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Evidence of a metastable form of fenofibrate

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Crystalline solids can be rendered amorphous by processes such as lyophilization, spray drying, milling, precipitation from solutions, supercooling of melt, milling and compaction of crystals [1]. From this amorphous form, a metastable one can appear. Despite the fact that in some cases obtaining of a metastable form could be interesting because of its better technological properties, in general its presence is undesirable [2]. Consequently, basic studies of crystallization form of the amorphous state are needed to better understand the underlying mechanisms of such transformations.

Fenofibrate is a hypolipidemic drug [3], whose chemical structure, physico-chemical properties, and degradation products were investigated by Shoji et al. [4]. In our study, we proved the existence of a metastable form of fenofibrate.

After melting fenofibrate powder, cooling of the liquid led to an amorphous form of the drug. TLC and NMR studies allowed to exclude the product's degradation under heating (data not reported). From this amorphous form, the crystallization of a metastable form II was revealed. In fact, by Differential Scanning Calorimetry (DSC), the melting endotherm of form I could be observed during the first heating (Fig. 1a). The calculated onset temperature was 353.4 K with a ΔH_F of 33.2 ($\pm$ 3.5) kJ $\cdot$ mol^{-1}. After slow cooling to room temperature (10 K $\cdot$ min^{-1}), a second heating showed an exothermic peak (approximately between 318 and 333 K), followed by the melting endotherm of the metastable form II. The calculated onset temperature was 346.62 K with a ΔH_F of 10.3 ($\pm$ 6.3) kJ $\cdot$ mol^{-1}. A further increase in temperature led to crystallization of the melt to the stable form I (Fig. 1c). In all experiments, the melting endotherm of the metastable form II was always followed by the form I melting endotherm. This could be due to the fact that some nuclei of form I always appeared during development of the amorphous form. It should be pointed out that brutal quenching of the melt (200 K $\cdot$ min^{-1}) gives a more stable amorphous form, that does not recrystallize during second heating. Its crystallization can occur spontaneously after two days at room temperature (Fig. 1b), or after grinding, or in the presence of a high relative humidity (86%). In all these experiments, a mixture of two forms was obtained. The existence of the metastable form was also shown by X-ray diffraction studies. Fig. 2 shows the X-ray spectra of form I and of the metastable form II, both after crystallization and after storage performed in order to check the stability of the metastable form II. The solid-solid transition of the metastable form II to form I began already after 15 days of storage. After 90 days, the metastable form could still be observed.

In conclusion, during some manufacturing processes a metastable form of fenofibrate could appear. This fact has to be taken into account when new processes are developed. Unfortunately, this new polymorphic form cannot be used in formulation because of its great instability.

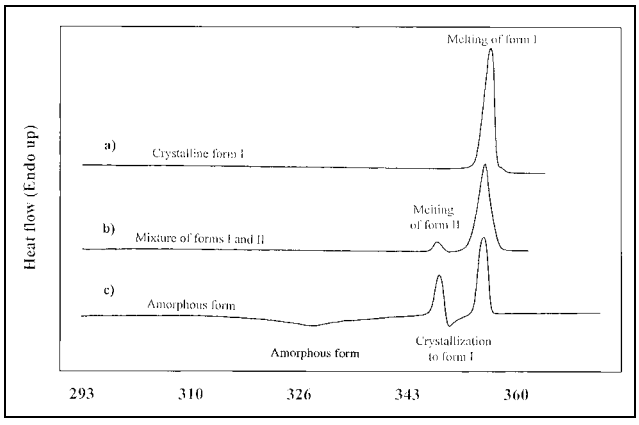

Fig. 1: Differential Scanning Calorimetry of fenofibrate. a) First heating run performed at 10 K $\cdot$ min^{-1}. b) Second heating run (10 K $\cdot$ min^{-1}) performed after brutal quenching of the melt (200 K $\cdot$ min^{-1}) to room temperature and after two days at room condtions. c) Second heating run performed immediately after slow cooling (10 K $\cdot$ min^{-1}) the melt to room conditions.

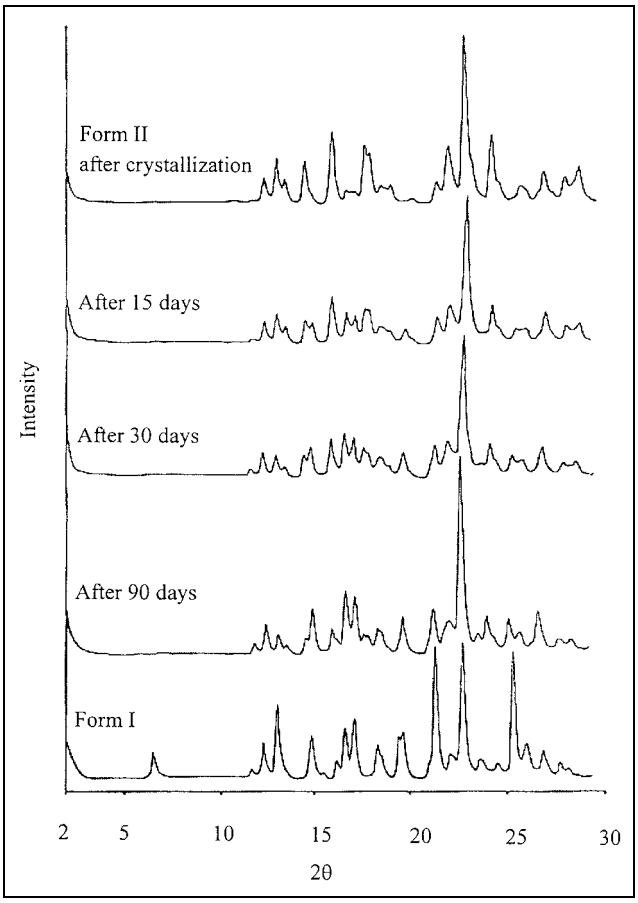

Fig. 2: X-ray diffraction patterns of form I, and metastable form II of fenofibrate, both after crystallization and after different periods of storage

Experimental

1. Materials

Fenofibrate was supplied by Sigma (USA). To make the X-ray diffraction study possible, a sufficient amount of the metastable form II was obtained by melting fenofibrate in an oven at 363 K, where only slow cooling to room temperature (10 K $\cdot$ min^{-1}) could be achieved. After crystallization, which occurred spontaneously at room conditions, the material was grounded.

2. Methods

2.1. Differential Scanning Calorimetry

DSC curves were recorded on a Perkin Elmer (Pyris 1, USA) equipment. The instrument was calibrated versus temperature and furnace with two standards (indium and zinc). Aluminium closed pans were used. An amount of 4–5.0 mg was weighed. A first heating run was performed from 273 K to 358 K at 10 K · min^{-1}. This step was followed by an isothermal 5-min step to avoid incomplete melting. Two cooling rates were tested: brutal quenching to room temperature (200 K · min^{-1}), or relatively slow cooling to room temperature (10 K · min^{-1}). A second heating run was then carried out at 10 K · min^{-1}. Mean ΔH_F values of the melting endotherms were determined from replicate experiments. 95% confidence intervals are given in parenthesis.

2.2 X-ray Diffraction study

X-ray diffraction studies were carried out to characterize fenofibrate modifications. A Philips PW 1730 diffractometer was used as X-ray generator of Cu Kα radiation (λ = 1.541838 Å). The experimental X-ray powder patterns were recorded on a PH 8203 instrument. The goniometer supply was a PW 1373 and the channel controle a PW 1390. The data were collected in the continuous scan mode using a step size of 0.01 2θ. The range scanned was 2θ to 30 . X-ray diffraction was also used to determine the physical stability of the metastable modification II. This form was stored for 90 days. X-ray analyses were performed and diffractograms were recorded at regular intervals.

Acknowledgements: The authors gratefully acknowledge the financial support of the Italian MURST – "Fondi 40% Progetto Nazionale Tecnologie Farmaceutiche".

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Received October 19, 1999
Accepted Febuary 26, 2000

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Metabolites of baicalein in human urine

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Baicalein is the aglycone of baicalin, which is one of the major constituents present in Traditional Chinese Medicine Radix Scutellariae. The crude drug and baicalin are widely used for the treatment of various inflammations including hepatitis. We have recently studied the metabolism of baicalin in human urine after oral administration and obtained some valuable results. In order to clarify the metabolic pathway of baicalin in human, further investigation of its metabolism in humans led to the identification of three main metabolites in urine after oral administration (Table).

Reversed-Phase HPLC of a sample from human urine showed three distinct peaks in the chromatogram due to the metabolites of baicalein, which were designated as M1, M2 and M3 in the order of increasing polarity (Fig. A).

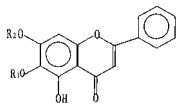
In the UV spectrum of M1, maximal absorptions were seen at 268.8 nm and 316.3 nm, which are characteristic of the flavone skeleton. By comparing the UV spectrum and the HPLC profile with an authentic sample, M1 was identified as baicalein-6-*O*-β-D-glucopyranuroside (Fig. B).

In the UV spectrum of M2, maximal absorptions were seen at 273.6 nm and 311.5 nm, which are characteristic of the flavone skeleton. The spectra were similar to those of 6-methyl-baicalein-7-*O*-β-D-glucopyranuroside [1]. These findings prompted us to synthesize the 6-methyl-baicalein-7-*O*-β-D-glucopyranuroside from baicalin (see experimental). The UV spectrum and HPLC profile of the synthetic compound were in agreement with M2 of the sample. (Fig. C). Thus, M2 was identified as 6-methyl-baicalein-7-*O*-β-D-glucopyranuroside.

In the UV spectrum of M3, maximal absorptions appeared at 278.3 nm and 316.3 nm, which are characteristic of a flavone skeleton and similar to those of baicalin. On the other hand, the chromatographic profile of M3 was in agreement with that of baicalin (Fig. D). Also, the UV spectrum and the HPLC profile of the sample kept unchangeable except for peak height enhancing of M1 as some baicalin was added to the sample. Thus, M3 was identified as baicalein7-*O*-β-D-glucopyranuroside (baicalin).

In the present study, three main metabolites of baicalein in human urine were identified to be in agreement with those of baicalin found in a previous study, which confirmed the

Table: Structures of the metabolites and derivative



	R_1	R_2
M_1	GlcUA	H
M_2	Methyl	GlcUA
M_{2a}	Methyl	GlcUA methyl ester
M_3	H	GlcUA

GlcUA: β-glucopyranuronosyl