

Introduction

Infrared and Raman spectroscopy are used in forensic analysis to identify pure chemicals but the techniques are also used to identify drug substances which are contained in tablet or capsule dosage forms. Due to sensitivity issues with both IR and Raman spectroscopy, detecting drug active(s) in low dose tablets can be problematic and in certain cases may require some type of extraction of the drug active from the dosage form prior to analysis. This includes tablets which contain benzodiazepine type drug actives (i.e. diazepam, lorazepam). These types of tablets can contain anywhere from 0.5 mg to 10 mg of drug active.

Although extraction methods can be used to extract these drug actives from the tablet matrices concentration of the dried extract can be problematic. This is especially true when using traditional glass slides and/or IR transmission windows (i.e. BaF₂). The dried material will disperse rather than dry down into a concentrated area (Figure 1A). As a result, larger volumes may be needed to build up a significant concentration of the analyte of interest to be able to detect it using IR and Raman spectroscopy.

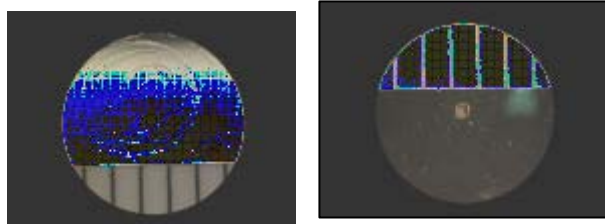


Figure 1A

Figure 1B

Figure 1: Photomicrograph of a 2 uL droplet of a lorazepam standard dried on (A) a glass slide and (B) on the Tienta substrate. The scale lines represent 1 mm.

In this application we look at the use of a new type of sampling substrate which allows for the deposition of small amounts of solvent and subsequent concentration when dried down (Figure 1B). The dried residue was analyzed using IR and Raman micro spectroscopy. The

sample used was a tablet containing 0.5 mg of lorazepam (Figure 2)

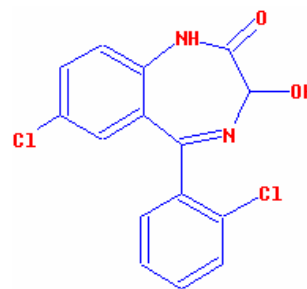


Figure 2: Chemical structure of lorazepam

Experimental

A 1.5 ug / uL (4.7 mM) standard solution of lorazepam (321.16 MW) in ethyl acetate was prepared by taking 1.5 mg of a lorazepam standard and dissolving it in 1 ml of ethyl acetate. 2 uL of the standard solution was then applied to the plate using a pipette. The plate with the 2 uL droplet was placed in an oven at 105 °C for 5 min. The resultant dried material was then analyzed using IR and Raman micro spectroscopy. The total amount of dried material analyzed was ~ 3 ug.

A single tablet containing 0.5 mg of lorazepam was ground using a small mortar and pestle. The ground powder was placed into a glass vial with 1 ml of ethyl acetate. The vial was shaken for 3 min to extract the lorazepam from the tablet matrix. The vial was then centrifuged for 5 min. Assuming 100 % extraction efficiency, the concentration of lorazepam in the ethyl acetate was ~ 0.5 ug/uL. 2 uL of the extractant was then deposited on the plate and placed into an oven at 105 °C and dried for 5 min. The resultant dried material was analyzed using IR and Raman micro spectroscopy. The total amount of dried material was ~1.5 ug.

The dried residue was then analyzed using IR and Raman micro spectroscopy. The IR measurement was performed using a diamond attenuated total reflectance (ATR) objective with a 100 um aperture, 128 coadds and a MCT detector. The

Raman measurements were made using a 633 nm laser with a 100 x glass objective (~1um spot size), 10 second integration time and averaged 2x.

Results and Discussion

Good agreement is observed when the Raman spectrum of a 3 ug deposit of a lorazepam standard is compared to the Raman spectrum of 1.5 ug deposit of lorazepam extracted from the tablet (Figure 3). The IR spectra also shows good agreement when the IR spectrum of a 3 ug deposit of a lorazepam standard is compared to the IR spectrum of 1.5 ug deposit of lorazepam extracted from the tablet (Figure 4). The agreement of the spectra provide strong evidence of the identity of the material extracted from the tablet.

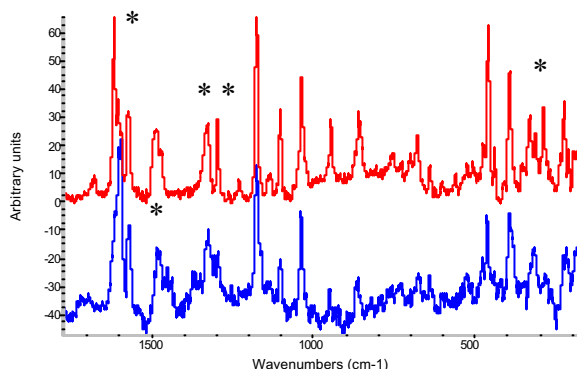


Figure 3: Raman spectrum of a 3 ug deposit of a lorazepam standard (red spectrum) compared to the Raman spectrum of 1.5 ug deposit of lorazepam extract from a 0.5mg lorazepam tablet (blue spectrum). These spectra were recorded on a LabRAM HR-IR using a 632 nm laser, 950 grooves /mm grating, 100X objective, 10 sec integration time averaged twice, and 200 um slit width.

There are however several differences in both the Raman and IR spectra, as indicated by the “*”. These subtle differences may be due in part to the crystal structure of the lorazepam standard and the extracted lorazepam crystals from the tablet. Different polymorph structures can arise from different crystallization conditions (i.e. using ethyl acetate extraction solvent), and differences in the source of the lorazepam standard vs. the lorazepam used in the manufacture of the tablet.

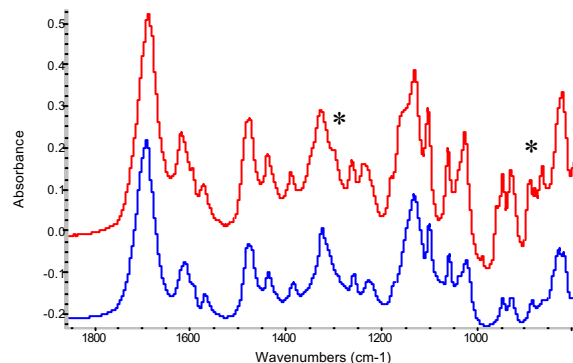


Figure 4: The Infrared spectrum of a 3 ug deposit of a lorazepam standard (red spectrum) compared to the Infrared spectrum of 1.5 ug deposit of lorazepam extract from a 0.5mg lorazepam tablet (red spectrum). Spectra were recorded on a LabRAM HR-IR using ATR objective, at 4 cm-1 resolution and 128 co-additions.

Conclusion

The use of a new type of sampling substrate allows for the extraction and concentration of a low dose drug substance. The concentration enhancement observed when using the substrate allows for techniques such as IR and Raman spectroscopy to be used to detect low concentration analytes.

References

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