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Solid-state characterization of zanoterone

William L. Rocco [a,*], Cynthia Morphet [b], Sharon M. Laughlin [a]

[a] *Sanofi Research Division, 1250 S. Collegeville Rd, Collegeville, PA 19426, USA*
[b] *Shaklee Inc., 1992 Alpine Way, Hayward, CA 94545, USA*

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Abstract

Differential scanning calorimetry (DSC) data, in conjunction with Fourier transform infrared (FTIR) spectroscopy and X-ray powder diffraction (XRPD), was used to discriminate various polymorphic forms of zanoterone. The solid-state degradation rate of form IV (hemihydrate A) was found to be greater than that of form III at 40° C/25% RH and 40° C/75% RH. At 40° C/75% RH, the rate of degradation was 4-fold higher for form IV vs form III. Mixtures of forms III and IV were observed following desolvation of the acetonitrile solvate at 40° C with humidity levels of 60 and 70% RH, while at 40° C and 20–40% RH pure form III was formed. At 60° C, a mixture of forms III and IV was observed at 70% RH but not at lower relative humidity. The concentration of each form of zanoterone in solution was measured as a function of time at room temperature in 3% sodium lauryl sulfate. Forms I, II, and V showed greater initial concentrations than form III, but the values decreased with time due to the precipitation of form VI (hemihydrate B). Therefore, concentrations measured for forms I, II, and V were not equilibrium solubilities for these forms. Forms IV and VI showed lower initial concentrations (than form III) which did not change significantly with time.

Keywords: Polymorphism; Physical characterization; Solubility; Stability

1. Introduction

It has been shown that the varying properties of the different crystal forms of a compound can influence the bioavailability, stability, and processibility of the drug product (Haleblian and McCrone, 1969; Haleblian, 1975; Shefter, 1981; Byrn, 1982; York, 1983). Complete characterization of the different polymorphs is an essential part of a preformulation program.

Several crystal forms of zanoterone exist; however, form III is the desired polymorphic form used in the clinical drug product. Form III was chosen due to the high reproducibility of isolation in manufacture. During the manufacture of zanoterone, the drug substance is first isolated as the acetonitrile solvate, then dried to remove the acetonitrile, thus converting the solvate to form III. This investigation was performed to provide an extensive study of the physical characteristics and to gain an understanding of the process parameters which are critical to obtaining the desired quality of zanoterone:

* Corresponding author. Tel. 610-983-7949.

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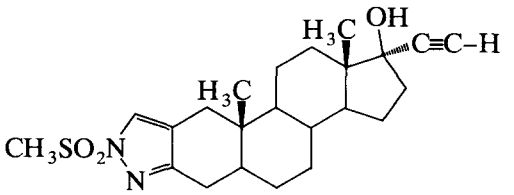


Several polymorphic forms were characterized by thermal analytical techniques, Fourier transform infrared spectroscopy, and X-ray powder diffraction. This study examines the physical/chemical stability and solubility of various polymorphs and illustrates the effects of desolvation conditions on the resulting crystal form.

2. Materials and methods

2.1. Preparation of polymorphs

Zanoterone was recrystallized from acetonitrile by dissolving in solvent at 70° C and cooling to 0° C in an ice bath. The product crystallized and was isolated by filtration as the acetonitrile monosolvate form of zanoterone.

Form I was obtained by heating form VI of zanoterone to 180° C for 30 min under nitrogen. Form II was obtained by recrystallization from ethanol and vacuum drying at 45° C. Form III was isolated by desolvating the acetonitrile solvate form at 80° C under vacuum. Samples of form IV (hemihydrate A) were initially isolated by suspending form III in acetonitrile, cooling to 0° C, filtering, and air drying under ambient conditions. Form IV was also observed in the desolvation study (below) by desolvating the acetonitrile solvate at 40° C/75% RH.

Form V was obtained by recrystallization from methanol, followed by recrystallization from acetonitrile, and air drying at 80° C. Form VI (hemihydrate B) was isolated by recrystallization from ethanol/water (70:30% v/v) (23° C crystallization temperature). Forms I, II, and III were identified by Houtaling (unpublished data) in 1985.

2.2. Characterization techniques

Differential scanning calorimetry (DSC) was performed on the Perkin Elmer DSC-7 system at 10° C/min heating rate under nitrogen purge. The samples were encapsulated in aluminum pans with pierced lids to allow for escape of volatiles. The system was calibrated with indium (m.p. 156.6° C) and tin (m.p. 231.9° C) prior to use.

Thermal gravimetric analysis was performed on the Perkin Elmer TGA-7 system at 10° C/min with nitrogen purge gas. The accuracy of the instrument was verified with barium chloride dihydrate (14.7% water) prior to use.

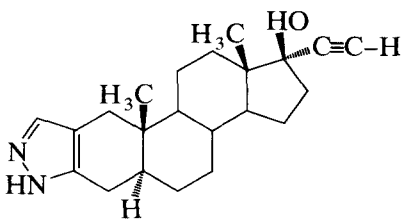
Fourier transform infrared (FTIR) spectra were obtained with the Nicolet 730 system equipped with an MCT detector. The samples were prepared as potassium bromide disks at approx. 1% drug. For quantitative analysis, the total drug concentration was increased to 3%, data were converted to absorbance, and the ratio of absorbance, $A(1642\ cm^{-1})/A(1581\ cm^{-1})$, was calculated. Plotting this ratio vs concentration allowed the determination of form IV concentration in mixtures with form III. The absorbance values were corrected for baseline changes at each wavelength. Each concentration (2, 4, 6, 8, and 16%) was examined a minimum of three times and a linear regression performed on average peak ratio as a function of concentration.

X-ray powder diffraction (XRPD) patterns were obtained on the Scintag XDS system with Cu-α radiation and a liquid nitrogen cooled solid-state germanium detector. Samples were ground with a mortar and pestle, spread onto a zero background quartz plate, and scanned at a rate of 5°/min.

The high-performance liquid chromatographic method utilized throughout this study consisted of a Waters 510 pump, a Waters WISP 710B autosampler, a Kratos 757 UV/Vis variable-wavelength detector, and a Waters 740 data module. The column was a 10 μm, 25 cm Whatman Partisil ODS-3, with a mobile phase of acetonitrile/methanol/water/85% (w/w) phosphoric acid (400:300:300:2), at a flow rate of 1.0 ml/min. Eluting peaks were monitored at 240 nm.

Two samples were prepared at each stability station, and duplicate injections were made for each sample. A 50 mg quantity of stressed drug substance was dissolved in 10.0 ml of acetonitrile and sonicated for 5 min to obtain a 5 mg/ml

solution. This solution was injected onto the column and the concentration of WIN 17782



(the primary degradation product) was calculated from peak area ratios against an external standard.

The results were reported as a percentage of the total drug content of the sample. An aliquot of the original sample was diluted with acetonitrile/water (60:40) to obtain a final concentration of 0.1 mg/ml. Dilution was necessary in the samples prepared for the determination of zanoterone to prevent column overload. This diluted sample was injected onto the column, and the concentration of drug substance was determined from peak area ratios against an external standard.

The solubilities of several crystal forms of zanoterone were determined in aqueous sodium lauryl sulfate (3% by weight). The solubility values were not equilibrium values for forms I, II, and V due to apparent phase transformations. Approx. 8 mg of each sample was added to a screw-capped vial containing 2 ml of solvent. The samples were mixed on a laboratory rotator. An aliquot was filtered (0.45 μm) at several time points and diluted 1:25 for HPLC analysis. The residual solid was recovered and analyzed by FTIR for several samples.

2.3. Physical / chemical stability studies

Forms III and IV were investigated for chemical/physical stability because these forms were the desired form and a crystalline impurity observed in production, respectively. The acetonitrile solvate was included since it was used in production to generate form III. Samples of polymorphic forms III, IV, and the acetonitrile solvate were held at 40° C (approx. 25% RH), 40° C/75% RH and 80° C/vacuum (the temperature used in the manufacturing process). The samples were analyzed for degradation level (% WIN 17782) by HPLC, polymorphic form by FTIR, and water content by Karl-Fisher (Mettler DL 20) analysis. Surface area data were obtained with the Quantasorb (Quantachrome Inc.). Moisture sorption studies were performed on the VTI MB 300W system (VTI Corporation), cycling samples from 40° C/25% RH to 40° C/75% RH.

The acetonitrile solvate also was held at 40 and 60° C over a range of relative humidity values to determine the effect of humidity and temperature on the polymorphic form that is obtained after desolvation. The FTIR spectra of these samples were used to determine polymorphic form and also qualitatively to assess whether desolvation was complete.

3. Results and discussion

3.1. Polymorph characterization

3.1.1. DSC

The DSC scans for polymorphic forms I–VI and the acetonitrile solvate are shown in Fig. 1. The form I scan shows a single melting peak at approx. 212° C and a heat of fusion of 60.6 J/g.

The scan for form II demonstrated an endothermic peak at approx. 195° C due to melting. The melting peak appeared to be preceded by a weak shoulder possibly indicative of a low level of form III. The XRPD data appear to confirm this hypothesis (section 3.1.3).

The DSC scan for form III, the polymorphic form used in the clinical formulation, shows two significant endotherms. The endotherms were observed at 190° C due to simultaneous melting and polymorphic transformation of form III to form I and at 212° C due to the melting transition of form I. Since the peak at 190° C was broader and of lower energy (28 J/g) than a typical melting peak, it was apparent that the transition was more complex than simple melting.

The scan for form IV (hemihydrate A) showed a broad endotherm at approx. 70–100° C followed

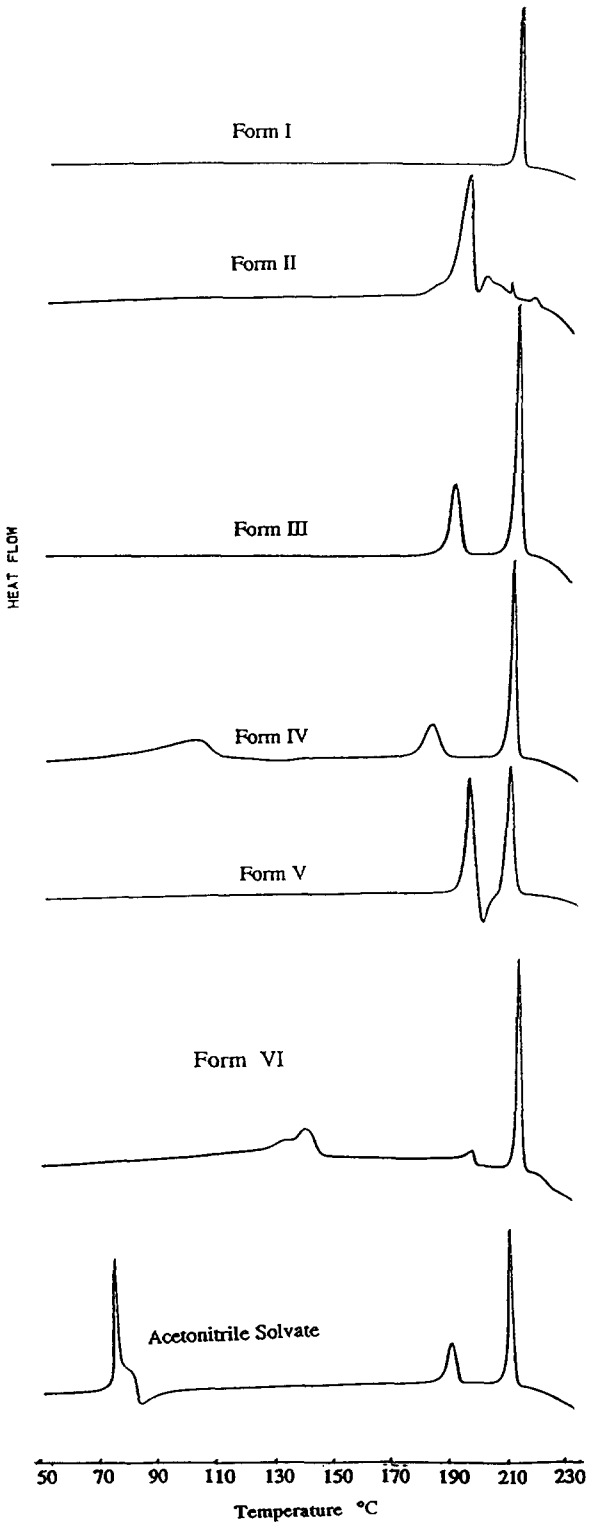


Fig. 1. Zanoterone DSC data for forms I–VI and acetonitrile solvate. Perkin Elmer DSC-7; scan rate, 10° C/min.

by endotherms at approx. 183 and 211° C. The broad endotherm appeared to be related to two separate events: the loss of moisture and the subsequent transformation of form IV to form III. The endotherms observed at 183 and 211° C were due to the transformation of form III to form I and the melting of form I, respectively. It should be noted that Karl-Fisher analysis of form IV samples indicated water levels of 1.7–2.0%, approximately equal to the theoretical value for a hemihydrate (2.1%).

The DSC scan for form V showed multiple peaks characteristic of melting/ recrystallization/remelting phenomena. The scan showed an endotherm at 195° C due to melting of form V, followed by an exotherm due to recrystallization. A second melting peak was observed at 209° C, presumably due to melting of form I.

The DSC scan for form VI (hemihydrate B) shows an endothermic peak from 120 to 140° C followed by peaks at approx. 195° C (weak) and 212° C (sharp). Thermal gravimetric analysis showed a weight loss of about 2.2%, approximately equal to the theoretical value for a hemihydrate of 2.1%. The data for water content was confirmed by Karl-Fisher analysis. It is important to note the differences in temperature for the peaks due to moisture loss for forms IV and VI. Form IV shows a DSC peak at 70–100° C while form VI demonstrates a peak from 120–140° C. Clearly the moisture in form VI is more tightly bound.

The initial peak of the acetonitrile solvate sample, due to desolvation, is followed by peaks characteristic of polymorphic form III. Thermal gravimetric analysis showed that the desolvation was associated with the presence of 8.7% of acetonitrile. This value agrees well with the theoretical value (9.0%) for a monosolvate of acetonitrile.

The DSC data illustrated differences in the thermal behavior of crystal forms I–VI and the acetonitrile solvate. The presence of solvate formation was evident in the initial peaks in forms IV, VI, and the acetonitrile solvate. However, since a single technique is insufficient to investigate polymorphic behavior, these samples were further examined by FTIR and X-ray powder diffraction.

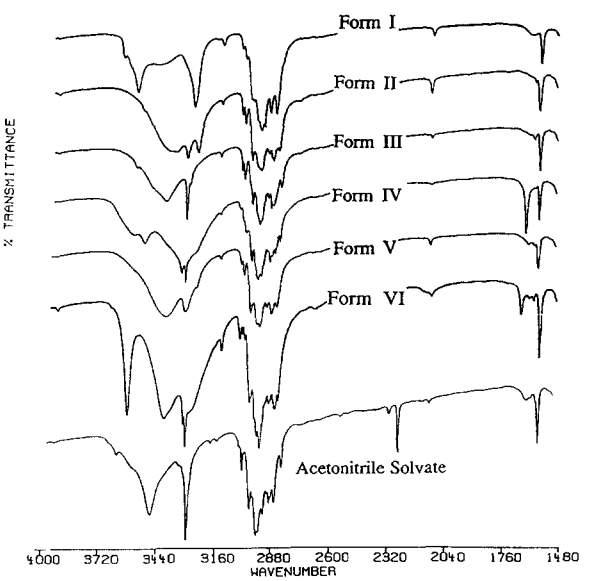


Fig. 2. Zanoterone FTIR data for forms I–VI and acetonitrile solvate. Nicolet 730 FTIR; 1% potassium bromide pellet.

3.1.2. FTIR spectroscopy

The FTIR spectra of forms I–VI of zanoterone are shown in Fig. 2. Each spectrum was unique and can be differentiated in several regions. In the wavenumber region between 3400 and 3700 cm^{-1}, where absorbance due to the hydroxyl bond was expected, the polymorphs show broad peaks typical of hydrogen bonding. A sharper hydroxyl peak was observed in the form VI sample at approx. 3600 cm^{-1}. In the region of 3250–3400 cm^{-1}, a different absorbance due to the alkyne hydrogen was observed for each polymorph.

The region between 2000 and 2500 cm^{-1} showed a weak peak for each polymorph at 2100–2110 cm^{-1}. Absorbance in this region is typical of a carbon-carbon triple bond. The peak appeared to be more intense for form II.

The acetonitrile solvate shows a unique peak at 2254 cm^{-1} due to the presence of a nitrile linkage. This peak was useful in qualitatively assessing the presence of a partial solvate.

The double bond region (1450–1650 cm^{-1}) showed a strong sharp peak at approx. 1642 cm^{-1} in the form IV spectrum. This peak was not observed in the spectrum of polymorph III and was therefore used in quantitative studies.

The ability to quantitate form IV in mixtures (section 3.2) with form III was determined by preparing mixtures at 2, 4, 6, 8, and 16% form IV in form III and plotting the peak ratio, A(1642 cm^{-1})/A(1581 cm^{-1}), vs form IV concentration. The absorbance at each wavenumber was corrected for baseline changes. A linear correlation was observed with a correlation coefficient of 0.993.

The FTIR data corroborated the DSC results, illustrating differences in the spectrum for each form. The hydroxyl, alkyne, and carbon-carbon double bond regions were particularly useful in distinguishing the different forms. X-ray powder diffraction patterns (below) confirm the DSC, and FTIR conclusions.

3.1.3. XRPD

The XRPD patterns for forms I–VI and the acetonitrile solvate are shown in Fig. 3. The patterns were unique and indicated that the samples were predominantly different polymorphic forms. The pattern for the form II sample showed several weak peaks (12.0, 14.5°) consistent with the form III pattern. These data suggested the presence of form III at a low level, thus corroborating the DSC results. The pattern observed for form

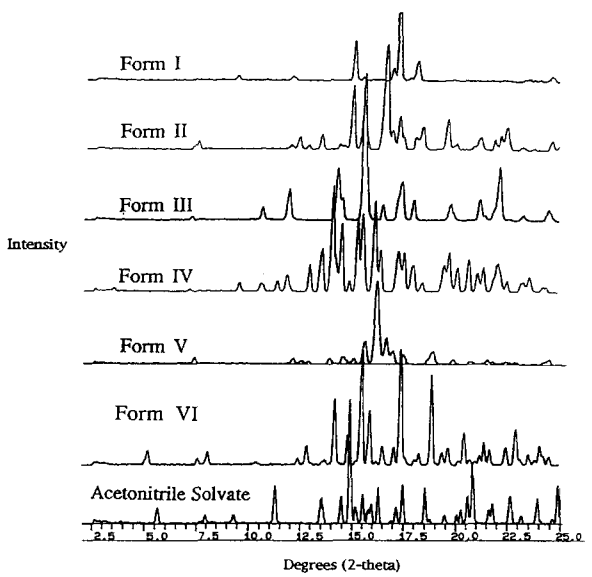


Fig. 3. Zanoterone XRPD patterns for forms I–VI and acetonitrile solvate. Scinta XDS system; 5° (2θ)/min.

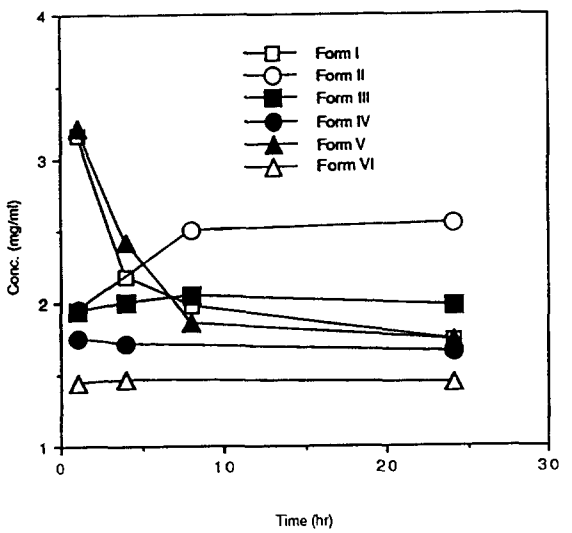


Fig. 4. Zanoterone solubility data. Solvent, 3% sodium lauryl sulfate; temperature, 23° C; HPLC analysis.

IV may also suggest the presence of some form III.

3.1.4. Solubility

The concentration in solution for forms I–VI of zanoterone was determined in 3% sodium lauryl sulfate as a function of time. Sodium lauryl sulfate was utilized to enhance the aqueous solubility and allow differentiation of the various forms. Two separate samples of form III were analyzed at 4 and 24 h to determine reproducibility. The replicate values agreed within 2–3%. The data (Fig. 4) showed significant differences between the samples. Forms I, II, and V, despite having higher melting points than form III, appeared to have higher initial concentrations in solution which then decreased with time. Form II was analyzed at 48 h to determine whether the solubility would eventually decrease with time. A concentration of 2.28 mg/ml was observed, indicating a concentration decrease. Since the concentrations of forms I, II, and V decreased with time it was apparent that the initial concentrations were not equilibrium solubilities. After 24 h, the concentrations of the form I and form V samples had decreased to a concentration similar to that of form IV. However, these samples appear to be still decreasing at 24 h and are supersaturated with respect to form VI. Since the FTIR spectrum of the residual solid was consistent with form VI (see below), one would expect the concentration for these forms to decrease to that of form VI eventually. It should be noted that a similar observation was made with stanozolol (Rocco, 1994). The concentration of form I of stanozolol in 2% SLS was observed to be higher than that of the monohydrate after 24 h despite showing conversion to the monohydrate by FTIR.

The form IV (hemihydrate A) sample had a lower solubility (1.7 mg/ml) than form III (2.0 mg/ml), while the form VI (hemihydrate B) sample was the lowest of all the samples at 1.5 mg/ml. Lower solubility and dissolution rate values for the hydrated form of a compound are common; this behavior has been observed with numerous compounds including succinylsulfathiazole (Shefter and Higuchi, 1963), theophylline (Shefter and Higuchi, 1963; De Smidt et al., 1986), and stanozolol, also a steroid (Rocco, 1994). The existence of multiple polymorphic forms for a particular hydrated state was also observed for amiloride HCl (Jozwiakowski et al., 1993). Equivalent solubilities were reported for the two hydrated polymorphs.

Samples of residual solid from the solubility studies for forms I, III, IV, and V were isolated at the end of the experiment and analyzed by FTIR to determine if polymorphic changes occurred during the experiment. The spectra of some (form I, form V) of these samples appeared to indicate conversion to form VI, while others (form III, form IV) were consistent with the spectrum of the sample prior to solubility analysis. The spectra of the residual solid in the form I and form V samples closely matched that of form VI. It is not surprising that the form I and V samples had converted to form VI, since form VI appears to be the most stable (least soluble).

The form II sample was not analyzed by FTIR due to poor recovery. However, observation by polarized light microscopy indicated the presence of long needle-shaped crystals, in contrast to the starting material which consisted of irregular shaped crystals; this suggests the conversion to a hydrated form had initiated, since a needle-like habit was also observed for form VI.

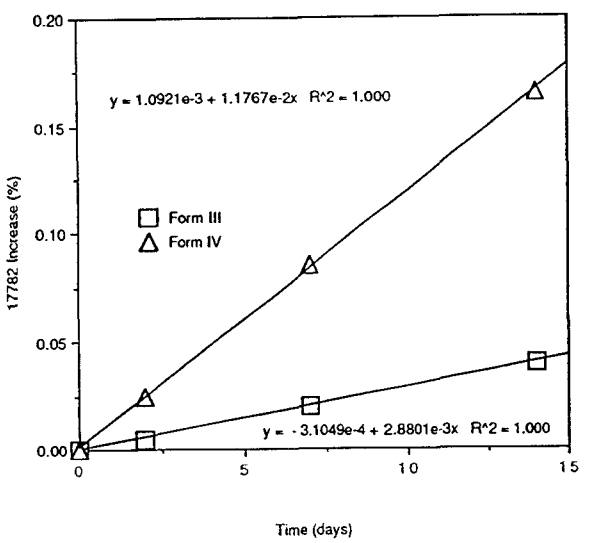


Fig. 5. Zanoterone solid-state stability at 40° C/75% relative humidity. % WIN 17782 increase vs time for form III vs form IV.

3.2. *Solid-state stability study-physical / chemical stability*

Samples of form III, form IV, and the acetonitrile solvate were held at 40° C (approx. 25% RH), 40° C/75% RH, and 80° C to determine the effect on chemical degradation (% WIN 17782 formation) and polymorphic form. It should be noted that WIN 17782 is a potent estrogen and therefore the drug substance specification for % WIN 17782 was set at a very low value (0.1%).

The data for % WIN 17782 are plotted in terms of increase (rather than actual value) for samples of forms III and IV held at 40° C/75% RH in Fig. 5. Each point is the average increase for two separate samples. The data showed a significantly higher rate of degradation for form IV vs form III. Linear regression of the data indicated that form IV degrades approx. 4-fold faster than form III under these conditions. Surface area analysis demonstrated that the average specific surface area was 0.6 m^2/g for both forms, indicating that the reaction rate differences cannot be explained by surface area differences.

The data for samples held at 40° C (approx. 25% RH) (Fig. 6) also suggested a higher degradation rate for form IV vs form III, but the increases were less substantial for these samples when compared with the 40° C/75% RH data.

The greater rates of degradation for form IV may or may not be related to the increased moisture levels for form IV. The moisture levels observed for form IV were about 1.8% at both 40° C (approx. 25% RH) and 40° C/75% RH. In contrast, the water levels for form III were 0.2% or less. Water participates in the reaction of zanoterone to form WIN 17782 by attack at the methane sulfonyl linkage. It is possible that the methane sulfonyl linkage is more vulnerable in the form IV crystal structure and therefore form IV would degrade more rapidly than form III.

It is interesting to note that both the form III and form IV samples degraded faster at 40° C/75% RH than at 40° C/25% RH despite having similar moisture levels under the two conditions (as measured by Karl-Fisher analysis). Ahlneck and Zografi (1990) discussed the ability of very small amounts of increased moisture to cause significant changes in chemical and physical properties through modification of molecular mobility. In order to test the theory that very small increases in the amount of water may be present at 40° C/75% vs 40° C/25% RH, samples were analyzed on the VTI moisture sorption

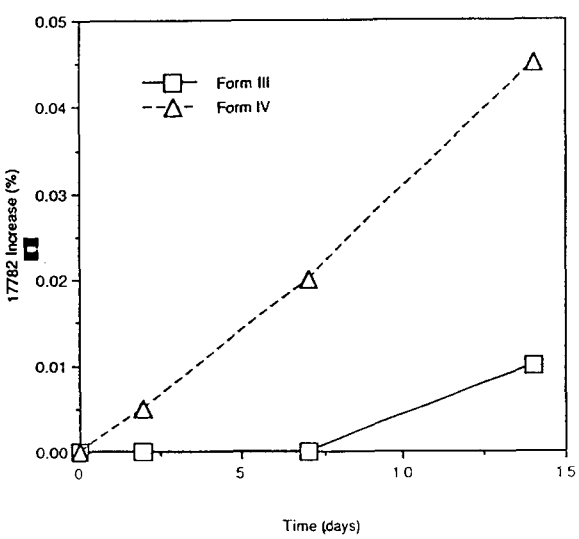


Fig. 6. Zanoterone solid-state stability at 40° C/25% relative humidity. % WIN 17782 increase vs time for form III vs form IV.

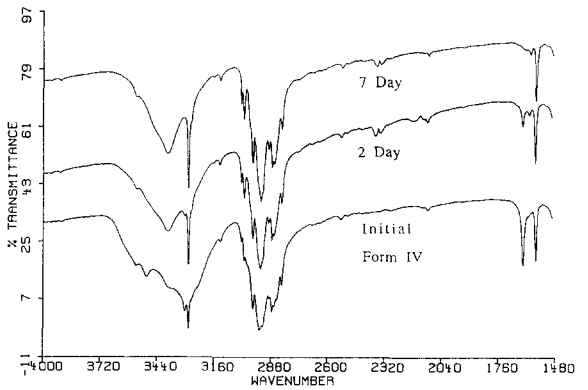


Fig. 7. Zanoterone physical stability for form IV stressed at 80° C. Nicolet 730 FTIR; 1% potassium bromide pellet.

system. Form III showed a reversible uptake of 0.12% moisture when cycled from 40° C/25% RH to 40° C/75% RH and back to 40° C/25% RH. Form IV showed a similar behavior, indicating that the mechanisms proposed by Ahlneck and Zografi may be a logical explanation for this phenomenon.

The form III samples held at 80° C (vacuum) were more chemically stable than the 40° C/75% RH station. The form IV samples initially showed degradation at 80° C, however, a change in form was observed in the form IV samples (below) and the rate of degradation decreased. This change in form during the 80° C stressing prevents meaningful interpretation of degradation for the different forms.

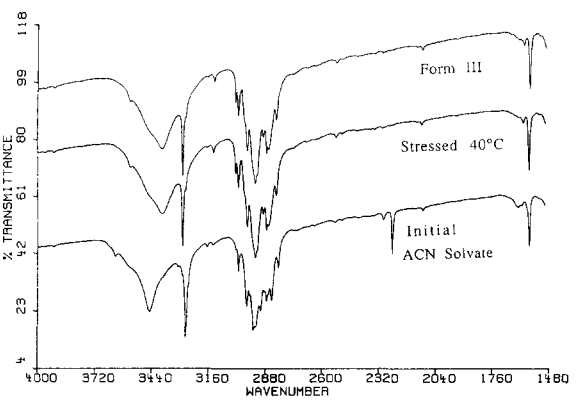


Fig. 8. Zanoterone acetonitrile solvate held at 40° C/25% relative humidity. Nicolet 730 FTIR; 1% potassium bromide pellet.

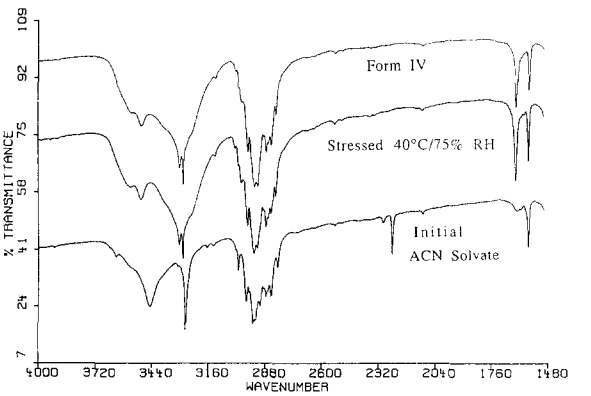


Fig. 9. Zanoterone acetonitrile solvate held at 40° C/75% relative humidity. Nicolet 730 FTIR; 1% potassium bromide pellet.

The form IV samples partially or completely transformed to form III with time at 80° C. Fig. 7 shows the FTIR spectra of a form IV sample held at 80° C as a function of time. The spectra clearly indicated a change for form IV under these conditions. Form III was stable with respect to polymorphic form at 80° C.

The acetonitrile solvate was observed to convert to form III at 40° C (approx. 25% RH) (Fig. 8) and 80° C but to form IV at 40° C/75% RH (Fig. 9). The form IV sample contained approx. 2% water. This observation indicated that the humidity should be held at a lower value in order to obtain form III. This subject is investigated in detail below.

3.3. Desolvation of acetonitrile solvate

Samples of the acetonitrile solvate of zanoterone were held at 40° C and 60° C at various humidity levels to determine the effect on polymorphic form. FTIR analysis after 3 days showed all samples to be completely free of acetonitrile.

The data indicated that at 40° C form III was obtained at lower humidity values (20–40% RH) while at higher humidity values (60–70% RH) significant levels of form IV with form III were observed. FTIR analysis indicated that at 40° C/60% RH approx. 15% form IV was observed after 3 days, while at 40° C/70% RH approx. 47% form IV was observed after 3 days.

At 60° C, the form IV levels were reduced. At 60% RH (3 days) there was not a measurable peak (< 2%) in the spectra due to form IV. At 60° C/70% RH the form IV level was approx. 10% after 3 days, significantly lower than the level of form IV (47%) at 40° C/70% RH.

At 80° C, acetonitrile solvate samples were desolvated at approx. 70% and 95% RH to determine if form III would be obtained as a pure polymorph. FTIR analysis showed the absence of form IV, indicating the presence of pure form III.

4. Conclusions

Zanoterone exists in multiple crystalline forms with unique physical and spectroscopic properties. These forms appeared to have unique FTIR spectra and X-ray diffraction patterns, useful in identifying each solid phase. FTIR was found to be useful in determining the polymorphic purity of form III when contaminated with form IV.

The higher degradation rate of form IV represents an example of the potential effects of crystal form on degradation rate. Thus, during the course of preformulation studies, if multiple polymorphs are discovered, it is important to determine the degradation rate of each form.

The isolation of the desired polymorphic form (form III) was shown to occur under conditions of lower relative humidity at 40–60° C. The isolation of form III appeared to be independent of humidity at 80° C. This study clearly illustrated the effect of humidity on the isolation of form III and indicated that, in general, humidity should be carefully controlled during drying or desolvation.

The concentration in solution for forms I–VI was determined at room temperature in 3% sodium lauryl sulfate. Forms I, II, and V, despite having higher melting points than form III, were found to have higher initial concentrations (than form III) which eventually decreased with time due to the precipitation of form VI. Form III had a solubility which was higher than the form IV and form VI samples after 24 h.

References

Ahlneck, C. and Zografi, G., The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. *Int. J. Pharm.*, 62 (1990) 87–95.

Byrn, S., *Solid State Chemistry of Drugs*, Academic Press. New York, 1982.

De Smidt, J., Fokkens, J., Grussels, H. and Crommelin, D., Dissolution of theophylline monohydrate and anhydrous theophylline in buffer solutions. *J. Pharm. Sci.*, 52 (1986) 497–501.

Haleblian, J., Characterization of habits and crystalline modifications of solids and their pharmaceutical applications. *J. Pharm Sci.*, 64 (1975) 1269–1288.

Haleblian, J. and McCrone, W., Pharmaceutical applications of polymorphism. *J. Pharm. Sci.*, 58 (1969) 911–929.

Jozwiakowski, M., Williams, S. and Hathaway, R., Relative physical stability of the solid forms of amiloride HCl. *Int. J. Pharm.*, 91 (1993) 195–207.

Rocco, W., Solid state characterization of stanozolol. *Drug Dev. Ind. Pharm.*, 20 (1994) 1831–1849.

Shefter, E., Solubility by solid state manipulation. *Techniques of Solubilization of Drugs*, Dekker. New York, 1981, Ch. 5.

Shefter, E. and Higuchi, T., Dissolution behavior of crystalline solvated and nonsolvated forms of pharmaceuticals. *J. Pharm. Sci.*, 52 (1963) 781–791.

York, P., Solid-state properties of powders in the formulation and processing of solid dosage forms. *Int. J. Pharm.*, 14 (1983) 1–28.