Vis spectra collected in 0.67 N H_2SO_4 for (**A**) Phenazepam, (**B**) Flubromazepam, (**C**) Bromazolam, and (**D**) Phenazolam.

- Due to the benzodiazepines being basic, the 0.67 N H_2SO_4 protonates drug molecules, altering their electronic structure (creating a chromophore) to enhance light absorption.
- **Figure 2** highlights the spectra produced in 0.67 N H_2SO_4 by Phenazepam (**A**), Flubromazepam (**B**), Bromazolam (**C**), and Phenazolam (**D**) which exhibited strong absorbance ranges and well-defined λ_{max}.
- In 0.67 N H_2SO_4, Clonazepam (**Figure 3A**) and 4'-Chloro Deschloroalprazolam (**Figure 3B**) each produced one absorption band at 275.5 nm and 276.0 nm, respectively.
- In MeOH, Clonazepam (**Figure 3C**) produced two absorption bands at 246.5 and 309.0 nm, while 4'-Chloro Deschloroalprazolam (**Figure 3D**) produced one at 249.5 nm.
- This spectral change could be due to the nitro substitution of these benzodiazepines and the hydrogen bonding capabilities of MeOH, which can influence the molecular energy levels of electronic transitions.
- In phosphate buffer, Alprazolam, Bromazolam, and 4'-Chloro Deschloroalprazolam produced absorption bands at 221.5, 224.0, and 251.5 nm, respectively.

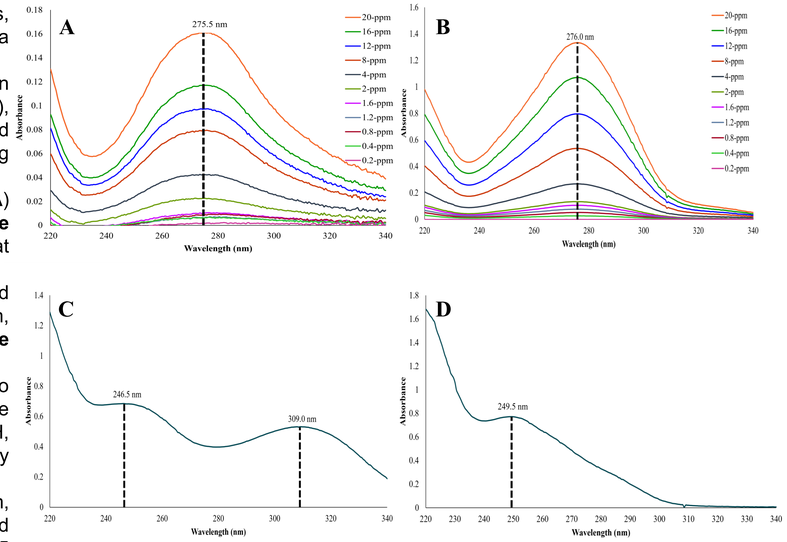

Figure 3: UV-Vis spectra collected in 0.67 N H_2SO_4 for (**A**) Clonazepam and (**B**) 4'-Chloro Deschloroalprazolam; in MeOH for (**C**) Clonazepam and (**D**) 4'-Chloro Deschloroalprazolam.

- In 0.45 M NaOH, 4'-Chloro Deschloroalprazolam (**Figure 4A**) produced an absorption band at 251.5 nm.
- **Figure 4B** highlights the spectra exhibited by Diazepam in 0.45 M NaOH.
- All benzodiazepines, besides 4'-Chloro Deschloroalprazolam, produced spectra in 0.45 M NaOH similar to the results seen with Diazepam, where there is no well-defined absorption band within the spectra.

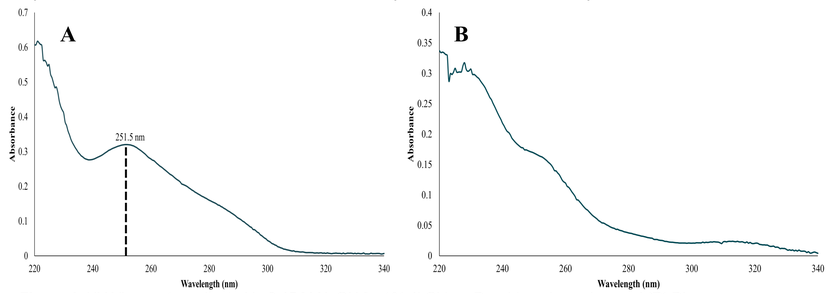

Figure 4: UV-Vis spectra collected in 0.45 M NaOH for (**A**) 4'-Chloro Deschloroalprazolam and (**B**) Diazepam.

Linearity Study

- As shown in **Table 1**, four traditional benzodiazepines out of six exhibited coefficient of determination (R^2) values greater than 0.99.
- Diazepam having the highest at 1 and 0.9999 for its first and second absorption bands, respectively.
- Four DBZDs displayed R^2 values greater than 0.99 (**Table 1**).
- 4'-Chloro Deschloroalprazolam and Flubromazepam exhibited the highest R^2 values.

Table 1: Coefficient of determination for all benzodiazepines included in the linearity study.

Type	Benzodiazepine	Coefficient of Determination (R^2)
Traditional	Alprazolam	0.9999
	Bromazepam	0.9995 & 0.9996
	Clonazepam	0.9797
	Diazepam	1 & 0.9999
	Flunitrazepam	0.9819
	Lorazepam	0.9984
DBZD	4'-Chloro Deschloroalprazolam	1
	Bromazolam	0.9990
	Flubromazepam	1 & 0.9999
	Phenazepam	0.9951 & 0.9932
	Phenazolam	0.9749

CONCLUSIONS

- Due to the basic nature of benzodiazepines, the strongly acidic solvent system 0.67 N H_2SO_4 enabled acquisition of characteristic UV-Vis spectra for all benzodiazepines included in this study.
- Clonazepam was differentiated from 4'-Chloro Deschloroalprazolam in MeOH.
- Only Alprazolam, 4'-Chloro Deschloroalprazolam, and Bromazolam could be differentiated in phosphate buffer.
- Of the traditional benzodiazepines and DBZDs examined, 4′-Chloro Deschloroalprazolam was the only compound distinguished by a distinct absorption band at 251.5 nm in 0.45 M NaOH.
- 4′-Chloro Deschloroalprazolam and Flubromazepam demonstrated high absorbance, enabling the acquisition of characteristic UV–Vis spectra at comparatively lower concentrations.
- Most benzodiazepines showed good linearity in 0.67 N H_2SO_4.
- No clear trends were observed between structural class and the ability to generate characteristic spectra, nor between traditional benzodiazepines and their DBZDs counterparts.

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