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RESEARCH PAPER

Characterization of Zopiclone Crystal Forms Found Among Generic Raw Materials

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ABSTRACT

This paper deals with the occurrence of polymorphs and pseudopolymorphs and their effect on the solid-state properties of zopiclone, a poorly water soluble nondiazepine sedative and hypnotic drug. X-ray powder diffraction (XRPD), infrared spectroscopy (IR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), particle size analysis, dissolution studies, and solubility determinations were used to characterize the zopiclone raw materials. An anhydrated form, a dihydrated form, and a mixture of these two crystal forms were found and characterized among the zopiclone powders.

Key Words: *Dissolution; Hydrates; Polymorphs; Solubility; Zopiclone.*

INTRODUCTION

Zopiclone is a cyclopyrrolone drug with sedative and hypnotic properties. It is chemically unrelated to the benzodiazepines, but has a similar spectrum of activity; it binds to sites on or closely linked to the benzodiazepine receptor complex (1). It is described as a benzodiazepine agonist (2). Figure 1 shows that zopiclone contains a 2-(5-chloropyridyl) ring (A) and a pyrazine ring (B) (3). Zopiclone is a white crystalline powder that is freely soluble in most organic solvents, such as alcohols, acetonitrile, chloroform, toluene, and dichloromethane. It is less soluble in acetone and slightly soluble in water (4).

Zopiclone is very unstable in nucleophilic solvents such as methanol and ethanol. The European Pharmacopoeia (5) reports that zopiclone has a ultraviolet (UV) absorption maximum at 303 nm, melts at 177°C with decomposition, and is sensitive to photon-induced degradation (light sensitivity).

In South Africa, generic zopiclone raw materials are available from numerous sources. Although zopiclone

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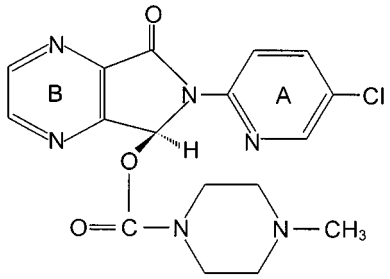

Figure 1. Chemical structure of zopiclone with A representing 2-(5-chloropyridyl) and B representing pyrazine (3,4).

might have the potential (due to its chemical structure) to crystallize as different polymorphs or pseudopolymorphs, little information is available on the solid-state properties of this drug. In this study, the solid-state properties of seven zopiclone powders randomly obtained from four different sources were studied to determine differences in crystal forms and the effect thereof on the physicochemical properties of the drug.

EXPERIMENTAL

Materials

Zopiclone powders were randomly obtained from four different sources (Fermion batches 96E14, ERM 817, and 96E120; Industriale Chimica batch 970327; Torcan Chemicals batch KH01604; and Chemo batches 960539 and PS12509). These powders were numbered samples 1 to 7, respectively, and the solid-state properties were measured. Grinding sample 7 to a finer powder produced sample 7a.

X-Ray Powder Diffractometry

X-ray powder diffraction (XRPD) patterns were obtained at room temperature with a Philips PM9901/00 diffractometer (Philips, Eindhoven, Netherlands). The measurement conditions were Co K_β target, iron filter, 40 kV voltage, 20 mA current, 0.2-mm slit, and 2° per min scanning speed. Samples of approximately 200 mg were weighed into aluminum sample holders, taking care not to introduce a preferential orientation of crystals.

Thermal Analysis

Differential scanning calorimetry (DSC) thermograms were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan). Samples weighing approximately 3–5 mg were heated in closed aluminum crimp cells at a rate of 10°C/min under a nitrogen gas flow of 20 ml/min.

Thermogravimetric analysis (TGA) thermograms were recorded with a Shimadzu TGA-50 instrument. The sample weight was approximately 5–8 mg, and heating rates of 10°C/min under nitrogen gas flow of 20 ml/min were used.

Infrared Spectrophotometry

Infrared (IR) spectra were recorded with a Shimadzu FTIR-4200 spectrophotometer over a range of 400–4000 cm^{-1} using the KBr disk technique. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) using an agate mortar and pestle. Disks were pressed using a Beckman 00-25 press (Beckman, Glasgow, Scotland) at a pressure of 15×10^3 kg/cm^2.

Particle Size Analysis

Particle size distributions in suspension of all samples were measured with a Galai-Cis-1 particle size analyzer (Galai, Tel Aviv, Israel). This instrument used dual-discipline analysis integrating laser diffraction and image analysis for particle sizing. Samples suspended in a suitable dispersing solution (liquid paraffin) were placed in small cuvettes and fitted into the analyzer. A small magnetic stirrer inside the cuvette prevented sedimentation of the particles during the measurement. The acquired data were used to compute means, medians, and standard deviations based on the total particle population.

Solubility Measurements

An amount of powder, enough to ensure that supersaturation was obtained (10 ± 1 mg), was measured in 10-ml test tubes with screw caps. To each test tube, 5 ml of high-performance liquid chromatography (HPLC) water was added, and the caps were screwed on tightly.

The test tubes were rotated at 60 rpm (Heidolph RZR-2000 rotator, Kelheim, Germany) in a thermostatically (Julabo EM/4 thermostat, Seelbach, Germany) controlled water bath at $30°C \pm 1°C$ for 24 hr. The concentration of the filtered (0.45-μm filter) samples were determined using the following ultraviolet (UV) spectrophotometric method.

Standard solutions of zopiclone in the concentration range 0.5 to 25 μg/ml were prepared, and the absorbance was measured using a Beckman DU 650I spectrophotometer (Beckman, Fullerton, CA). A standard curve of ab-

sorbance versus concentration was plotted, and the absorptivity was calculated (6) (absorptivity $[a] = 35.9706$ $L \cdot gram^{-1} \cdot cm^{-1}$, with a 95% confidence interval of 35.4541, 36.4872). The absorbances of the filtered sample solutions were converted to concentration using the calibration curve.

Powder Dissolution Studies

Powder dissolution was performed according to the described method (7) using method 2 (paddle) of USP 23 (8). The paddles were rotated at 50 rpm, and samples were drawn from the dissolution medium (0.1 M HCl) at 7.5, 15, 30, 45, and 60 min. Powder samples (75 mg) and glass beads (40 mg) with a mean size of 0.1 mm were weighed into 10-ml test tubes. Dissolution medium (2 ml) was added to the test tubes, and the mixture was agitated for 2 min using a vortex mixer. The contents of the test tubes were transferred to the dissolution medium (900 ml), and the dissolution rate was measured. The similarities between the dissolution curves were calculated (9). The concentration of dissolved powder was measured using the same method as described for solubility determination.

RESULTS AND DISCUSSION

Microscopic examinations showed three different crystal habits among the samples:

1. Plates, for samples 1 (rectangular), 2 (square), 3 (mixture of rectangular and square), and sample 6
2. Shapeless, for sample 4
3. Thin needles, for samples 5 and 7

X-Ray Powder Diffraction Analysis

It was clear from the XRPD traces (Fig. 2) that samples 2, 3, 4, 5, 7, and 7a were comparable with respect to number of peaks and peak positions. These samples represented the same polymorphic form (form A), although there were slight differences in both the peak intensity and maxima, especially in the °2θ range of 14.9 to 20 (Table 1). These differences were due to variations in the crystal habit (crystal morphology) of the different samples. Since both sample 7 and 7a were polymorphic form A, it showed that processing stresses during grinding did not cause transformation of the polymorphic form.

The XRPD pattern of sample 6 can be seen in Fig. 2. The XRPD peak positions and corresponding peak intensities are listed in Table 1. The characteristic XRPD peaks of sample 6 were at °2θ values of 5.66, 13.58, 16–17.76, around 20, and from 24 to 27. There were definite differences between the XRPD patterns of sample 6 and the samples representing crystal form A. These XRPD results indicated that sample 6 was a second distinct crystal form (form B). DSC, TGA, and IR data confirmed this.

Sample 1 exhibited an XRPD pattern that contained characteristic peaks from both form A and form B (Fig. 2 and Table 1). Sample 1 was therefore a mixture of these two crystal forms.

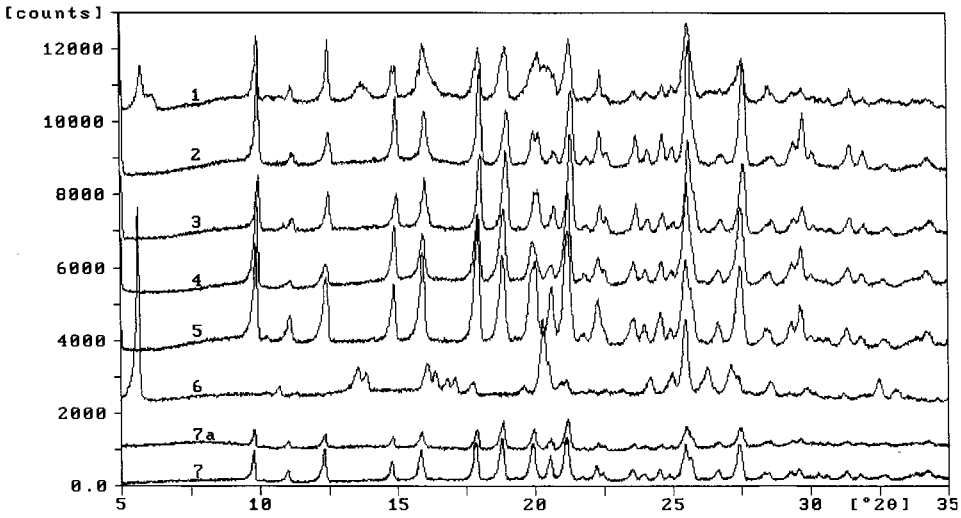

Figure 2. X-ray powder diffraction patterns of eight zopiclone samples.

Table 1

Intensity Values (I/Io) at Main X-Ray Powder Diffraction Peak Angles °2θ of Zopiclone Samples

Main Peaks	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7	
	°2θ	*I/Io*	°2θ	*I/Io*	°2θ	*I/Io*	°2θ	*I/Io*	°2θ	*I/Io*	°2θ	*I/Io*	°2θ	*I/Io*
1	5.0	100	5.0	93	5.0	66	4.9	100	4.9	100	5.7	100	5.0	66
2	9.9	47	10.0	75	10.0	58	9.9	68	9.9	58	13.6	9.5	12.5	65
3	12.5	45	14.9	54	16.0	56	14.9	47	15.9	55	16.1	9.6	15.9	62
4	15.9	40	16.0	44	18.0	79	15.9	38	17.9	75	20.4	27.7	18.0	78
5	17.9	39	18.0	73	19.0	79	17.9	53	18.8	53	20.6	9.4	18.9	87
6	19.0	37	21.3	61	20.1	43	18.8	59	20.0	50	25.1	8.9	20.0	66
7	20.1	36	25.6	100	21.3	100	21.2	68	21.2	68	25.5	26.0	21.2	100
8	21.2	45	27.6	81	25.6	97	25.5	80	25.4	53	26.3	10.6	25.5	72
9	25.6	52	29.7	46	27.6	71	27.5	47	27.5	46	27.1	10.0	27.5	74

After close inspection of the XRPD patterns of all samples with regard to peak intensity and peak position, the following crystal forms were identified:

- form A, for samples 2, 3, 4, 5, 7, and 7a (a true polymorph)
- form B, for sample 6 (a dihydrate)
- a mixture of forms A and B, for sample 1

Thermal Analysis

The DSC and TGA results are shown in Figs. 3 to 5. The thermograms (Fig. 3) of samples 2, 3, 4, 5, and 7 were nearly identical (melting point of 177°C–178°C) and support the XRPD findings that all five samples were the same polymorphic form (form A). It was clear that these samples were not pseudopolymorphs (solvates or hydrates) because of the absence of any characteristic desolvation peaks (DSC traces) or weight loss (TGA traces).

The DSC thermogram of sample 6 (Fig. 4) showed a desolvation endotherm at 97°C and two major endotherms, at 146°C and 173°C. The endotherm (melting process) at 146°C was immediately followed by a small recrystallization exotherm and then by the endotherm at 173°C (melting point).

The desolvation endotherm at 97°C confirmed that sample 6 was a pseudopolymorph (solvate). TGA weight loss data (Fig. 4) indicated an experimental weight loss close to the theoretical weight loss (8.8%) calculated for a dihydrate. Recrystallization of zopiclone from different solvents (e.g., acetone, ethyl acetate, *n*-butanol, acetonitrile, etc.) produced powders that exhibit a TGA weight loss varying from 8% to 8.8% independent of the solvent used (Fig. 4). These findings strongly indicate the presence of a dihydrated form of zopiclone rather than solvates.

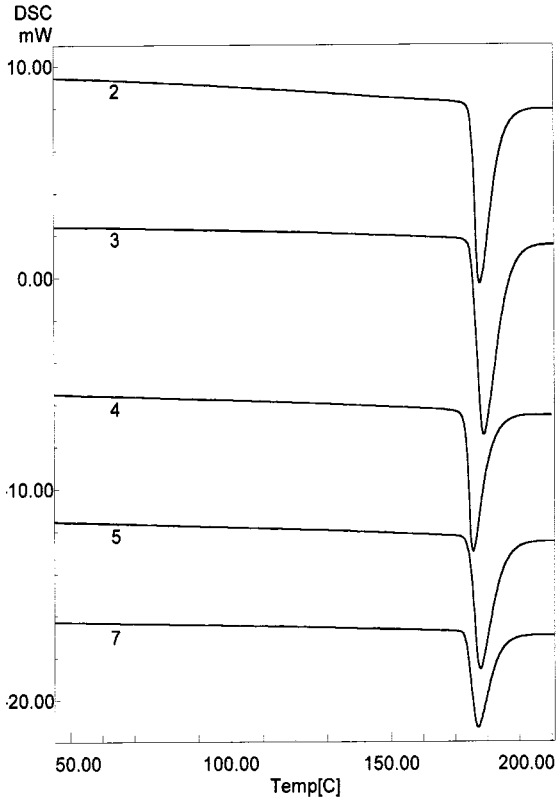

Figure 3. DSC thermograms of zopiclone samples 2, 3, 4, 5, and 7.

The DSC trace of sample 1 (Fig. 5) showed a desolvation endotherm at 108°C; a small, but significant, exo-endothermal process at 146°C; and an endotherm at

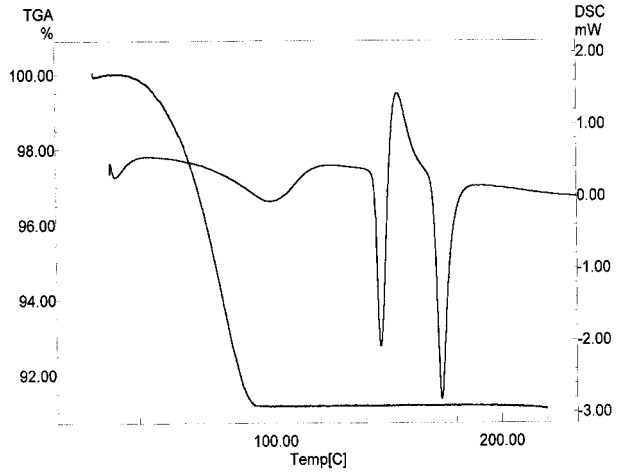

Figure 4. DSC and TGA curves of zopiclone sample 6.

176°C. Sample 1 clearly also contained the dihydrate because the thermal process at 146°C (Fig. 5) was the same as the process seen in sample 6 (Fig. 4). The slight differences in melting points among sample 1 (176°C), polymorph A (177°C–178°C), and form B (173°C) were due to sample 1 being a mixture of forms A and B.

Infrared Analysis

The IR spectra of samples 2, 3, 4, 5 (Fig. 6), and 7 (Fig. 7) were clearly identical with respect to chemical structure. All five samples exhibit identical peaks at the same wavenumbers (cm^{-1}).

There were significant differences between the IR spectra of sample 6 (form B) (Fig. 7) and the other samples. There were extra peaks in the IR spectra of sample 6 at 3499, 3391, 995, 871, and 744 cm^{-1}, and it lacked important peaks at 2978, 2839, 1010, 976, and 754 cm^{-1}. This clearly supports the XRPD.finding that sample 6 represents a different crystal form.

The IR spectrum of sample 1 (Fig. 7) differs slightly from the other spectra. Combination of the IR spectra of samples 2 and 6 in comparison with that of sample 1 suggest that sample 1 was a mixture of forms A and B.

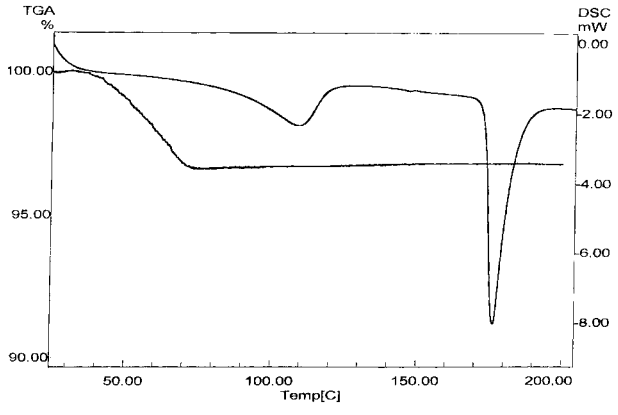

Figure 5. DSC and TGA curves of zopiclone sample 1.

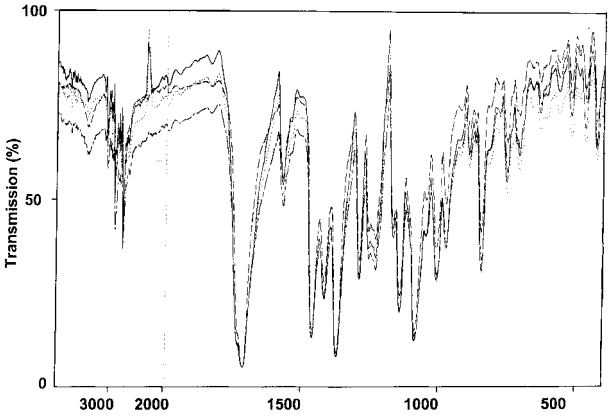

Figure 6. IR spectra of zopiclone samples 2 (—), 3 (----), 4 (----), and 5 (-·-·).

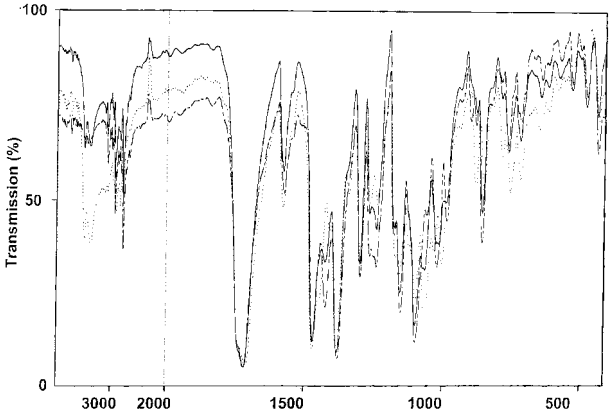

Figure 7. IR spectra of zopiclone samples 1 (—), 6 (----), and 7 (-·-·).

Solubility Determination

The results of the solubility study can be seen in Table 2. At first glance, no significant solubility differences could be detected, but statistical comparisons between

Table 2

Solubility Data of Seven Zopiclone Powders in HPLC Water at 30 ± 1°C

Sample	Solubility (mg/ml)
1	0.1243 ± 0.0012
2	0.1255 ± 0.0014
3	0.1249 ± 0.0009
4	0.1320 ± 0.0004
5	0.1254 ± 0.0020
5	0.1254 ± 0.0020
6	0.1209 ± 0.0009
7	0.1234 ± 0.0002

the results using the Tukey HSD test (Statistica for Windows 5.1B, StatSoft, Inc., Tulsa, OK), showed some differences.

Careful examination of solubility results showed that, during solubility determination, the anhydrous powders (samples 2, 3, 4, 5, and 7) were transformed to the dihydrate (Fig. 8). This was confirmed by TGA results (Fig. 4). The values listed in Table 2 represent the equilibrium solubility in HPLC water of this dihydrate, and not the anhydrous, form of zopiclone.

The solubility of sample 4 (same polymorphic form as samples 2, 3, 5, and 7) was statistically significantly higher than all the other samples, as indicated by a mean p value of .00013. Microscopic examination shows that sample 4 consists of very fine, almost shapeless, particles, thereby increasing the particle's surface-to-volume ratio.

The small shapeless particles of sample 4 dissolved relatively faster in comparison to the larger particles of the other samples, thus explaining the statistically higher solubility. It could be that more of sample 4 went into solution before transformation to the dihydrate (Fig. 8) occurred.

Sample 6, the dihydrate, seemed to be less soluble in comparison to the other samples ($p \leq .01218$), suggesting that the anhydrate might be more soluble. However, this difference was not statistically significant ($p = .24597$) when the solubility of sample 6 was compared to that of sample 7 (form A, true polymorph).

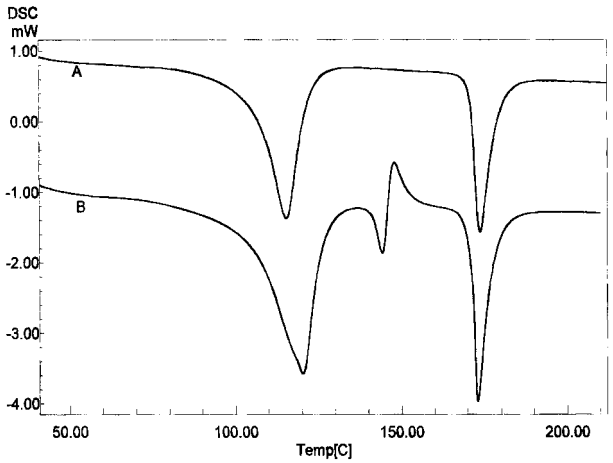

Figure 8. DSC thermograms of zopiclone polymorph A and form B after solubility determination.

Powder Dissolution

Dissolution profiles were compared using a mathematical method described by Moore and Flanner (9). The equation used to calculate a similarity factor is

$$f_2 = 50 \cdot \log\left(\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t(R_t - T_t)^2\right]^{-0.5} \cdot 100\right)$$

where n is the number of dissolution time points, R_t and T_t are the reference and test dissolution values at time t, respectively, and w_t is an optional weighting factor. The value of f_2 is 100 when the test and reference mean profiles are identical. The mean f_2 value of zopiclone powder dissolution was 87 ± 9.3, ranging from 75 to 97 for all possible comparisons among the dissolution profiles.

Dissolution results (Table 3) indicated that all seven samples dissolved practically immediately in the dissolution medium (0.1 M HCl). There were, however, slight differences in the total amount (percentage) of powder dissolved after 60 min. Again, it was found that conversion to the dihydrate took place.

The speed of this conversion depended on the surface area exposed to the dissolution medium. Smaller particles dissolved more quickly, leading to more drug being in solution before conversion to the dihydrate occurred. These results also suggest that the anhydrate is more soluble than the dihydrate.

CONCLUSION

A range of characterization methods was used to characterize the crystal properties of seven zopiclone powders

Table 3

Dissolution Rates of Zopiclone Samples in 0.1 M HCl

Time (min)	Amount Dissolved (%)						
	1	2	3	4	5	6	7
7.5	99	100	101	100	101	100	104
15	99	101	103	101	102	100	104
30	100	101	103	101	102	100	104
45	100	101	103	101	102	100	103
60	99	100	103	101	102	99	103

obtained from four different suppliers. The results obtained clearly indicate that zopiclone exists at least as an anhydrate and a dihydrate.

These two distinct crystal forms (i.e., form A [true polymorph] and form B [dihydrate]) and a mixture of forms A and B were found among the raw materials. These crystal forms were identified and characterized using XRPD, IR, and thermal analysis. During solubility and dissolution measurements, the anhydrated powders changed to dihydrated zopiclone, and no significant difference in aqueous solubility could be detected. Results suggest that the zopiclone dihydrate was less soluble compared to the anhydrated crystal form.

ACKNOWLEDGMENTS

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