# Differentiation of polymorphic forms of alprazolam and fluconazole by near infrared Fourier transform Raman spectroscopy

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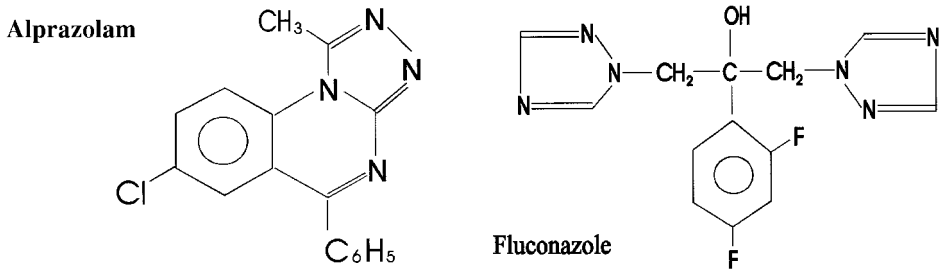
## Introduction

Characterization of polymorphic forms constitutes an important aspect of drug development. Different polymorphs of a drug may exhibit significantly different biological activities due to their different solubility and dissolution rate.[1] The stability and acceptability of the formulation may also be affected by different polymorphs. There are several techniques for analyzing polymorphs including differential scanning calorimetry (DSC), optical microscopy, infrared (IR) spectroscopy, solid state NMR[2] and x-ray powder diffraction which is the most widespread method. Recently, Fourier transform (FT) Raman spectroscopy has emerged as a new technique for differentiation and quantitative analysis of polymorphs.[3,4] The main advantage of FT-Raman is that no sample preparation is required and the polymorphic form of the sample will not be changed. The Raman spectrum is obtained by collecting back scattered light from either a powder or a formulation. It is well known that some polymorphic forms may interconvert under high pressure, by the grinding involved in sample preparation, or by the heating process used in DSC measurement.[5] Therefore, the FT-Raman technique is more suitable for studying certain polymorphs.

There are other advantages of FT-Raman spectroscopy over IR spectroscopy. Vibration modes involving symmetric and non-polar bonds, normally IR inactive, give strong intensities in Raman spectra. The interference from water, usually strong in IR, is minimal in Raman spectra. The spectral region between 40 and 400 $cm^{-1}$, normally not available with an IR spectrometer, can easily be measured with an FT-Raman spectrometer. In fact, most lattice vibrational modes in crystals fall in this region.

## Experimental

The Raman spectra were measured using a MB157 based FT-Raman/FT-IR spectrometer (Bomem, Hartmann & Braun Inc.). The excitation source was a Nd:YAG laser (Antares 76-s, Coherent Inc.) operating at 1.064 μm with a power stability of better than 1% rms. An InGaAs detector used in the spectrometer covers a Raman shift range from 150 to 3700 $cm^{-1}$. The laser beam is focused down to a 0.5 mm spot at the sample which is placed at the focus of an ellipsoidal




mirror. The scattered light is collected by this mirror and directed into the MB 157 near infrared (NIR) spectrometer. A laser power of about 100 mW at the sample was used. The samples (a few mg of powder) were placed in a glass capillary tube and their spectra were collected with an instrumental resolution of 4 $cm^{-1}$ for 200 scans.

Alprazolam, 8-chloro-1-methyl-6-phenyl-4H-[1,2,4] triazolo [4,3-a][1,4] benzodiazepine, is an anxiety-sedative drug that belongs to the benzodiazepine family.[6] Sample A was recrystallized in ethyl acetate solvent and sample B in either ethanol or propanol.

Fluconazole, 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-propan-2-ol, classified as an antifungal agent, has high oral efficacy and water solubility.[7] Sample (A) and sample (B) were obtained from two different manufacturers as supplier A and supplier B respectively.

## Results and discussions

### Fluconazole

FT-Raman spectra of two fluconazole samples over the frequency range of 150–1700 $cm^{-1}$ and 2700–3200 $cm^{-1}$ are compared in Figures 1(a) and (b) respectively. Both spectra appear complex with more than 60 well resolved peaks in the fingerprint region of 500–1700 $cm^{-1}$. Such complex spectra are not unexpected since fluconazole contains several functional groups such as triazole, 2,4-difluorobenzyl and hydroxy, in addition to the propyl backbone. The different Raman spectra of fluconazole for samples A and B indicated that they belong to two different polymorphic forms. The possibilities of pseudopolymorphs due to solvation or hydration were excluded based on the thermogravimetric analysis of both samples.

The strongest Raman band of fluconazole is the ring breathing mode of the 2,4- difluorobenzyl group at 734 and 737 $cm^{-1}$ for samples A and B respectively. Since the ring structure is relatively rigid, only a small shift was observed for the two different polymorphs. Another characteristic band in Figure 1(a) is the ring breathing mode of the triazole group at 1135 and 1136 $cm^{-1}$ for sample A and B respectively.

There are two spectral regions which show clear differences in the Raman spectra of sample A and B. The first region of interest is 1000–1150 $cm^{-1}$. The band at 1026 $cm^{-1}$ in the spectrum of sample A is shifted to 1018 $cm^{-1}$ in the spectrum of sample B with an enhanced intensity. This band was assigned to the C–(OH) stretching vibration. This shift was expected considering that polymorphs often exhibit different hydrogen bonding network.[8] Another band at 1117 $cm^{-1}$ in the spectrum of sample A is shifted to 1106 $cm^{-1}$ with a reduced intensity in the spectrum of sample B. This band was assigned to the C–C stretching mode in the propyl backbone. These two bands show larger shifts in the spectra of the two polymorphic forms as compared to the shifts of breathing modes of the triazole and difluorobenzyl ring groups. This is due to the fact that when molecules are packed together in different arrangements in crystals (i.e. polymorphism) the

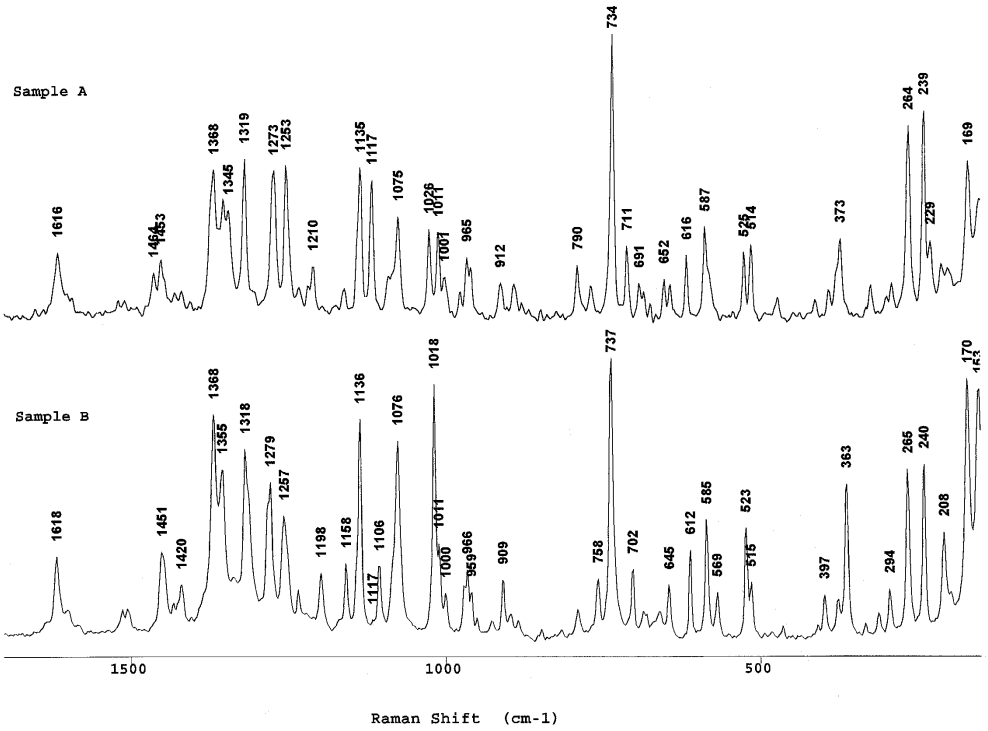



**Figure 1(a). FT-Raman spectra of fluconazole sample A and sample B from 150 to 1700 $cm^{-1}$.**

vibrational mode involving less rigid bonds will be affected more than those of rigid structures. The second region of interest is that of the C–H stretching bands between 2950–3140 $cm^{-1}$. The C–H stretching modes tend to be weak in the IR due to low polarity, however, they are relatively strong in the Raman spectrum. The bands at 3089 and 3015 $cm^{-1}$ in the spectrum of sample A, assigned to the C–H stretches of the difluorobenzyl group, are shifted to 3097 and 3022 $cm^{-1}$ respectively in the spectrum of sample B. The C–H stretch band of $CH_2$ in the propyl backbone at 2968 $cm^{-1}$ in the spectrum of sample B is split into two bands at 2963 and 2975 $cm^{-1}$ respectively in the spectrum of sample A.

Other features which show the differences in the Raman spectra of the two polymorphic samples are the band splittings. Bands at 1451 and 2968 $cm^{-1}$ in the spectrum of sample B are split into doublets of ~12 $cm^{-1}$ apart in the spectrum of sample A. This kind of band splitting can occur when the symmetry of the molecule is violated, leading to the decoupling of normally degenerative vibrational modes. These band splittings occur in the vibrational modes of $CH_2$ in the propane backbone which is less rigid than the triazole and difluorophenyl rings. The low-frequency modes, arising largely due to lattice vibrations, are very sensitive to structural changes in the solid state. For example, the band at 229 $cm^{-1}$ in the spectrum of sample A cannot be found in the spectrum of sample B. The intensity of the 208 $cm^{-1}$ band in the spectrum of sample B is substantially reduced in the spectrum of sample A. The spectral differences in this region between

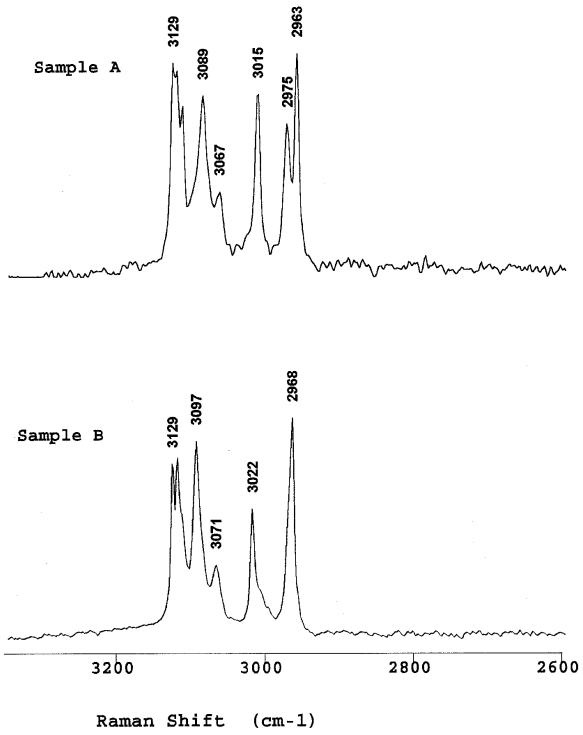



**Figure 1(b). FT-Raman spectra of fluconazole sample A and sample B from 2700 to 3200 $cm^{-1}$.**

the two polymorphs can be explained by the differences in intermolecular interactions and differences in crystal symmetry in the two forms.

The FT-Raman results agree well with those of DSC and x-ray powder diffractometry. The DSC thermogram of sample A has one endothermic peak at 139.7°C, indicating a single polymorphic form I. Sample B exhibits a main endothermic peak at 138.9°C and a smaller peak at 139.7°C, indicating that it consists mainly of another polymorphic form II and possibly a small amount of the form I. However, it is also possible that a small amount of form II transformed into form I during the heating process. The x-ray diffraction pattern of sample A exhibits main diffraction peaks at $2\theta$ = 10.0, 15.0, 16.0, 16.6 and 20.0°, and those of sample B exhibit main diffraction peaks at $2\theta$ = 11.6, 14.7, 15.8, 17.3, 18.4, 19.5 and 24.4°. The x-ray results also indicate that sample A consists of only one polymorphic form (I), however, sample B consists of mainly (90%) of another polymorphic form (II) and a small amount (10%) of polymorphic form I since the strong peaks at 10.0, 16.6 and 20.0° in pattern A also appear in pattern B although with much reduced intensities.

The Raman bands in Figure 1 have an average bandwidth at half of their intensity of ~8 $cm^{-1}$. At 10% of their intensity, the bandwidth is broadened to ~20 $cm^{-1}$. Since most of the band shifts and splittings due to polymorphism are about 10 $cm^{-1}$ or less, the 10% of polymorph I in sample B would contribute only to the shoulders and tails of the Raman bands of polymorph II and consequently would be difficult to quantify. The band at 1117 $cm^{-1}$ in the spectrum of polymorph

I (A) is an exception. Because of its large shift (11 $cm^{-1}$) in the spectrum of polymorph II, it can still be observed in the spectrum of sample B. The quantitative analysis of binary polymorphic mixtures by FT-Raman spectroscopy was reported recently.[9] However, it requires pure polymorphic samples which are not available for fluconazole polymorphs.

### Alprazolam

FT-Raman spectra of two alprazolam samples are compared in Figures 2(a) and (b). In the spectral region of 150–1700 $cm^{-1}$ there are a number of bands in the spectrum of sample B, such as those at 259, 406, 686 and 1311 $cm^{-1}$ split into doublets in the spectrum of sample A. In the spectral region of 2700–3200 $cm^{-1}$, the band at 3072 $cm^{-1}$ in the spectrum of sample A, assigned to the aromatic CH stretching, splits into a doublet at 3076 and 3069 $cm^{-1}$ respectively in the spectrum of sample B. Another band at 2908 $cm^{-1}$ for sample A also splits into a doublet in the spectrum of sample B. Three bands at 3002, 2994 and 2981 $cm^{-1}$ respectively in the spectrum of sample A show substantial changes in their intensities in the spectrum of sample B. These differences indicate that sample A and B belong to two different polymorphic forms, I and II, respectively. Since alprazolam consists of relatively rigid rings the band shifts are small for two different polymorphs.

The FT-Raman analysis agrees well with those of DSC and x-ray powder diffractometry. The DSC thermogram of sample A has one exothermic peak at 228.3°C, indicating a single polymor-

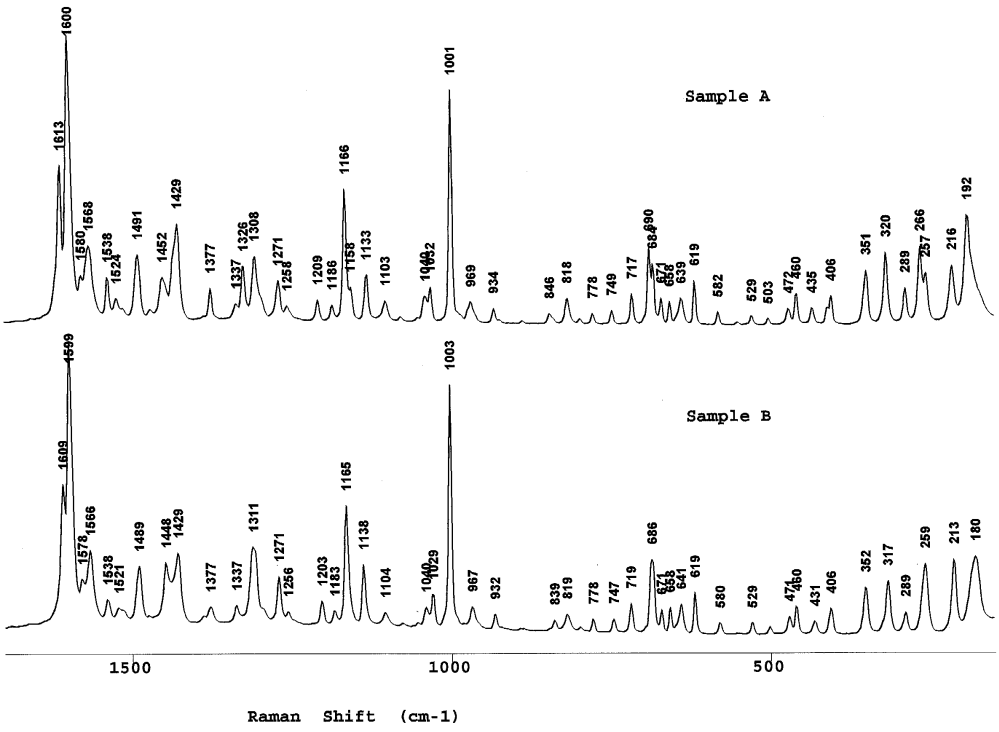



**Figure 2(a). FT-Raman spectra of alprazolam sample A and sample B from 150 to 1700 $cm^{-1}$.**

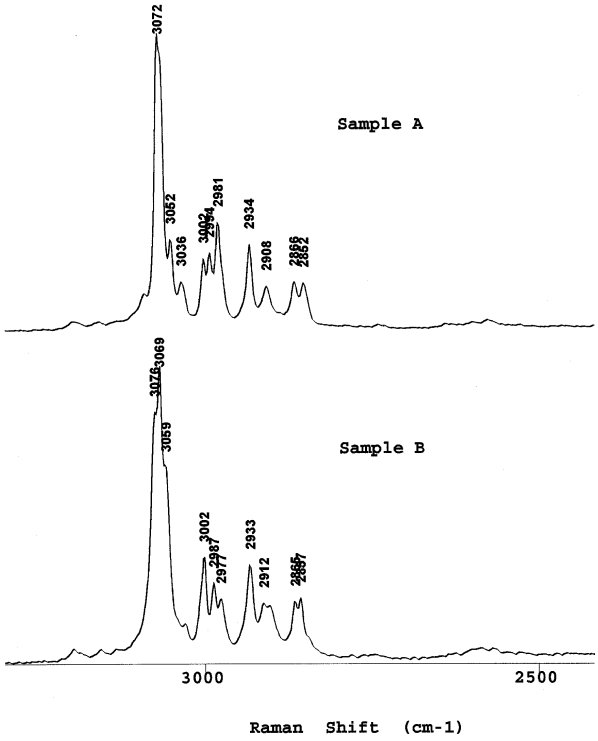



**Figure 2(b). FT-Raman spectra of alprazolam sample A and sample B from 2700 to 3200 $cm^{-1}$.**

phic form I. Sample B exhibits two exothermic peaks at 221.0 and 227.1°C and one endothermic peak at 224.5°C, indicating that it consists of another polymorphic form II. The x-ray diffraction patterns of sample A exhibits main diffraction peaks at $2\theta$ = 6.1,7.1,13.1,15.8,18.4 and 20.2° and those of sample B exhibits main peaks at $2\theta$ = 9.5,12.2,14.9,19.0 and 24.2° respectively, confirming that they belong to two different polymorphic forms.

## Conclusions

From the data presented in this paper, it is evident that polymorphs of alprazolam and fluconazole can be readily differentiated using FT-Raman spectroscopy. The differences of the polymorphs are characterized by the band shiftings and splittings in their Raman spectra. The existence of polymorphs in alprazolam and fluconazole is also confirmed by the DSC thermograms and by the x-ray powder diffraction patterns. The fact, however, that the polymorphs can be readily characterized without the need for sample preparation shows the great potential of FT-Raman spectroscopy in pharmaceutical analysis.

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