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TECHNICAL NOTE

V. C. Bass,[1] *Ph.D.*

Identification of Benzodiazepins by Raman Spectroscopy

The Raman effect, first reported nearly 50 years ago, was of greater interest theoretically than practically until recent years. The comparative inefficiency of mercury vapor lamps for stimulating the Raman effect, and the corresponding necessity for large quantities of sample, precluded any general interest in the use of this technique in the laboratory. Recently, the availability of high-powered lasers for light sources and the increased sophistication of spectrophotometric instrumentation have stimulated new interest in Raman spectroscopy. A large body of literature has evolved, relating chiefly to the elucidation of molecular structure [*1-3*]. Thus Raman spectra can extend and complement the type of structural information available from infrared spectroscopy. However, infrared spectra have also long been used in the forensic laboratory to identify organic compounds such as drugs on a "fingerprint" basis with little or no assignment of bands to specific portions of a molecule. In the same way, a Raman spectrum is unique for a particular compound and thus provides positive identification of that compound.

The Sadtler Research Laboratories' collection of Raman spectra contains 4000 entries as of this writing. However, comparatively few of these are drugs. Therefore it seemed useful to examine this technique as an aid to forensic science by building up a library of Raman spectra of drugs. Work has already been done along these lines for pesticides [*4-6*] and barbiturates [*7*]. We took as our first group of compounds the four benzodiazepins diazepam, chlordiazepoxide, flurazepam, and oxazepam. A group of sympathomimetic amines is also under examination. Some preliminary work has been done in this laboratory and elsewhere [*8*] on the identification of thin-layer chromatography spots.

Experimental Procedure

Apparatus

Raman spectra were obtained with a Beckman Model 700 Raman spectrophotometer equipped with a Spectra-Physics Model 165 2-W argon ion laser. The 488.0-nm laser line was used throughout. The spectral slit width was 8 wavenumbers, as was the intermediate slit width; the slit height was 12 mm.

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[1] Research chemist, FBI Laboratory, Washington, D.C. 20535.

Reagents

Diazepam and chlordiazepoxide were obtained from Applied Science Laboratories. Oxazepam and flurazepam were obtained as *tert*-butyl alcohol solutions from Theta Corp. These drugs were also obtained as the pharmaceutical preparations Valium®, Librium®, Serax®, and Dalmane®, respectively.

Extraction Procedure

To one or two capsules or finely ground tablets add 30 ml distilled water and one drop concentrated hydrochloric acid; warm slightly and filter into a separatory funnel. Make alkaline with concentrated ammonium hydroxide and add 70 ml chloroform; shake. Discard aqueous layer. Filter organic layer and evaporate to dryness on a steam bath.

Results and Discussion

The spectrum of diazepam is shown in Fig. 1. The spectrum of a 10-mg Valium tablet, run as-is with a tablet holder, is shown in Fig. 2. Figure 3 is a spectrum of a solid extract prepared from a Valium tablet and run in a glass capillary. These three spectra illustrate some of the strengths and weaknesses of this technique. The ability to run opaque samples without further preparation results from the fact that Raman spectroscopy uses a scattering, rather than an absorption, effect. However, in a tablet whose ratio of active to inactive ingredient is less than about 1:10 the spectrum of the active substance is usually not clear enough to produce a good identification. Thus in Fig. 2 the major peaks for diazepam can be seen but it is not certain that the spectrum is that of the pure compound. On the other hand, the Valium extract (Fig. 3) provides a perfect match with the spectrum of pure diazepam.

An advantage that Raman spectroscopy has over infrared analysis is in the ability to analyze substances in water solution. In the region 0 to 2000 cm^{-1} the only water band, a minor one, occurs around 1640 cm^{-1}; there is also a rising baseline at low frequencies

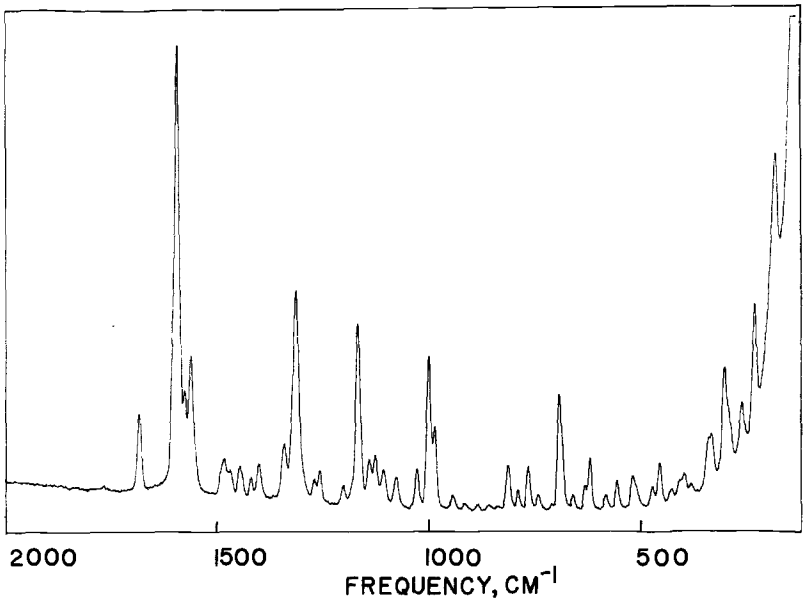

FIG. 1—*Raman spectrum of diazepam (solid).*

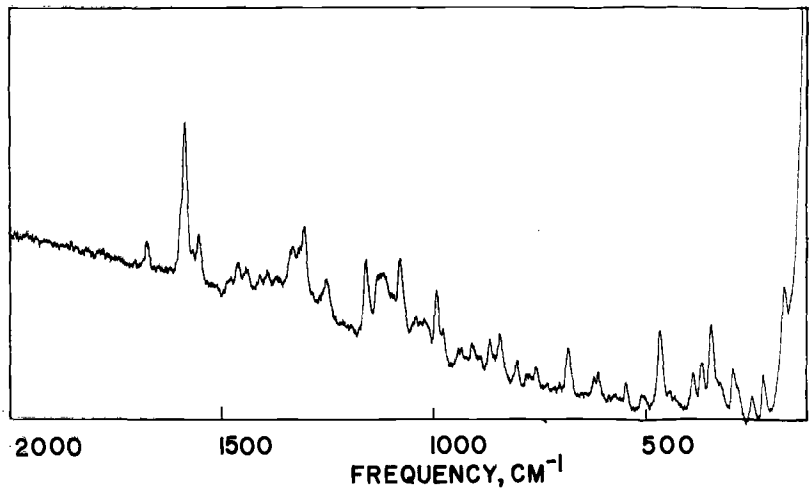

FIG. 2—*Raman spectrum of diazepam (as-is Valium tablet, solid).*

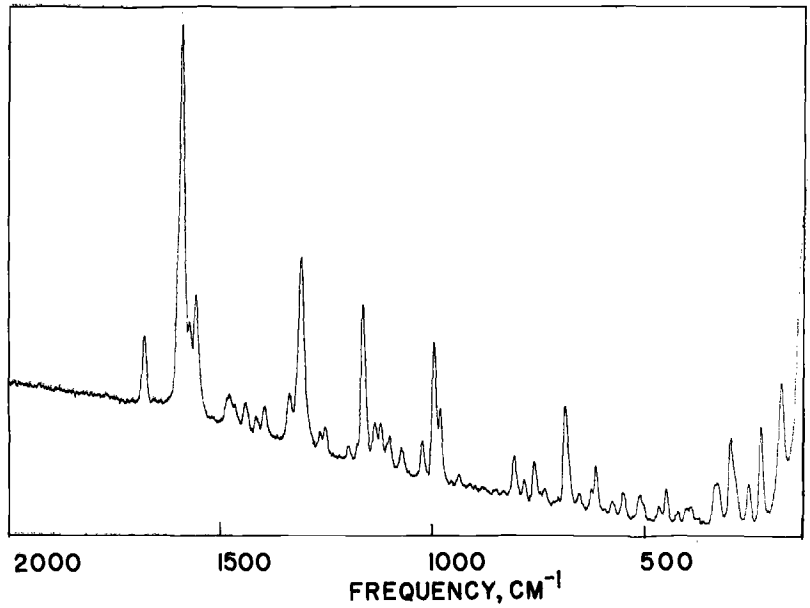

FIG. 3—*Raman spectrum of diazepam (Valium extract, solid).*

because of the increased Rayleigh scattering of the water. Neither of these effects presents serious interference, as the aqueous spectrum of diazepam in Fig. 4 shows. As might be expected, the aqueous spectrum differs slightly from that of the solid; the presence of a polar solvent causes changes in the vibrational and electronic relationships within the molecule.

Often the as-is approach is more successful with solutions than with solids. Figure 5 is the spectrum of a Valium tablet put into water solution and filtered; all of the peaks from the pure sample are present and the shifting baseline, a result of fluorescent material in the tablet, does not impede identification. The aqueous spectrum of a Valium tablet extract is very similar to that of pure aqueous diazepam, as seen in Fig. 6.

Another difficulty that sometimes arises is charring of the sample from the intense heat of the laser beam. Colored tablet fillers are the most frequent culprit, but sometimes pure white crystals will also char. This was the situation with chlordiazepoxide; it was therefore not possible, under the experimental conditions established, to obtain a spectrum of this drug in solid form. However, the aqueous spectrum of chlordiazepoxide (Fig. 7) was obtained and shows the intense bands in the region 1500 to 1600 cm^{-1} characteristic of this

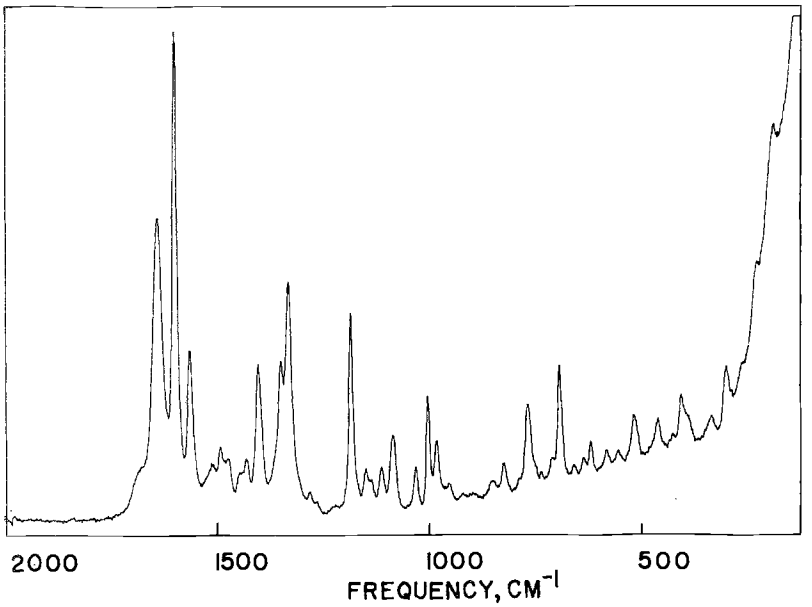

FIG. 4—*Raman spectrum of diazepam (aqueous).*

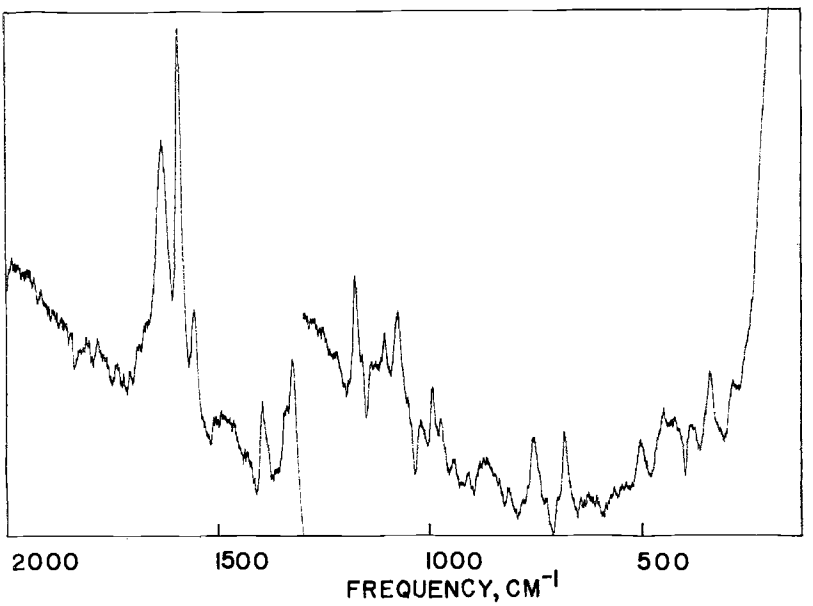

FIG. 5—*Raman spectrum of diazepam (as-is Valium tablet, aqueous).*

group of compounds. A Librium capsule dissolved in water and filtered produced a similar, identifiable spectrum (Fig. 8).

There are several methods that sometimes prevent charring: rotating the sample or using a different laser line, for instance. Neither of these approaches was attempted at this time.

Charring was also a problem with oxazepam and flurazepam. It was not possible to obtain solid-state spectra of these compounds in pure form or as extracts. There was also some difficulty in getting them into solution. However, the pharmaceutical products Serax (oxazepam) and Dalmane (flurazepam) did not char and produced satisfactory spectra

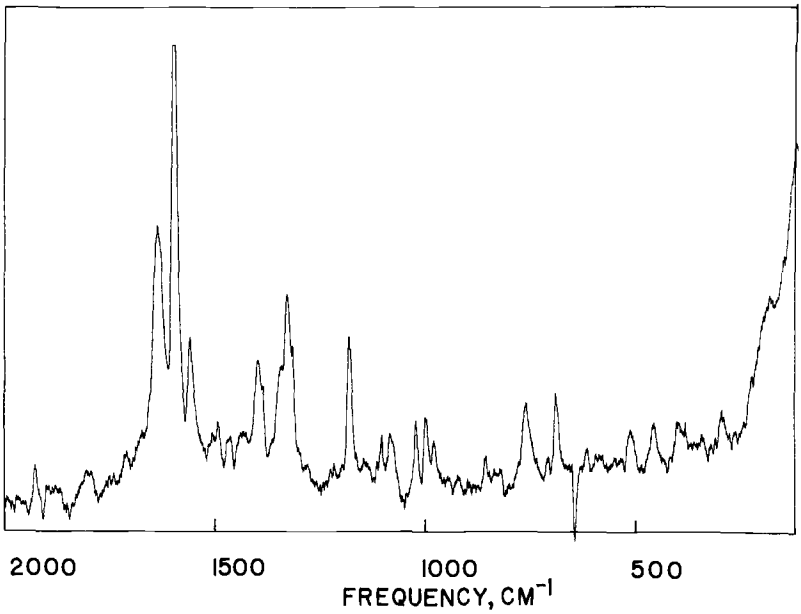

FIG. 6—*Raman spectrum of diazepam (Valium extract, aqueous).*

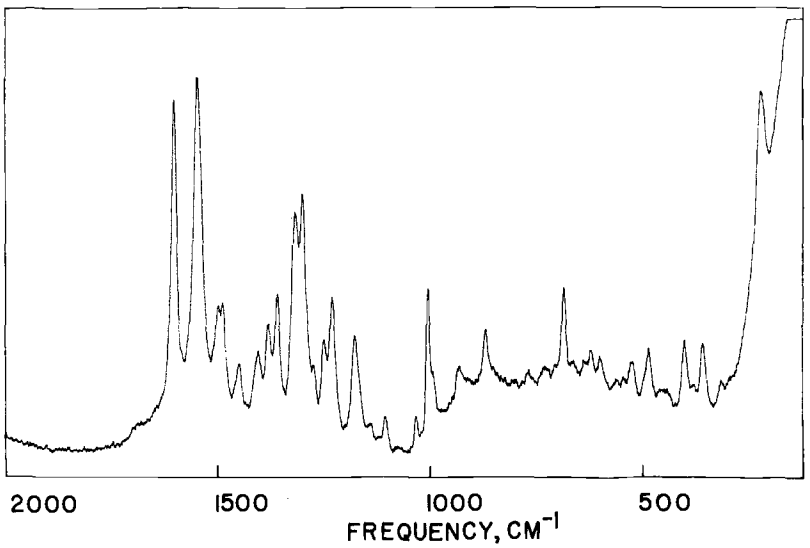

FIG. 7—*Raman spectrum of chlordiazepoxide (aqueous).*

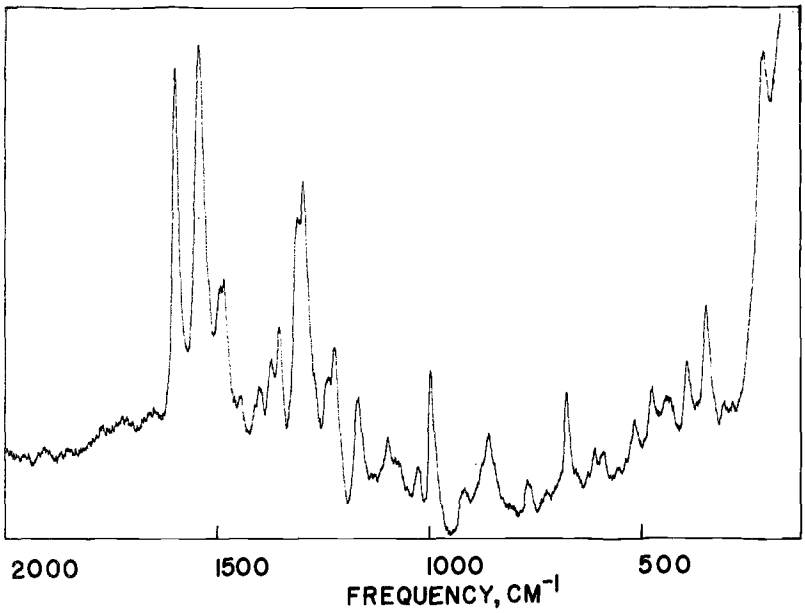

FIG. 8—*Raman spectrum of chlordiazepoxide (as-is Librium tablet, aqueous).*

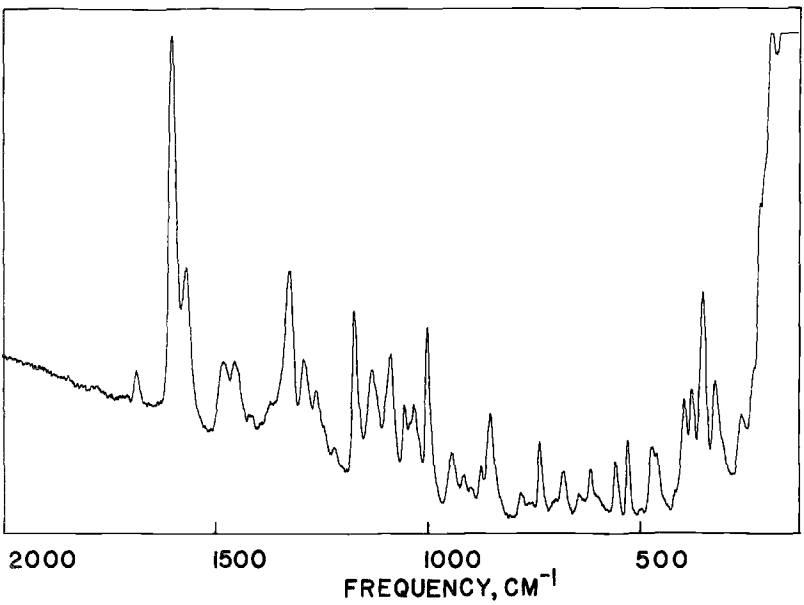

FIG. 9—*Raman spectrum of oxazepam (as-is Serax capsule, solid).*

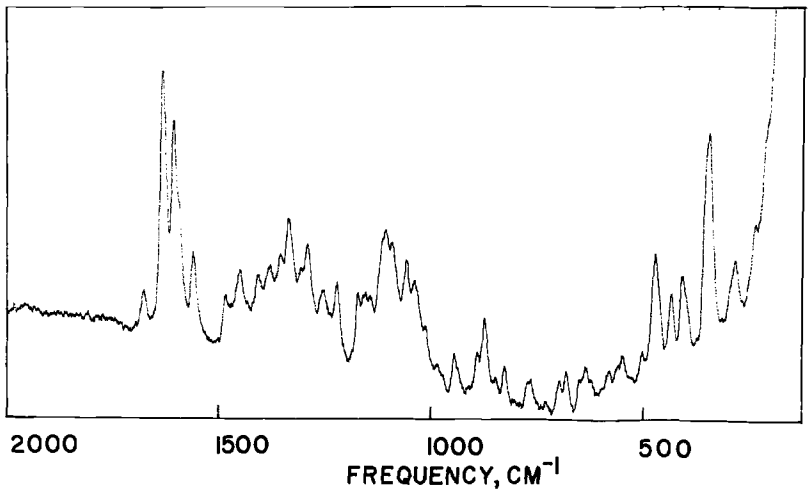

FIG. 10—*Raman spectrum of flurazepam (as-is Dalmane capsule, solid).*

showing characteristic bands, especially in the benzodiazepin region 1500 to 1600 cm^{-1} (Figs. 9 and 10). Thus these two drugs can also be identified through their Raman spectra.

Conclusions

From this study it is apparent that Raman spectra can be used successfully to identify small amounts of drugs such as the benzodiazepins. Group similarities exist among the spectra, and individual differences make it possible to distinguish each compound.

In this study no attempt was made to ascertain lower levels of sensitivity. This will be pursued at a later date. Reported levels are 3 μg of solid and 4 nl of a pure liquid [*9*]. This sensitivity, together with the ability to obtain spectra of substances without prior sample preparation and to work with aqueous solutions, makes Raman spectroscopy a useful technique in the field of forensic analysis.

Summary

The identification of drugs by means of Raman spectroscopy has been discussed, including the strengths and weaknesses of the technique. Spectra of a group of benzodiazepins, in pure form and as pharmaceutical preparations, have been presented.

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Address requests for reprints or additional information to
Virginia C. Bass, Ph.D.
FBI Laboratory
3256 Hoover Bldg.
Washington, D.C. 20535