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RESEARCH PAPERS

SOLID STATE CHARACTERIZATION OF STANOZOLOL

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ABSTRACT

A study was undertaken to illustrate the ability to characterize various crystal forms of Stanozolol by by thermal analysis (differential scanning calorimetry-DSC), X-ray powder diffraction (XRPD), and Fourier transform infrared spectroscopy (FTIR). Mixtures of forms I and II were prepared and analyzed by each technique to investigate the strengths and weaknesses of the three methods. The detection of a contaminant polymorph in mixtures of forms I and II was possible by both FTIR and XRPD but not possible by DSC.

Various solvated forms were prepared by recrystallization from methanol, ethanol, 2-propanol, and were shown by thermal gravimetric analysis to exist in a 1:1 stoichiometry with Stanozolol. XRPD analysis of solvates indicated that each solvated form (monohydrate, ethanol solvate, methanol solvate, 2-propanol solvate) exists in a crystal unique with respect to forms I and II.

The concentration of drug in solution for the different crystal forms was determined at room temperature in 2% SLS at 5 and 24 hours. A significantly higher concentration was observed for the form II sample at 5 hours versus the form I and the monohydrate samples. It is likely that the form I sample had converted to the monohydrate form after 5 hours and a maximum concentration would be observed at an earlier time. At 24 hours, both the form I and II samples had converted to the monohydrate form and concentrations of 0.5-0.6 mg/mL were observed for each form. Due to the transformations to the hydrate during solubility studies, meaningful comparisons were difficult.

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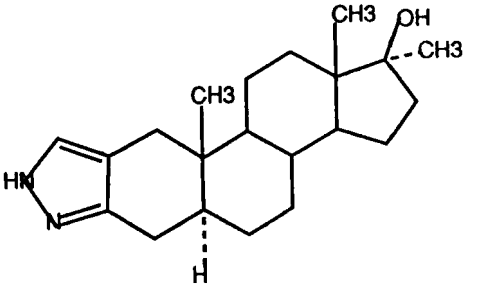
INTRODUCTION

Stanozolol, an anabolic steroid derived from testosterone, is used in the treatment of hereditary angioedema.



It is known that Stanozolol exhibits polymorphism and exists in several solvated forms (1-3). Differences in polymorphic form influence the bioavailability, stability, and processibility of a compound (4-6). Brandstatter showed FTIR spectra of forms I, II, and the monohydrate form (1). However, unambiguous and accurate characterization of mixtures of crystal forms by typical techniques has not been accomplished. In particular, mixtures of forms I and II are difficult to analyze by DSC due to the transformation of form II to form I. In this study, characterization of forms I, II, and mixtures of forms I and II by differential scanning Calorimetry, X-ray powder diffraction, and FTIR spectroscopy was attempted to illustrate the strengths and weaknesses of these analytical methods in characterizing polymorphic mixtures for this compound. Characterization of form III was omitted in order to limit the scope of this study. Form III has not been observed in various Stanozolol samples.

In addition, solubility characteristics of the different crystal forms were investigated. The concentration at 5 and 24 hours of each form in 2% SLS was determined.

Finally, samples were recrystallized from ethanol, methanol, and 2-propanol to form solvated crystals. These samples and a monohydrate form were characterized by thermal gravimetric analysis and X-ray powder diffraction in order to show stoichiometry of solvation and determine whether the solvates exist as polymorphic solvates or pseudopolymorphic solvates.

MATERIALS AND METHODS

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) data was obtained for Stanozolol form I, form II, mixtures of form I/II, the monohydrate form, and the ethanol solvate form on the Perkin Elmer System-7 at 10°C/min with nitrogen

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purge gas. Crystal form II was run at a series of heating rates: 10, 20, 40, 60, and 100°C/min. Mixtures of forms I/II at 10/90 and 90/10 ratios were prepared by mixing with a spatula for several minutes. The DSC instrument was calibrated with indium (m.p. 156.6°C) and tin (m.p. 231.9°C) prior to each set of samples.

Thermal Gravimetric Analysis (TGA)

Thermal gravimetric analysis (TGA) data was obtained on the monohydrate form and various solvated forms with the Perkin Elmer-TGA-7 at 10°C/min with nitrogen purge gas. The system accuracy was verified with Barium Chloride Dihydrate (14.7% water prior to analysis).

X-Ray Powder Diffraction (XRPD)

X-ray powder diffraction (XRPD) data was obtained with the Scintag XDS system using Cu K-alpha radiation and a liquid nitrogen cooled germanium solid state detector. Samples were run at 2° (2-theta)/min from 2-30°. Patterns were obtained on Stanozolol form I, form II, mixtures of form I/II, the monohydrate form and the several solvated forms. Mixtures of form I/II at 10/90 and 90/10 ratios were prepared by mixing with a spatula for several minutes. The samples were run on zero background quartz plates.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) results were obtained with the Nicolet 730 FTIR on Stanozolol form I, form II, mixtures of form I/II, the monohydrate form, and the ethanol solvated form. Samples were prepared as 1% KBr dispersions. Mixtures of forms I/II at 10/90 and 90/10 ratios were prepared by weighing the necessary weights of each form to obtain 2 mg total compound and then grinding with approximately 200 mg KBr.

Preparation of Solvated Forms

Stanozolol was recrystallized from ethanol, methanol, and 2-propanol by dissolving at 60-70°C at appropriate concentrations and then cooling to 0°C in an ice bath to induce crystallization. The crystals were collected with a Buchner funnel and dried at low temperatures (30-40°C).

Preparation of Crystal Forms I and II

Form II was obtained by recrystallization from ethanol and drying at approximately 130°C. Form I was obtained by heating solvated samples or mixtures of form I and II to 205°C.

Solubility

The concentration of forms I, II and the monohydrate form in 2% SLS was determined at room temperature by adding excess solid to 2 mL of solvent and mixing for 24 hours. A sample was removed from each vial at 5 hours and the concentration determined at this point for comparison with the 24 hour values. Solids were removed by filtration through a 0.5 micron filter. The samples

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were diluted with 2% SLS by 1/20 or 1/40 and analyzed by UV (Hewlett Packard-diode array) with the standards described below.

Standards were prepared at 8, 20, 40, and 60 micrograms/ml in 2% SLS and the absorbance measured at 228 nm. A standard curve was constructed by linear regression.

It should be noted that due to the transformations observed during solubility studies, the measured solubilities were not equilibrium values.

Materials

Methanol, ethanol, and 2-propanol were obtained from Fisher Scientific.

RESULTS AND DISCUSSION

DSC Data-Differential Scanning Calorimetry

DSC data obtained on Stanozolol form I at 10°C/min and form II at 10, 20, 40, and 60°/min is shown in Figures 1 and 2A-D. The scan for form I shows a single melting peak at 245°C with an enthalpy of melting of 89.7 J/g. In contrast, the data for form II shows multiple melting peaks. The first peak appears at approximately 231°C and the second at approximately 243°C (similar in location to form I). If the form II scan at 10°C/min was viewed independently one may be inclined to postulate that the sample contains a small concentration of form II. However, as heating rate is increased, the peak area for form II increases dramatically. It appears that a melting/ recrystallization/ remelting phenomena is occurring as evidenced by the exotherm observed at a rate of 10°C/min. A scan for form II at 100°C/min shows no evidence of form I (Figure 3) but at this rate resolution is reduced significantly. Thus, it appears from the DSC heating rate study that the concentration of form II is very high. XRPD and FTIR data verify this hypothesis.

DSC data on form I/II mixtures of 90/10 and 10/90 are shown in Figures 4 and 5. The scan of the form I/II 90/10 mixture shows no evidence of melting for form II and suggests that form II may transform prior to melting when present in a mixture. The striking appearance of the scan for the form I/II 10/90 mixture (Figure 5) appears to confirm this hypothesis. Despite the presence of 90% form II, no melting is observed for form II but a rather broad endotherm at approximately 215°C characteristic of transformation. The energy of transformation was approximately 9 J/g. The broad endotherm was followed by a sharp melting peak consistent with form I. In unknown mixtures, one may be tempted to view the weak broad endotherm as transformation of a "small" concentration of form II. This characterization is clearly incorrect. This experiment indicates the

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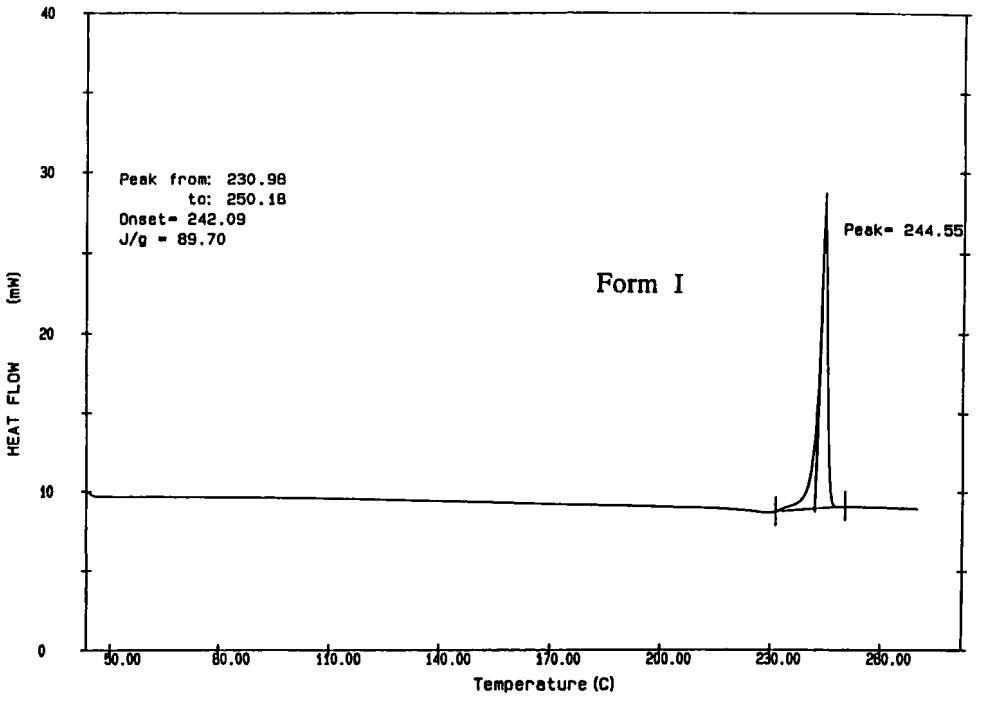

FIGURE 1
DSC Data Stanozolol Form I

difficulty in using DSC to elucidate polymorphic composition of Stanozolol. It should be noted that the particle size of the polymorph(s) may also influence the DSC data.

DSC data on the ethanol solvated and monohydrate forms of Stanozolol are shown in Figures 6 and 7. The ethanol solvate scan shows the desolvation peak at 128°C followed by recrystallization and the melting peaks for forms III, II, and I. The existence of each form was observed by Brandstatter (1972) in microscopy studies (2). The appearance of forms I, II, and III in a scan of the ethanol solvate may not be observed in repeat samples and should not be regarded as typical. This experiment shows the utility of DSC in detecting the presence of solvation but not the stoichiometry. Determination of stoichiometry by TGA is illustrated below.

DSC data on the monohydrate form (Figure 7) shows a desolvation peak at 134°C, followed by a broad exotherm due to recrystallization, an endotherm characteristic of a transformation, and finally melting.

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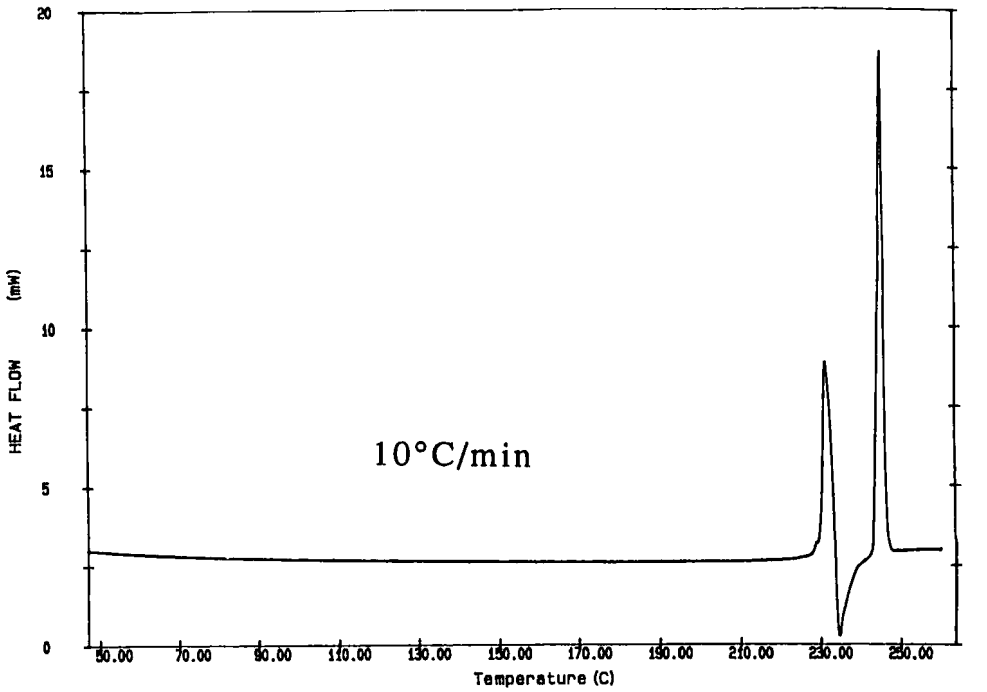

FIGURE 2A
DSC Data Stanozolol Form II 10°C/min

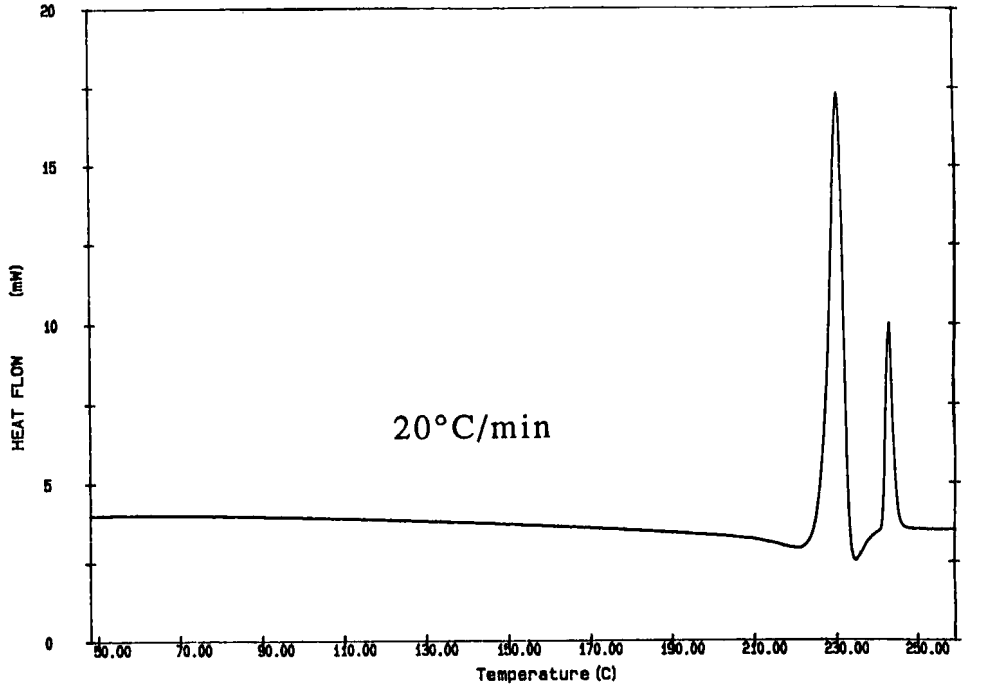

FIGURE 2B
DSC Data Stanozolol Form II 20°C/min

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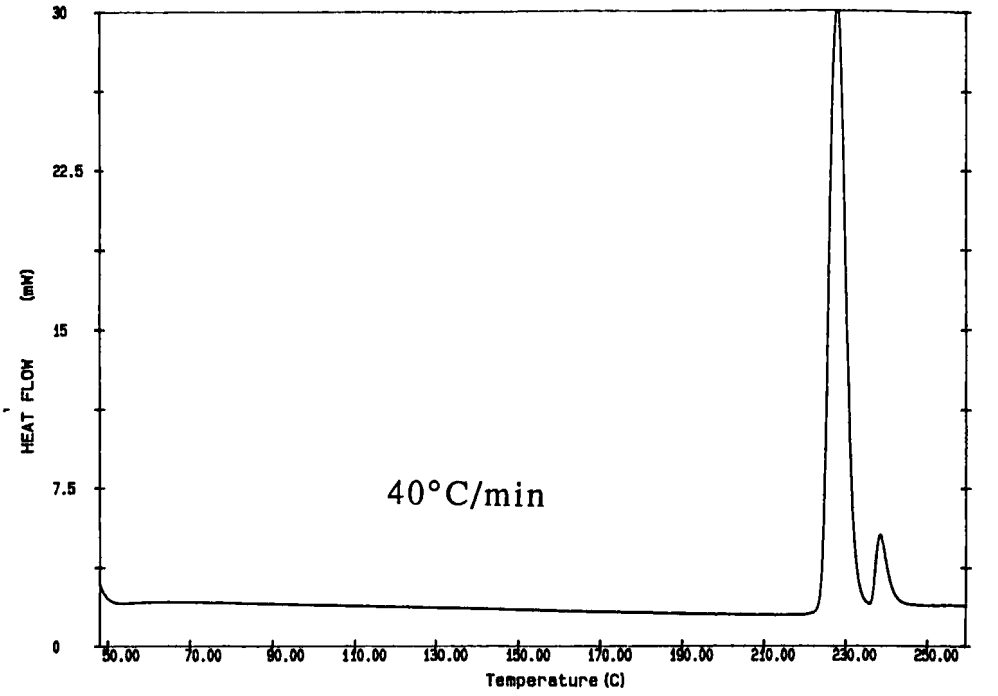

FIGURE 2C
DSC Data Stanozolol Form II 40°C/min

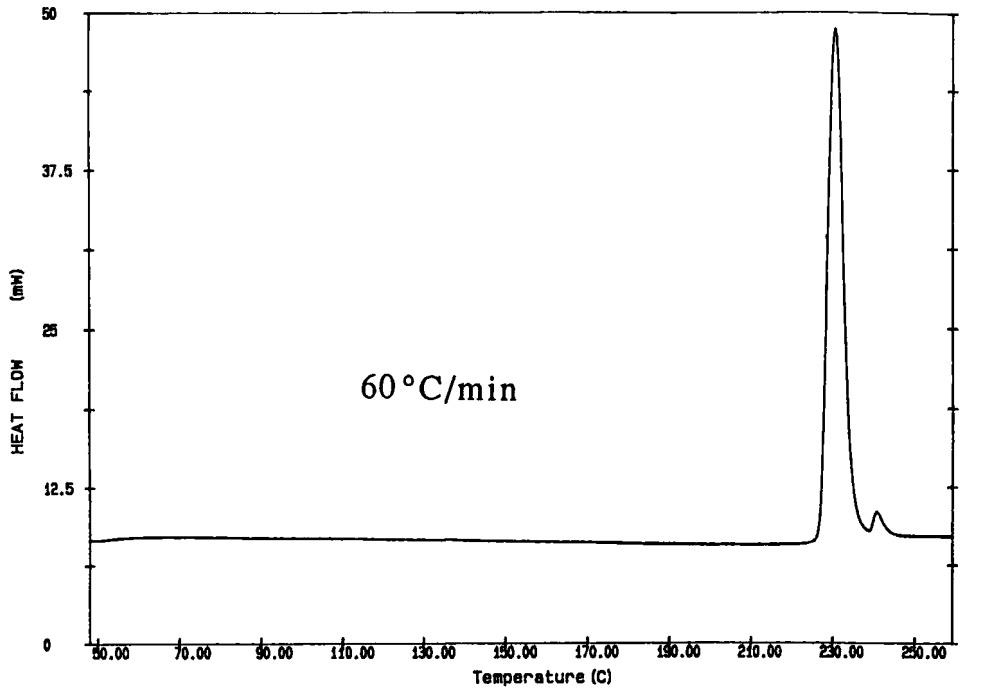

FIGURE 2D
DSC Data Stanozolol Form II 60°C/min

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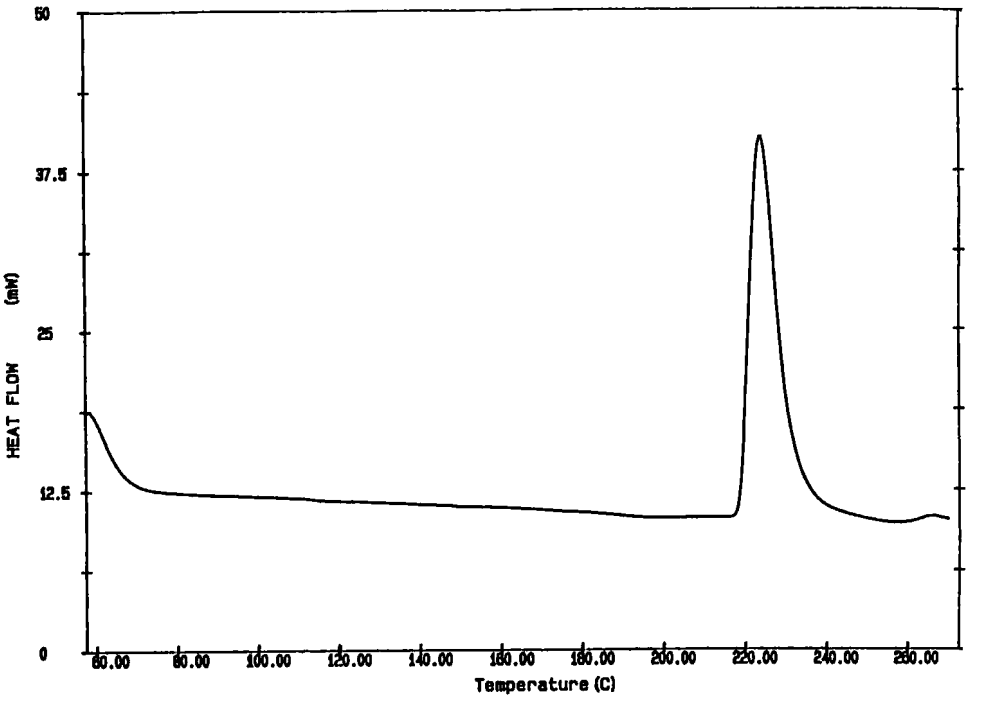

FIGURE 3
DSC Data Stanozolol Form II 100°C/min

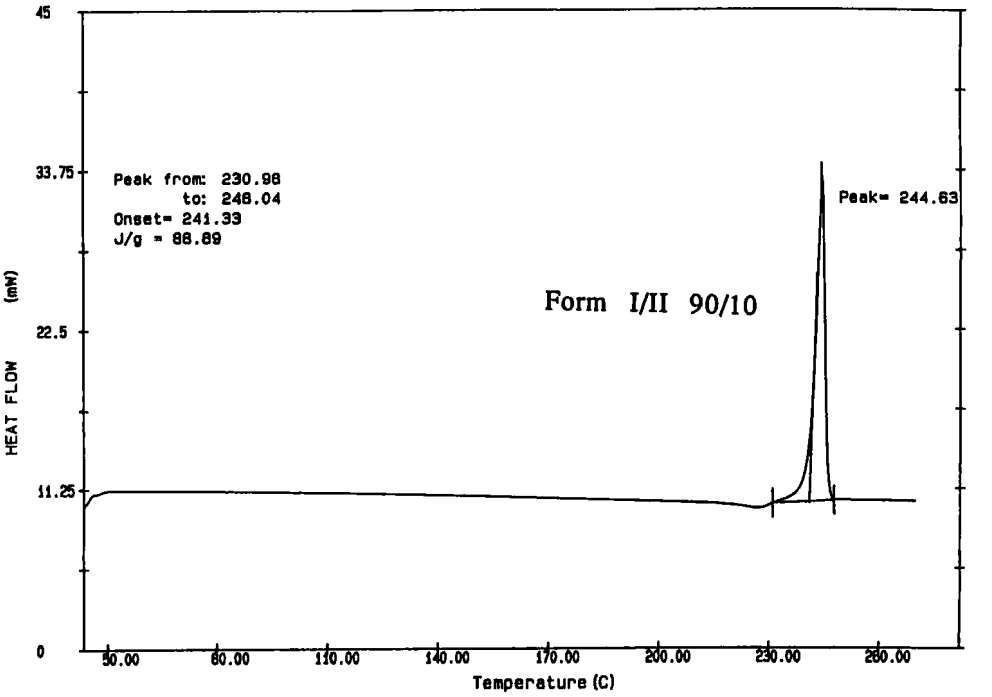

FIGURE 4
DSC Data Stanozolol Form I/II Mixture 90/10 Ratio

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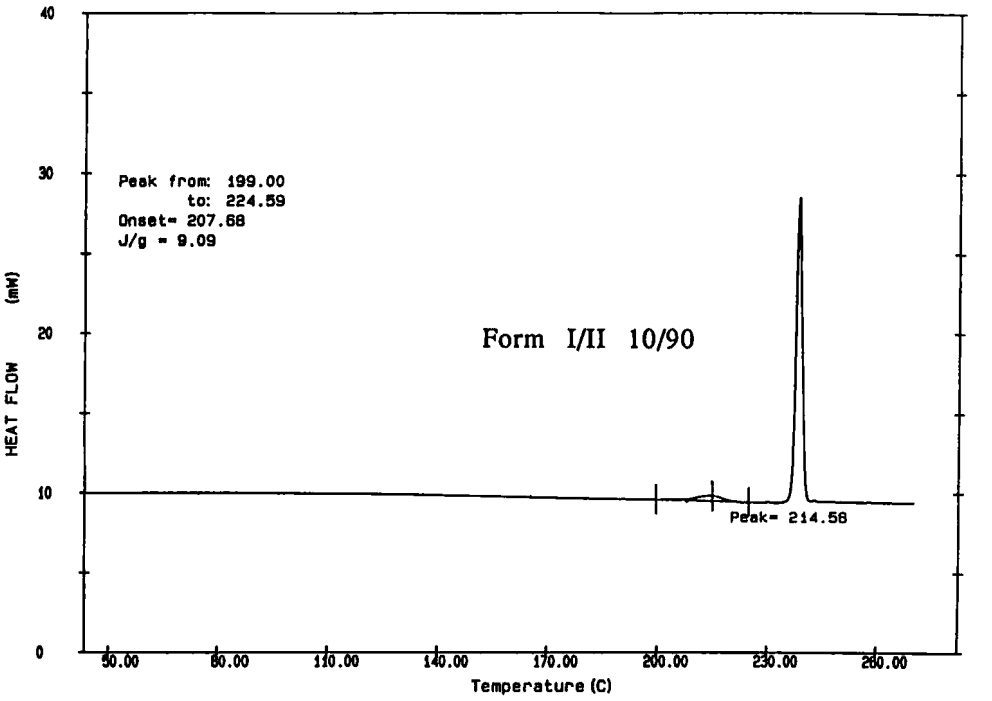

FIGURE 5
DSC Data Stanozolol Form I/II Mixture 10/90 Ratio

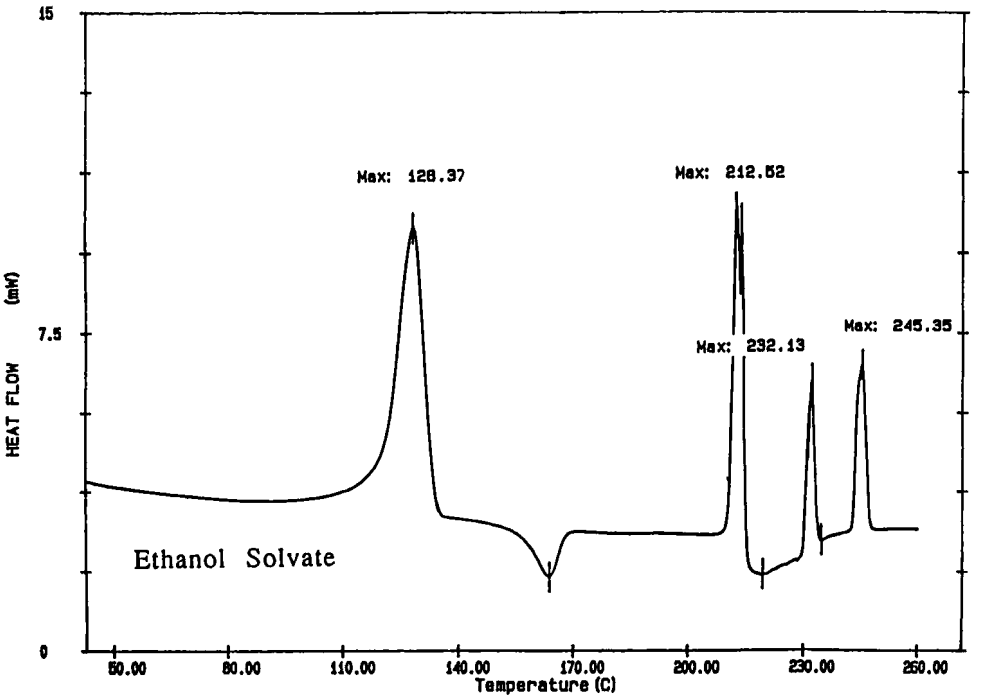

FIGURE 6
DSC Data Stanozolol Ethanol Solvate

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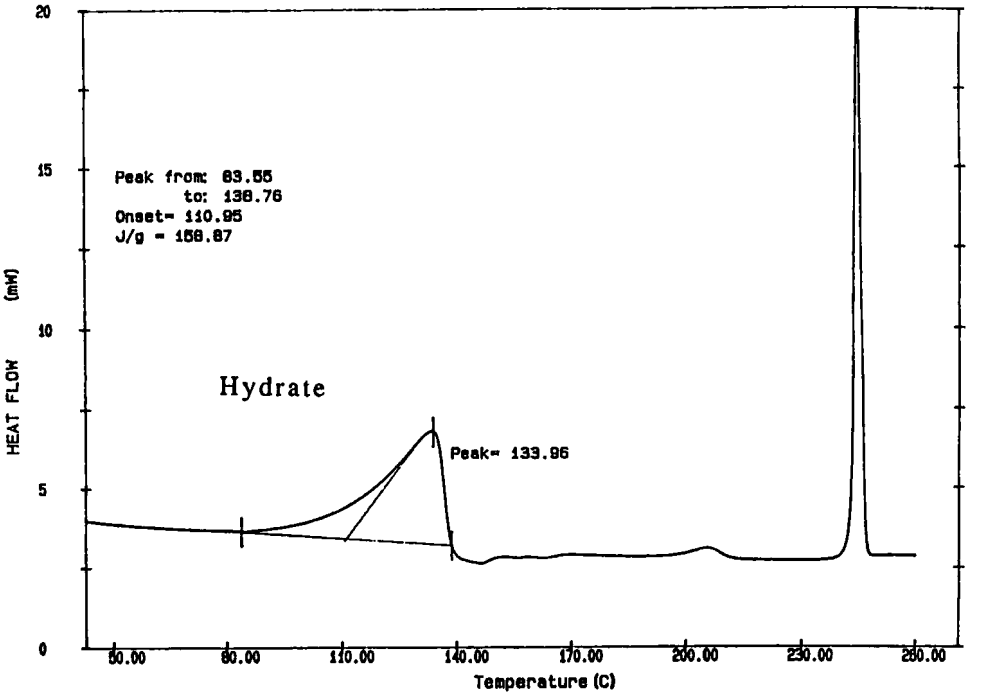

FIGURE 7
DSC Data Stanozolol Monohydrate

Thermal Gravimetric Analysis

TGA data on the ethanol solvate indicated a weight loss of 12.2% was associated with the broad endotherm observed in the DSC. This value compares well with the theoretical value of 12.3% for a monoethanolate.

TGA data on the monohydrate indicated a weight loss of 5.0%; the expected value for a monohydrate is 5.2%.

TGA data for the methanol and 2-propanol recrystallized samples showed a sharp weight loss of 8.9% and 16.2% respectively. This data compares well with the theoretical weight loss of 8.9% for a monosolvate of methanol and 15.5% for a monosolvate of 2-propanol. Thus, TGA data has shown each solvate to exist in a 1:1 ratio of solvate to drug molecule.

X-Ray Powder Diffraction

XRPD data for forms I and II are shown in Figures 8 and 9. The well defined patterns show significant differences throughout the pattern and are unique. It was very useful to examine the XRPD data for form I/II mixtures since DSC appears to be inconclusive in

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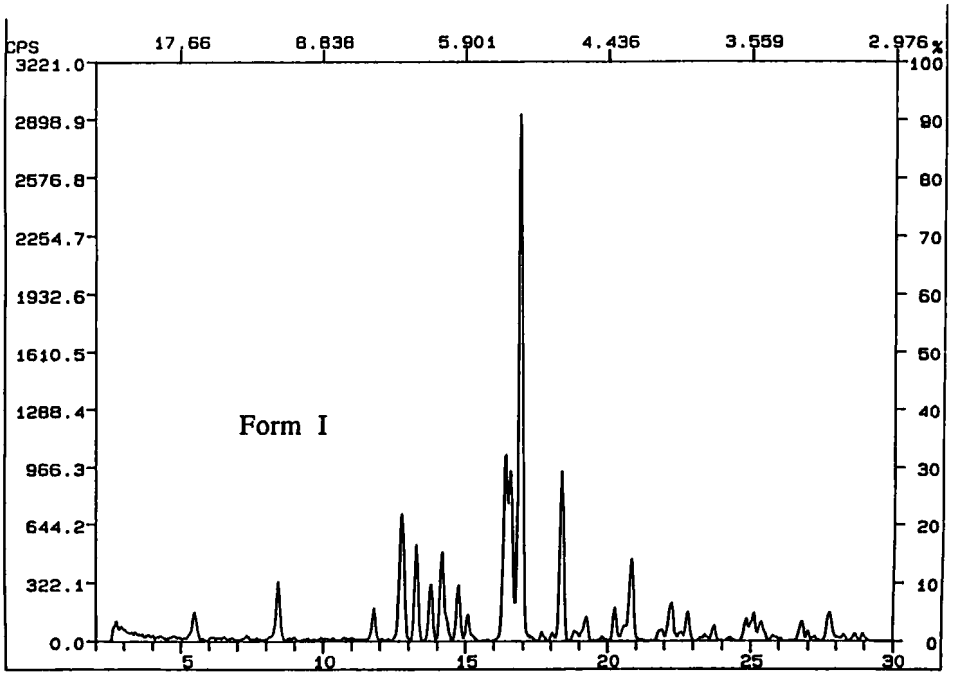

FIGURE 8
XRPD Pattern Stanozolol Form I

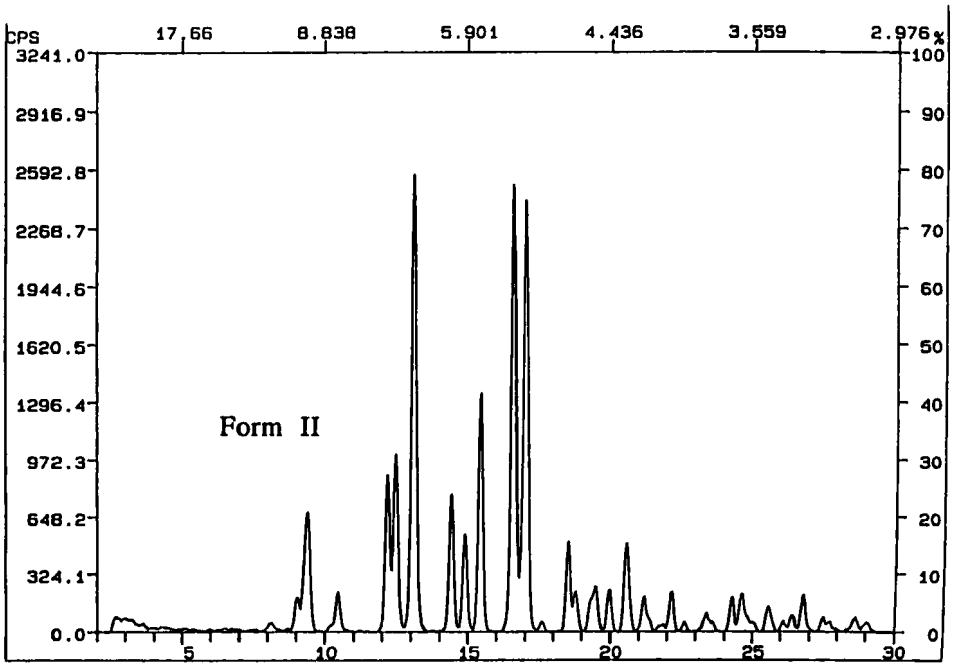

FIGURE 9
XRPD Pattern Stanozolol Form II

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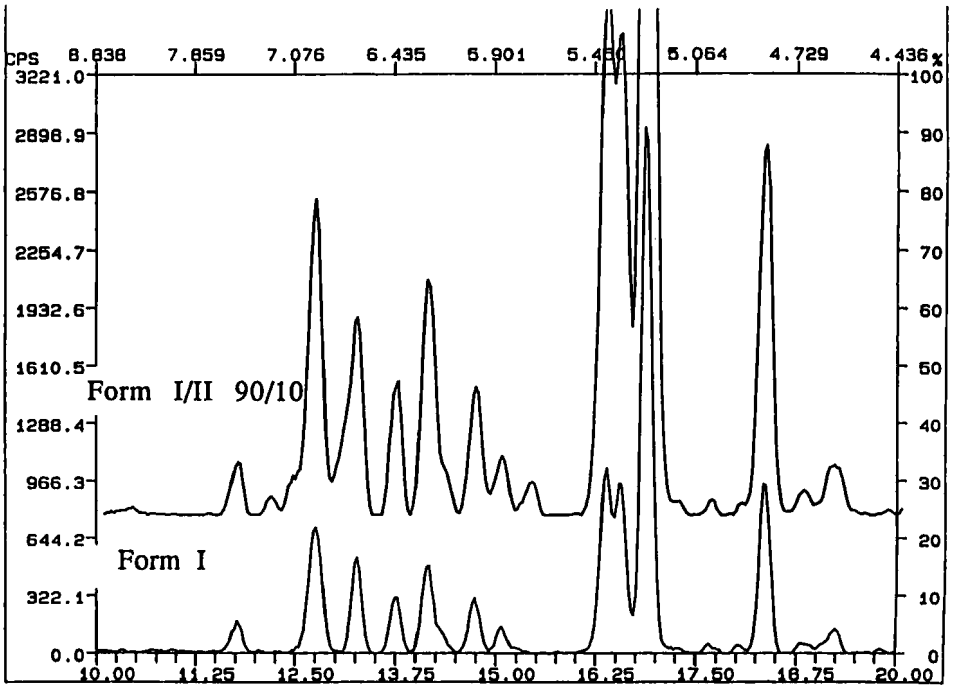

FIGURE 10
XRPD Pattern Stanozolol Form I/II Mixture 90/10 Ratio

estimating concentrations of forms I and II in mixtures. The 90/10 form I/II mixture is overlaid in Figure 10 with a sample of form I. At 10% concentration, the presence of form II is clearly observed. Peaks were observed at approximately 12.25 and 15.5° (2-theta) due to the presence of form II. The development of a semi-quantitative method may be possible by plotting form II concentration versus peak area. The XRPD pattern of the 10/90 form I/II mixture is compared with form II in Figure 11. The presence of a weak peak at approximately 5.5° indicated the presence of form I as a polymorphic contaminant.

The XRPD patterns of the monohydrate and ethanol solvated forms of Stanozolol, shown in Figures 12-13, are unique and differ from the patterns of form I and form II. In addition, the patterns of the methanol and 2-propanol solvated samples were unique. This observation indicates that the forms containing a solvent molecule have a different crystal structure from the non-solvated forms and can be described as polymorphic solvates. This observation assumes that the unknown pattern for form III is also unique. In some compounds the XRPD patterns of

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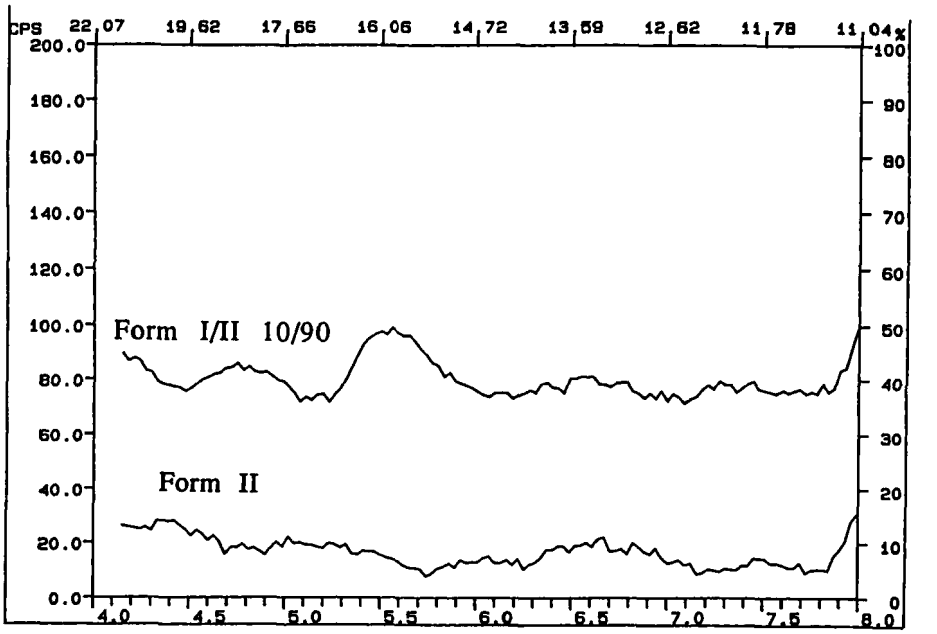

FIGURE 11
XRPD Pattern Stanozolol Form I/II Mixture 10/90 Ratio

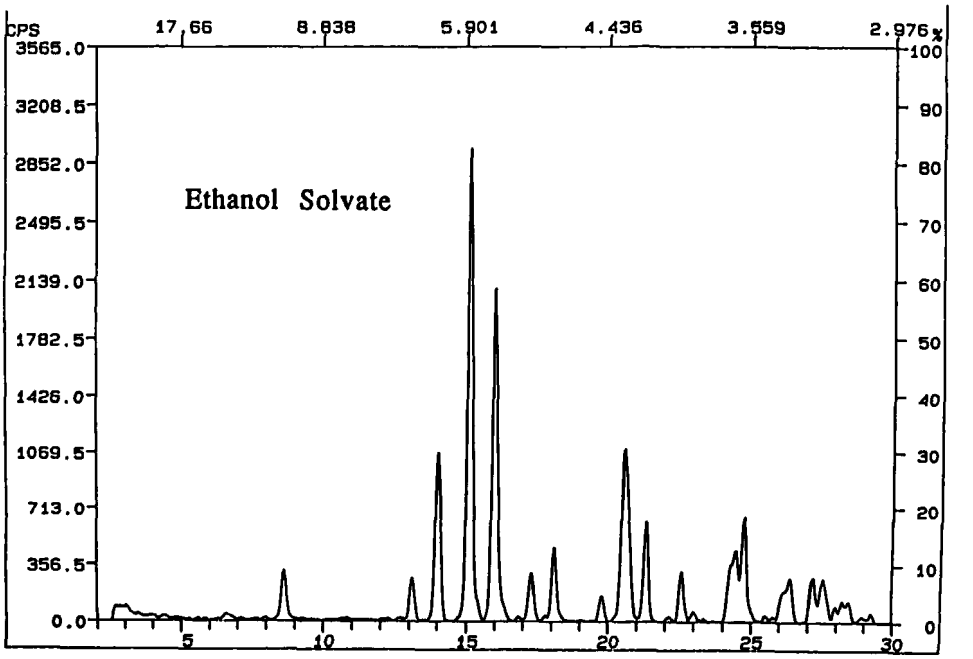

FIGURE 12
XRPD Pattern Stanozolol Ethanol Solvate

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the solvate and non-solvate are the same and can be termed pseudopolymorphic solvates. In pseudopolymorphic solvates the solvent molecule will occupy a void or space in the crystal lattice of the drug and will give a similar XRPD pattern.

FTIR

FTIR spectra of Stanozolol forms I and II (Figures 14-15) show interesting differences in the spectra of these forms (1). At approximately 3620 cm-1, the spectra of form I exhibits a sharp peak characteristic of free (non-hydrogen bonding) -OH stretch. This peak is found to be on the edge of a broad -OH stretch, characteristic of hydrogen bonding, from approximately 3200-3600 cm-1. In contrast, the FTIR spectra of form II shows only a broad -OH stretch from approximately 3200-3600 cm-1. This difference may be useful as a means to quantitate the concentration of polymorphs in a mixture.

The FTIR spectra of the form I/II 10/90 mixture is compared with the spectra of form II in Figure 16. The region of interest, from 3000-4000 cm-1, is expanded. The mixture clearly shows the presence of a sharp peak at approximately 3620 cm-1. This comparison indicates the ability to easily detect form I in a mixture at 10% concentration.

The FTIR spectra of the form I/II 90/10 mixture (Figure 17) was compared with form I. The region of 600-1000 cm-1 is shown in this case. A peak at 793 cm-1, due to the presence of form II (which absorbs strongly at 793 cm-1), is apparent.

The FTIR spectra of the monohydrated form of Stanozolol is shown in Figure 18. The appearance of a strong -OH peak at 3481 cm-1 is a feature which is not found in either form I or form II. Other differences throughout the spectrum are apparent.

Solubility

The concentration of drug in solution for samples of the various forms of Stanozolol in 2% SLS is shown below in Table 1 for samples analyzed at 5 and 24 hours.

The concentration of Stanozolol in the form II sample was significantly higher at 5 hours versus both the form I and the monohydrate samples. The form I sample was slightly higher than the monohydrate but FTIR spectra at 24 hours showed conversion to the monohydrate had occurred. It is very possible that this conversion had occurred after 5 hours. The data at 24 hours shows a significant decrease in concentration for form II due to precipitation (FTIR analysis) of the monohydrate form. The observed transformations make comparisons difficult since it is likely that the rate of conversion is a function of many characteristics including: polymorphic form, particle size, crystal habit, solvent composition, degree of agitation, and temperature. Therefore,

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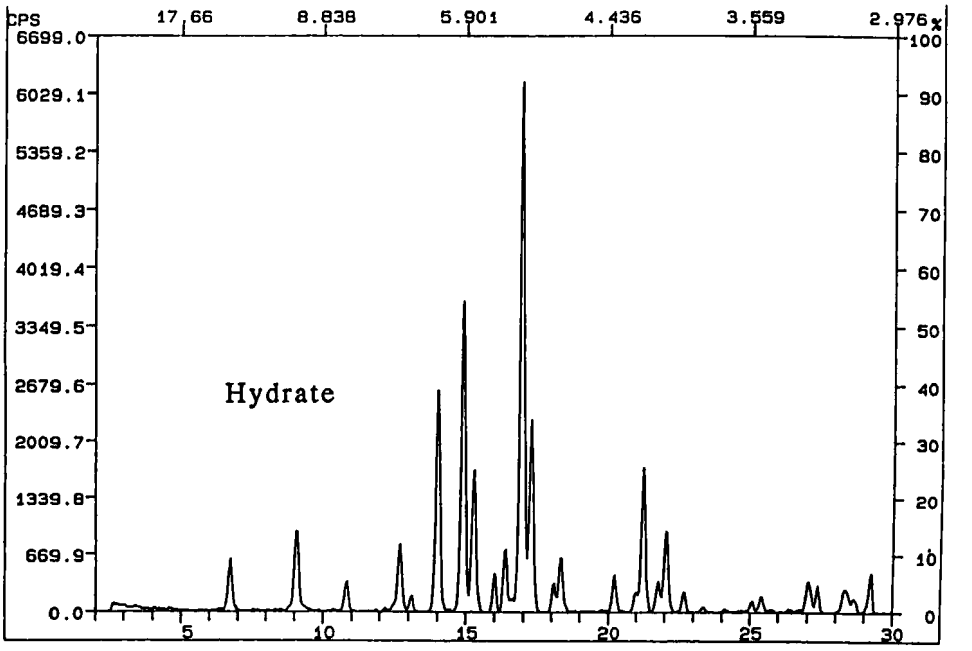

FIGURE 13
XRPD Pattern Stanozolol Monohydrate

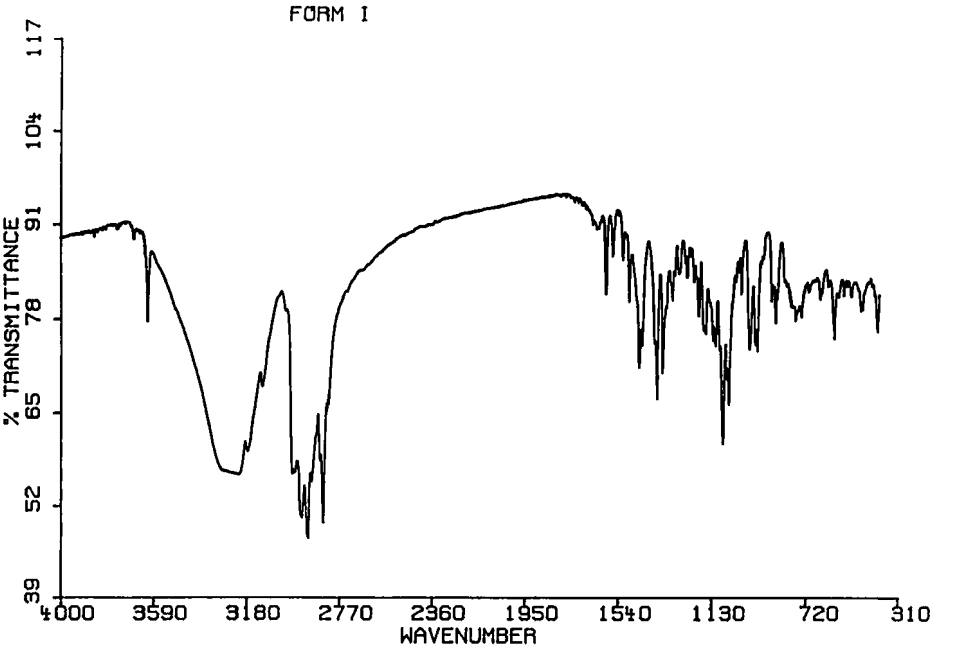

FIGURE 14
FTIR Spectra Stanozolol Form I

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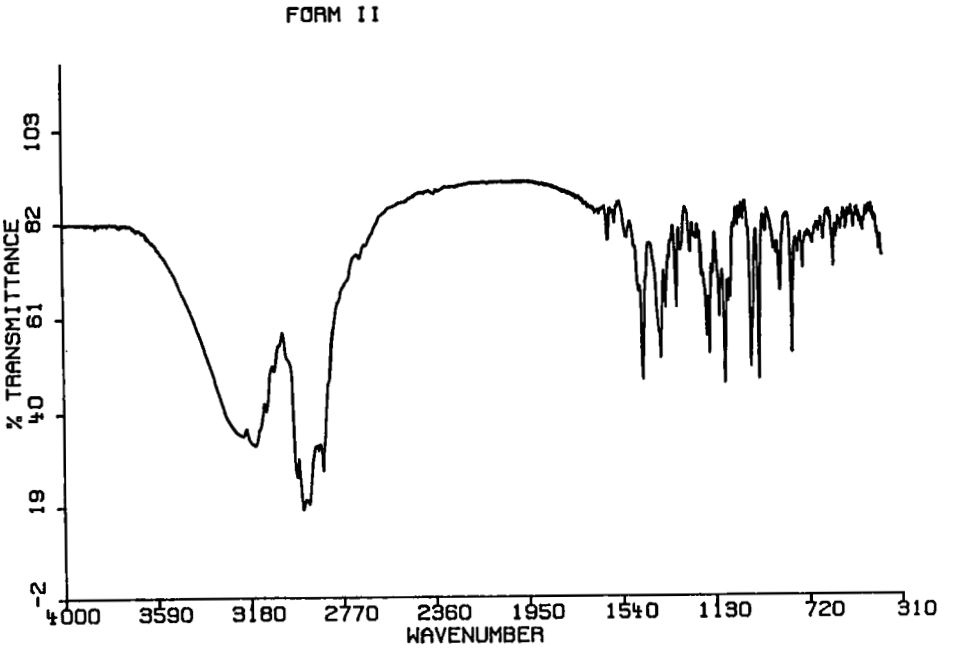

FIGURE 15
FTIR Spectra Stanozolol Form II

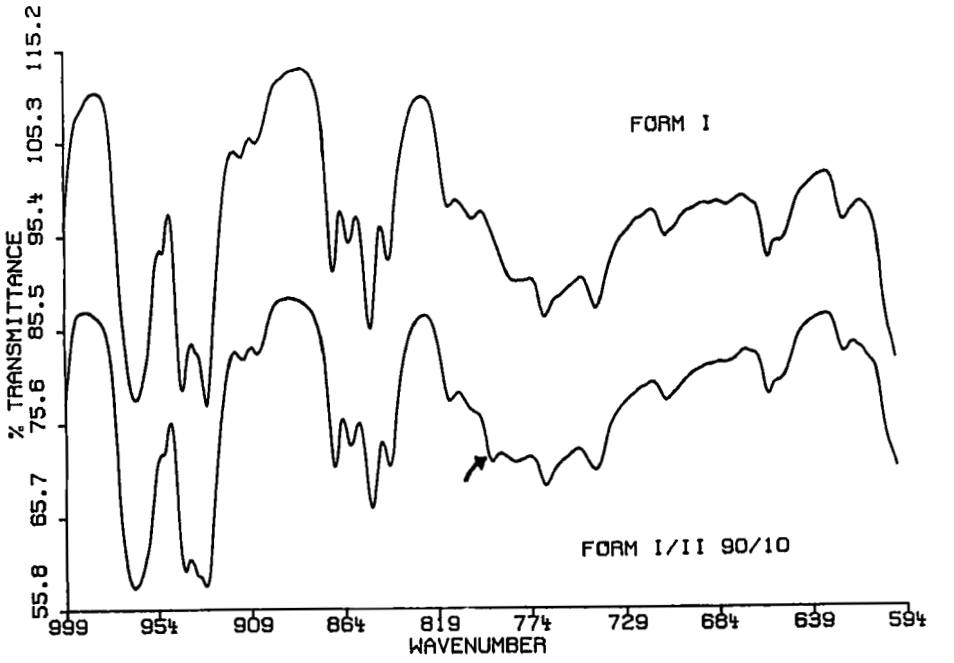

FIGURE 16
FTIR Spectra Stanozolol Form I/II Mixture 90/10 Ratio

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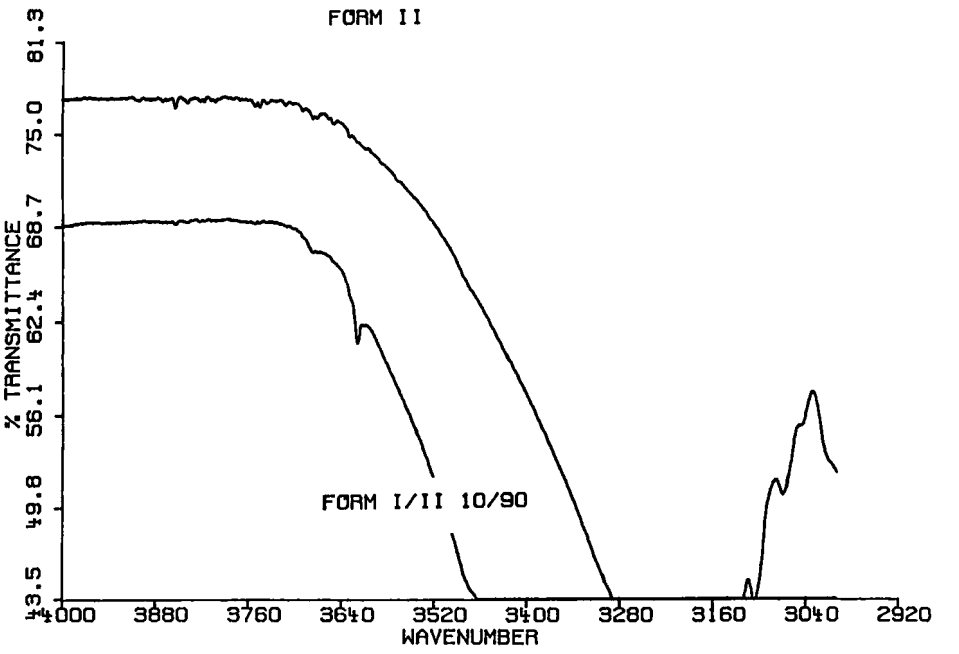

FIGURE 17
FTIR Spectra Stanozolol Form I/II Mixture 10/90 Ratio

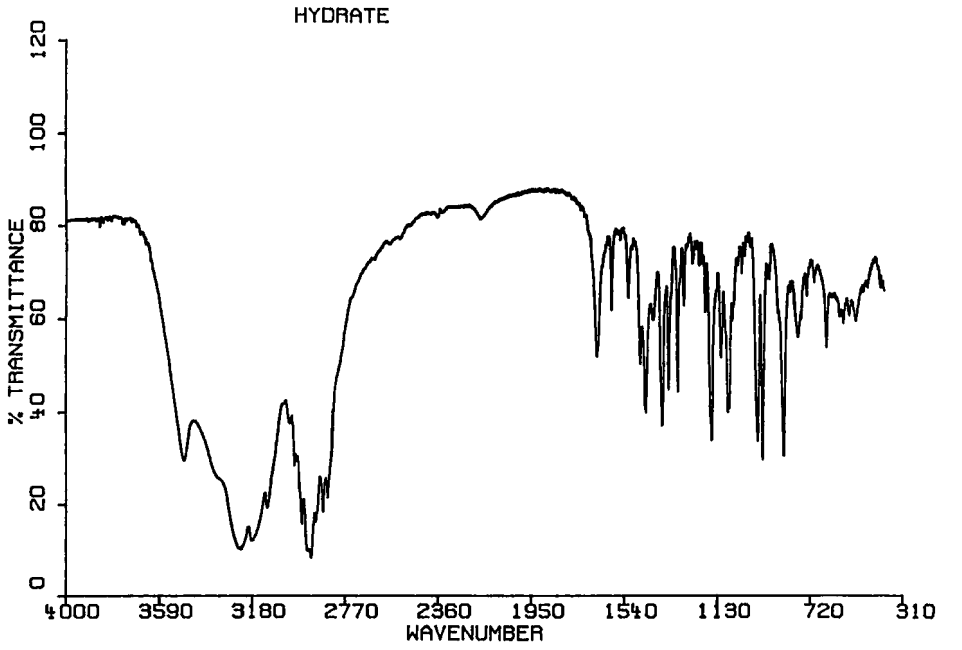

FIGURE 18
FTIR Spectra Stanozolol Monohydrate

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TABLE 1.
Concentration of Stanozolol in 2% SLS at 23°C

Form	Conc. (5 hr)	Conc. (24 hr)
Form I	.61 mg/ml	.60 mg/ml
Form II	1.40 mg/ml	.56mg/ml
Monohyd.	.47 mg/ml	.53mg/ml

different solubility and dissolution versus time values would be expected as these variables are changed.

Lower solubility values are typical for hydrated forms versus anhydrous forms of a compound. This behavior has been observed for theophylline and glutethimide (7).

CONCLUSIONS

The ability to detect forms I and II of Stanozolol and mixtures of these crystal forms was shown to be very difficult with thermal analytical techniques due to the ability of form II to transform to form I. This transformation was shown to occur with a 10/90 mixture of form I/II. When transformation occurs prior to melting there is a tendency to misinterpret DSC data and, therefore, DSC is not recommended as a primary tool in discerning polymorphic mixtures of Stanozolol.
The strength of thermal techniques for Stanozolol is in the ability to detect the presence of a solvate and determine the stoichiometry. Solvates of water, ethanol, methanol, and 2-propanol were shown to exist in monosolvated forms.

Analysis of forms I and II by X-ray powder diffraction was very useful and well resolved patterns of each form were obtained. The ability to detect the contaminant polymorphic form in 90/10 and 10/90 mixtures of form I/II was illustrated. XRPD may be the best technique for detecting low levels (<10%) of form II in form I. The XRPD patterns of the monohydrate and other solvated forms were shown to be unique and were not similar to the patterns of form I and form II. This observation indicates that the solvated forms are polymorphic solvates rather than pseudopolymorphic solvates.

FTIR was also very useful in the analysis of form I, form II, and the 10/90 and 90/10 mixtures thereof. The appearance of a strong peak, due to non-hydrogen bonded -OH, was present in form I and easily allowed detection at a level of 10% in form II. Examining the spectra for this peak may be the best technique for detecting low levels (<10%) of form I in form II.

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The FTIR spectra of the monohydrate form was unique and shows the ability to discriminate this form from forms I and II.

The concentration observed for Stanozolol in the form II sample in 2% sodium lauryl sulfate after 5 hours was significantly higher than both the form I and the monohydrate samples. However, the form I sample converted to the hydrate after 24 hours and may have converted to the hydrate after 5 hours. Since the form I sample appeared to convert more rapidly to the hydrate than the form II sample, a comparison between forms I and II is difficult to assess.

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