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# The effect of recrystallization on the crystal growth, melting point and solubility of ketoconazole

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**Abstract**

Variations in the physico-chemical properties of ketoconazole have been studied, following polymorphic changes caused by fusion-cooling processes or by recrystallization in solvents commonly used in the pharmaceutical industry. Changes in physico-chemical properties were measured by differential scanning calorimetry (DSC), infra-red, X-rays and HPLC. Results revealed changes in the peak temperature of the different recrystallizations and changes in their X-ray diffraction patterns. Infrared spectra of the samples indicated no changes in the chemical structure of ketoconazole. HPLC results indicated a decrease in the solubility except in one case. No degradation products were detected.

*Keywords:* Ketoconazole; Recrystallization; Solubility

## 1. Introduction

Ketoconazole is an oral antifungal agent used for the treatment of mucocutaneous infections [1]. Many studies outline the shortcomings of the active principle in this therapy with respect to biological factors (biopharmaceutical inequivalents), the most common of which is the gastric pH of the individual [2, 3].

The active substance is a derived imidazolic. Its structure presents two basic groups, a piperazine and an imidazole with $pk_a$ values of 2.94 and 6.51 respectively. Its water solubility and antimicotic activity are dependent on the percentage protonation of the piperazine group. This decreases drastically when the medium pH is greater than the

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isoelectric pH of the piperazine group, thus justifying the importance of the gastric pH [4].

Up to now, however, possible technological inequivalents in the product have not been studied in depth. These could, in part, be caused by possible differential crystalline growth of ketoconazole and the affect this could have on its physico-chemical properties and bioavailability.

Unlike inorganic substances, many organic molecules exist in more than one crystalline form. The biopharmaceutical or technological importance of this fact depends on the type of crystal formed by the active principle, because this determines its physical properties [5]. Polymorphism could result from the differential conditions used in preparation of the pharmaceutical form leading to accidental recrystallization after fusion or after dissolution [6, 7].

The aim of this study is to investigate the physico-chemical modifications of ketoconazole characteristics that could originate during the preparation of the pharmaceutical form and that could cause unexpected changes in the biopharmaceutical properties of the end product.

Polymorphic changes were induced in the active principle by fusion-cooling or recrystallization in solvents commonly used in the pharmaceutical industry. Samples were then analysed by differential scanning calorimetry (DSC), X-rays, infrared spectroscopy (IR) and high-performance liquid chromatography (HPLC).

## 2. Experimental

### *2.1. Apparatus*

Differential scanning calorimetry was performed using a differential scanning calorimeter (Model FP 85, Switzerland). The system was calibrated with a high-purity sample (5 mg) of indium.

The infrared spectrum of KBr pellet samples of each crystal form was measured with an Perkin-Elmer double-beam IR spectrophotometer (model 983).

X-ray diffraction spectra of powder samples were obtained using an X-ray diffractometer (PW1710, Phillips, The Netherlands).

A Waters Associates Inc. (Milford, MA, USA) liquid chromatograph was used to analyse ketoconazole. The analytical apparatus consisted of an M-45 high-pressure pump, and a 994 programmable photodiode Array detector. Injections were made by a Rheodyna (Berkeley, CA, USA) injector loop (20 µl).

Prepacked 30 cm × 3.9 mm I.D. µbondapak $C_{18}$ Waters columns (Waters division of Millipore Co. Milford, MA, USA) were used. The back pressures of the columns ranged between 1200 and 1500 p.s.i. separations were performed at room temperature.

### *2.2. Materials*

Ketoconazole was supplied by Jannsen Pharmaceuticals N.V. (Beerse, Belgium). Solvents were purchased from Panreac (Barcelona, Spain). Orthophosphoric acid,

reactive grade monobasic sodium phosphate and methanol LiChrosolv$^{R}$ were all purchased from Merck Co. (Darmstadt, Germany).

### 2.3. Preparation of crystalline forms

Appropriate quantities of ketoconazole were dissolved in the solvent depending on their solubility [8]. Solutions were then left to rest at room temperature (25°C), taking care that the surrounding environment did not become saturated with the solvent. After complete evaporation, samples were dried at reduced pressure and kept in containers isolated from both light and humidity.

Phase transitions were also induced by heating ketoconazole above its heat of fusion and cooling it at (A) 25°C, (B) 0°C and (C) in a desiccator at room temperature (25°C).

### 2.4. Identification and characterization

The active principle and the recrystallizations were heated over the temperature range from 30 to 180°C at a rate of 5°C min$^{-1}$, and in samples ranging in weight from 5 to 6 mg. The peak transition and heat of fusion were determined for all samples. IR spectra for all samples were then registered.

Samples were analysed again after 6 and 12 months to verify the persistence of the crystalline forms obtained.

X-ray diffraction studies were performed using the following experimental conditions: Cu K$\alpha$ radiation, automatic slit, and a scan rate of 6° per minute between 2° 2$\theta$ and 60° 2$\theta$. The obtained diffractograms were computerized in order to compare them.

### 2.5. Solubility studies

Sample solubility was determined by suspending excessive quantities of the crystals in water and maintaining the temperature at 37°C for 24 h. Changes in ketoconazole water solubility were determined by HPLC after filteration of samples with 0.45 μm Durapore filters (Waters division of Millipore Co. (Milford, MA, USA).

### 2.6. Chromatographic conditions

The mobile phase consisted of 75% (v/v) methanol and 25% (v/v) 0.02 M monobasic sodium phosphate, pH 6.0.

Serial dilutions of standard solutions of ketoconazole were freshly prepared in the concentration range of $5.14 \times 10^{-1}$–$5.14 \times 10^{-6}$ mg ml$^{-1}$, and a calibration curve was produced. Standard solutions were injected into the $C_{18}$ column five times so that final values could be obtained from the means of the five measurements.

## 3. Results and discussion

The DSC curves of ketoconazole showed one endothermic peak of fusion, with a melting point of 149.6°C. DSC studies revealed differences in the peak temperatures

and heats of melting of the different ketoconazole samples recrystallized in different solvent systems (Figs. 1 and 2, and Table 1). The greatest variation corresponds to the crystal formed in ethyl alcohol (137.3°C). In accordance with previous studies [9], changes of more than two degrees in the peak temperatures are usually associated with new crystalline forms or extreme changes in the growth of the faces preservation of the same crystalline form. It is well documented in the pharmaceutical literature that the rate of evaporation of the solvent can affect the crystal habit and degree of

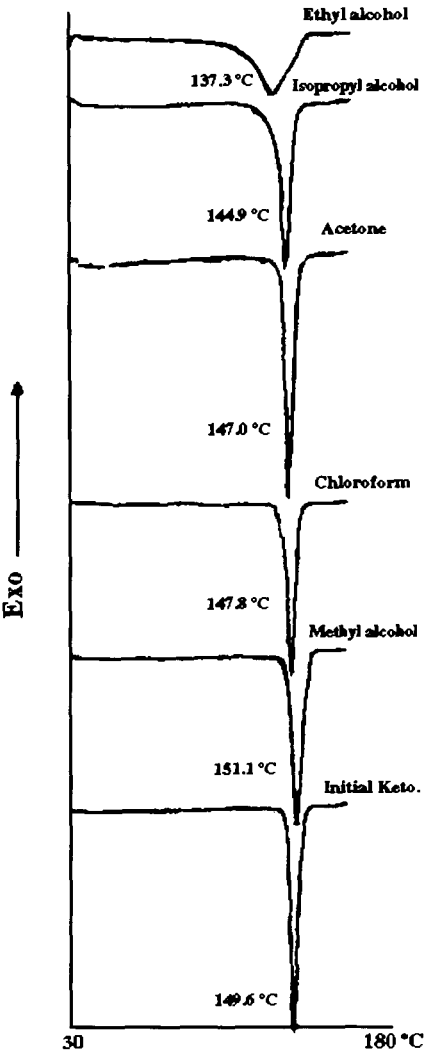


Fig. 1. Differential scanning calorimetric data for ketoconazole and ketoconazole recrystallized in different solvent systems.

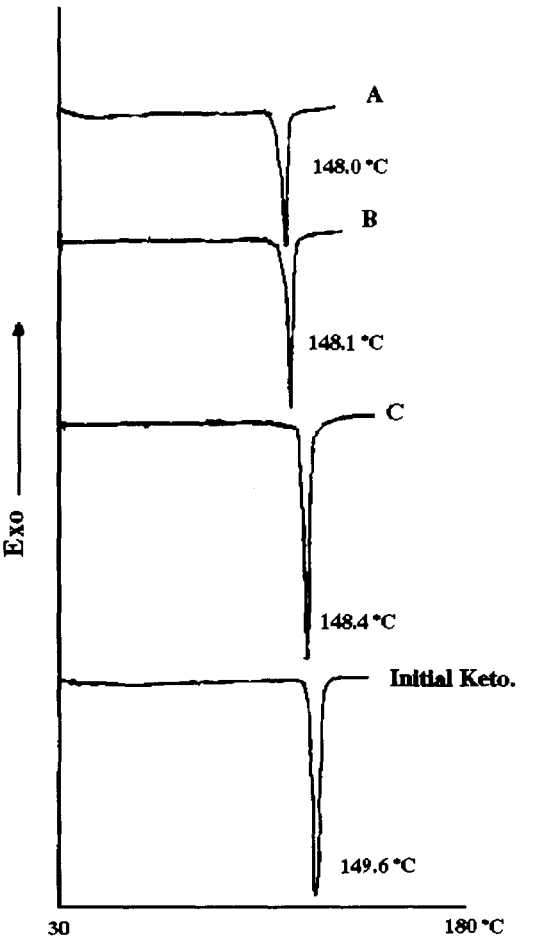


Fig. 2. Differential scanning calorimetric data for ketoconazole and recrystallizations obtained by fusion-cooling.

crystallinity. In this study various solvents with different vapour pressures have been used. So it was expected that the rate of evaporation of all these solvents was different at room temperature; therefore, the crystal habits of these crystals were not the same. In order to clarify this, recrystallization was carried out by a conventional method (dissolving a maximum amount of ketoconazole in a minimal amount of the same solvents at their boiling points and then recrystallizing the samples with cooling). A comparison of the crystal properties of ketoconazole crystals obtained from these two methods demonstrated that there were no significant differences.

These modifications (differences in the peak temperatures of the different recrystallizations) did not imply chemical changes in the ketoconazole structure, as confirmed by comparing their IR spectra to the initial drug. Fig. 3 clearly shows that wavelengths characteristic of ketoconazole, 1507, 1640, 1240, 1258 and 1221 $cm^{-1}$, remain un-

Table 1
Experimental heats of melting obtained for the different samples studied (average of three determinations in each case)

| Samples | $\Delta H_{average}$ $J g^{-1}$ |
|---|---|
| Commercial ketoconazole | 103.00 |
| Isopropyl alcohol | 114.00 |
| Ethyl alcohol | 85.20 |
| Methyl alcohol | 100.00 |
| Acetone | 97.61 |
| Chloroform | 95.71 |
| **A** recryst. at 25°C | 44.80 |
| **B** recryst. at 0°C | 64.63 |
| **C** recryst. at 25°C in absence of humidity | 55.85 |

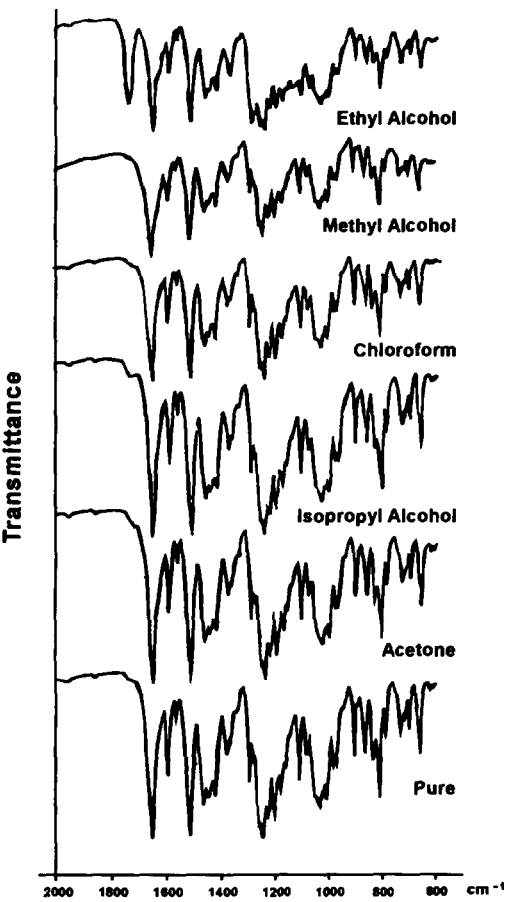


Fig. 3. Infrared spectrograms of commercial product and recrystallizations.

changed in all the recrystallizations. An absorption band was found at 1740 $cm^{-1}$ in form A, attributed to a carboxyl group from impurities in the ethyl alcohol [10].

Comparisons of the diffractograms of the commercial product with those of the recrystallizations are shown in Fig. 4; they reveal significant differences in the intensity of some of the peaks indicating that some of the crystal faces undergo variations in growth. The presence of the same characteristic peaks of ketoconazole in every case implies that new crystal forms are not produced. The only exception is in the sample obtained by recrystallization in isotropyl alcohol where an increase in baseline height is recorded, probably indicating a decrease in its degree of crystallinity.

### 3.1. *Solubility and degradation study*

Five concentrations of the standard solution were tested. The analysis was run over two days, and straight line calibration plots were obtained, with the correlation coefficient $r = 0.9866$ ($r^2 = 97.35\%$).

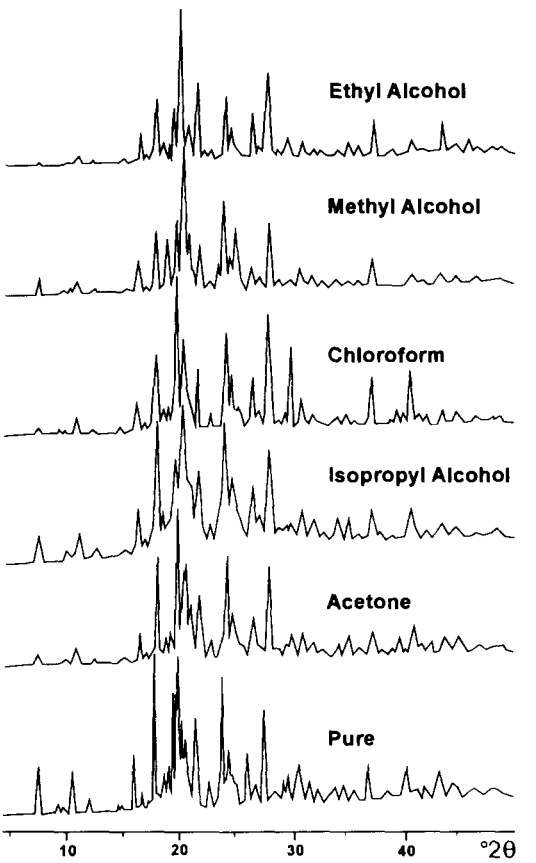


Fig. 4. Powder X-ray diffraction patterns of crystals of ketoconazole.

A study of the precision of the HPLC assay was performed over a 2-day period, calculating the variation coefficient for 5 determinations per day of two different solutions of ketoconazole standard ($5.14 \times 10^{-2}$ mg ml$^{-1}$). The resulting coefficient of variation (CV) was 1.70%.

The limit of detection was defined as the lowest detectable, but not reproducible or accurately quantifiable, concentration with a signal-to-noise ratio > 3:1 [11]. A value of $2.92 \times 10^{-4}$ mg ml$^{-1}$ was obtained. The limit of quantification was defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Limits of $8.75 \times 10^{-4}$ mg ml$^{-1}$ were found for ketoconazole.

The solubility of the samples was determined by calculating the area under the chromatographic peak and applying the calibration curve obtained from the minimum square model. The results of the solubility tests are shown in Table 2. As observed, the solubility was reduced drastically on using methyl alcohol or chloroform.

The already low ketoconazole solubility is reduced by 68.13% and 79.44% after its recrystallization in some of the solvents (methyl alcohol and chloroform respectively). Thermal treatment also produced changes in solubility; an increase of 103.95% was produced on recrystallizing ketoconazole at 0°C (B). This was detected by HPLC, the most suitable method for determining small changes in ketoconazole concentration and for carrying out stability studies.

Retention times of ketoconazole in the recrystallization chromatograms confirm the absence of physico-chemical modifications in the active principle in all cases. Similarly, no ketoconazole degradation products are detected in the chromatogram under the experimental conditions used.

Products analysed after 6 and 12 months showed no notable changes in the previously determined properties. No degradation products were found in the stability study performed with HPLC during a 12-month period.

Table 2
Results of the solubility tests

| Sample | Conc. (mg/100 ml) | Conc. (%) | Increment (%) |
|---|---|---|---|
| Commercial product | 0.9129 | 100 | |
| Isopropyl alcohol | 0.8649 | 94.74 | −5.26 |
| Ethyl alcohol | 0.7115 | 77.94 | −22.06 |
| Methyl alcohol | 0.2909 | 31.87 | −68.13 |
| Acetone | 0.6607 | 72.37 | −27.44 |
| Chloroform | 0.1877 | 20.56 | −79.44 |
| A | 1.8619 | 203.95 | +103.95 |
| B | 0.3554 | 38.93 | −61.07 |
| C | 0.5901 | 64.64 | −35.36 |

## 4. Conclusions

The use of solvents or high temperatures (fusion cooling) on ketoconazole do not lead to a change in the crystalline form. However, a change in the growth of the crystal faces is observed, affecting the physico-chemical properties and bringing about changes in the peak temperatures of the crystals. It is clearly evident that all materials consist of the same polymorphic form. From this finding, it is equally clear that the other experimental variations noted among the sample preparations are associated with a combination of habit modification and varying degrees of crystallinity. That such effects would accompany solvent-mediated recrystallizations is not surprising. The preliminary results shown in this work suggest that the shortcomings of the active principle in antifungal therapy are caused by biological factors more than technological inequivalents.

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