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Crystal structure and solid-state studies of aged samples of tienoxolol, an API designed against hypertension

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ABSTRACT

Aging of drug molecules is generally studied following regulatory procedures, i.e. under forced conditions and for relatively limited storage time; therefore naturally aged samples are rare and provide scientific reference data beyond regulatory considerations. Tienoxolol was studied after 25 years of storage in the dark under ambient conditions. About 86% of the samples still consisted of tienoxolol and the main impurity (13%) was caused by the hydrolysis of the ester moiety. Protection from humidity is therefore important. Other sensitive groups containing nitrogen and sulfur appear to be quite stable with less than 0.8% conversion over 25 years. In addition, the crystal structure has been solved. Tienoxolol orange needles were found to crystallize in the orthorhombic non-centrosymmetric space group *Iba*2, indicating that the crystal is a racemic compound. The unit cell parameters at room temperature are $a = 10.069(5)$ Å, $b = 45.831(10)$ Å, and $c = 9.822(5)$ Å and the unit cell volume is 4533(3) Å^3 with $Z = 8$.

1. Introduction

Samples of tienoxolol (TXL) stored in the dark for about twenty-five years, were fortuitously found in the laboratory, together with related documents from the former research centre of UPSA laboratories. Because at that time no solid-state studies or crystal structure solution had been performed, they were undertaken together with stability studies after its unusually long storage time.

TXL was designed to combine a diuretic and a β-adrenoreceptor antagonist into a single molecule (Fig. 1) as a therapeutic approach for the treatment of hypertension (Bouley and Teulon, 1984; Bouley et al., 1986). Generally, these two types of activity are produced by administration of different active pharmaceutical ingredients (API) complicating control over simultaneous release and activity. Although the approach stems from the early eighties (Berdeaux et al., 1988; Bouley et al., 1986; Cloarec et al., 1984, 1987; Leary et al., 1993), there is still potential for APIs that combine two pharmacological activities in a single molecule.

2. Materials and methods

2.1. Materials

As stated in the introduction, TXL (420.51 g mol^{-1}) samples were provided by UPSA Laboratories and stored for 25 years in the dark. The samples consisted of single crystals as shown in Fig. 2.

2.2. Single crystal X-ray diffraction

Diffraction data were collected with an Enraf-Nonius CAD4 diffractometer using Mo Kα radiation ($\lambda = 0.71073$ Å). Data collection and reduction was done with the CAD4 Express Enraf-Nonius programs package and XCAD4 (Enraf-Nonius, 1994; Harms and Wocadlo, 1995). All data were corrected for Lorentz polarization effects. The structure was solved by direct methods with SHELXS97 and refined by least-squares methods on F^2 SHELXL-97 (Sheldrick, 2008). The positions of the non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were given idealized positions and were constrained to ride on their parent atoms: CH distances 0.93, 0.98, 0.96, and 0.97 Å for $CH_{(aromatic)}$, CH, CH_2, CH_3 groups respectively and with $U_{iso}(H) = 1.5U_{eq}(C)$. For OH and

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Table 1
Crystal and experimental data of tienoxolol.

Chemical formula	$C_{21}H_{28}N_2O_5S$
Formula weight	420.51
T (K)	298(2) K
Crystal size (mm)	0.80 × 0.20 × 0.10
Crystal system	Orthorhombic
Space group	*Iba* 2 (no. 45)
Unit cell dimensions	a = 10.069(5) Å
	b = 45.831(10) Å
	c = 9.822(5) Å
Volume (Å^3)	4533(3)
Z	8
D_x (g cm^{-3})	1.232
μ (Mo Kα) (mm^{-1})	0.175
F(0 0 0)	1792
θ range for data collection	2.07–25.00°
No. of reflections collected	8454
No. of independent reflections	4281
No. parameters	272
Absorption correction	Multi-scan
T_{min}; T_{max}	0.902; 0.983
Goodness-of-fit on F^2	1.093
R indices [$I > 2\sigma(I)$]: $R1$	0.0750
R indices (all data): $R1$	0.1444
$(\Delta/\sigma)_{max}$	0.000
CCDC deposition number	727239

NH bonds, hydrogen atoms were located in a difference Fourier map for their positions and isotropic displacement parameters were set to U_{iso}(H) = 1.2U_{eq}(O) and U_{iso}(H) = 1.2U_{eq}(N). The final refinements converged to $R1$ [$I > 2\sigma(I)$] equal to 0.0750. The crystal and structure-refinement data are summarized in Table 1.

CCDC-727239 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

2.3. High resolution X-ray powder diffraction

X-ray powder diffraction was performed on a transmission mode diffractometer using Debye–Scherrer geometry equipped with a cylindrical position-sensitive detector (CPS120) from INEL (France) containing 4096 channels (0.029° 2θ angular step) (Ballon et al., 1983) with monochromatic Co Kα_1 (λ = 1.78897 Å) radiation.

Ground specimens were introduced in a Lindemann capillary (0.5 mm diameter) rotating perpendicularly to the X-ray beam during the experiments to improve the average over the crystallite orientations.

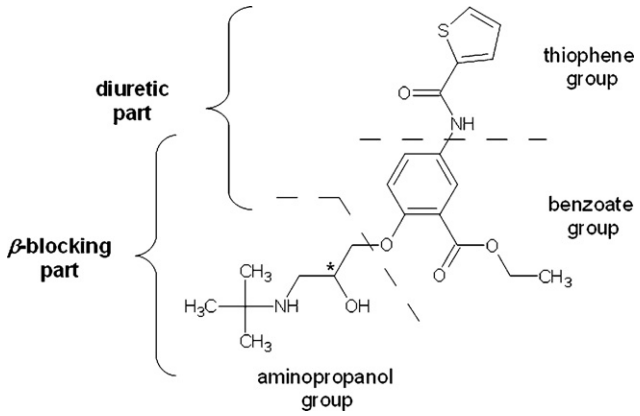

Fig. 1. Chemical structure of tienoxolol ($C_{21}H_{28}N_2O_5S$).

2.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) experiments were performed with a Q100 analyzer from TA-Instruments. Indium was used as a standard for the calibration of temperature and enthalpy change. Specimens were weighed with a microbalance sensitive to 0.01 mg and sealed in aluminum pans of 30 μL inner volume

2.5. Polarimetry

To verify that TXL was obtained from a non-stereospecific synthesis, the optical activity was measured with an ADP220 Digital Polarimeter (Bellingham & Stanley Ltd.) at the sodium D line using a solution in ethanol of 12.27 g L^{-1} and a cell length of 20 cm. The measured value was 0.00 ± 0.02° giving rise to a specific optical activity of 0.0 ± 0.5° dm^{-1} g^{-1} cm^3.

2.6. High-pressure liquid chromatography coupled electrospray ionization tandem mass spectrometry (HPLC–ESI-MS/MS)

Analytical HPLC–ESI-MS/MS was performed on a LCQ Quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) interfaced with a Variance Polaris C 18-A column (Ansys Technologies, Lake Forest, CA, USA) 150 mm in length, 4.6 mm in internal diameter and 3 μm in particle size. Analytes were eluted at a flow rate of 1 mL min^{-1} with a gradient of 0.1% (v/v) trifluoracetic acid (solution A) and acetonitrile (solvent B) at the ratio of 75:25 v/v for the initial 25 min, then gradually increasing to 30:70 v/v for 15 min, and maintained at this ratio for another 15 min. The column effluent was directed to the ESI-MS/MS. The instrumental parameters of the mass spectrometer were as follows: the electrospray ionization source

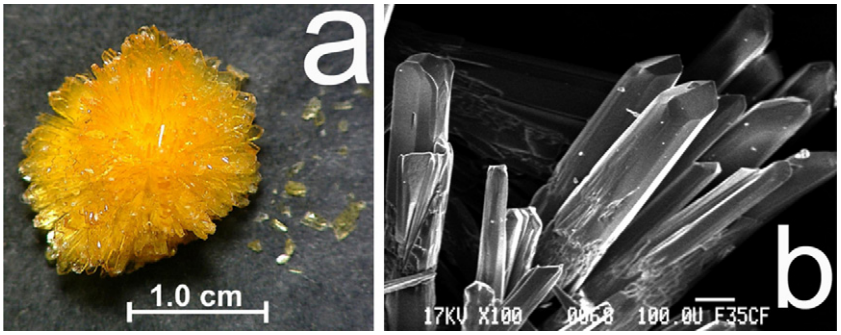

Fig. 2. Tienoxolol crystals from the samples provided by UPSA Laboratories: (a) Optical microscopy image; (b) Scanning electron microscope (JEOL JSM-35 CF), the scale bar represents 100 μm.

was operating in the positive ionization mode with a 1/3 split, the spray voltage was 4.5 kV and the source current was 80 μA. High purity hydrogen was used as sheath/auxiliary gas (flow rate 80/20). Capillary voltage was 3.0 V and the temperature was set at 270 °C. The analysis of related compounds and impurities was conducted by scanning a mass range of 50–1000 mass-to-charge ratio (m/z). The data acquisitions were analyzed using Xcalibur software (Thermo Finnigan), version 1.2. The collision energy in MS/MS mode, concurring with the full argon induced fragmentation of the parent ions, was 1.5 V (pressure reading 8.05×10^{-6} mbar). Processing of mass spectrometry data was optimized by ACD Labs software version 10 (ACD Labs, Toronto, Ontario, Canada). The UV detector was a photodiode array TSP UV 6000 (Les Ulis, France) controlled by Chromquest® software (TSP). The detection wavelength was set at 287 nm. LC–MS conditions were set up to ensure detection of significantly present impurities. A 0.05% threshold was chosen in accordance with ICH recommendations and the concentration of the test samples was adjusted according to the detection limit of the method. Therefore, each sample was diluted in 50/50 v/v ultrapure water/acetonitrile to obtain a final concentration of 300 μg mL^{-1}.

Table 2
Selected bond lengths (Å) and bond angles (°).

N2–C17	1.39(1)
N2–C18	1.47(1)
N1–C6	1.414(7)
N1–C5	1.346(6)
C5–O1	1.224(7)
C12–O2	1.218(7)
C12–O3	1.311(8)
C16–O5	1.41(1)
N1–C5–O1	122.1(5)
O2–C12–O3	122.3(6)
C9–O4–C15	118.2(5)
C15–C16–O5	104.4(7)
C17–N2–C18	116.4(8)

Table 3
Important hydrogen bonds in the tienoxolol structure (Å,°).

D–H···A	D–H	H···A	D···A	D–H···A
N1–H1A···O1(i)	0.85(4)	1.99(3)	2.824(6)	166(5)
N2–H2A···O5(ii)	0.9(1)	2.7(1)	2.93(1)	115(7)
O5–H5···N2(ii)	1.01(8)	2.1(1)	2.93(1)	135(10)

Symmetry codes: (i) $2-x, y, 1/2+z$; (ii) $1-x, -y, z$.

3. Results and discussion

3.1. Crystallographic study

TXL orange needles (Fig. 2a) were found to crystallize in the orthorhombic non-centrosymmetric space group *Iba*2. The unit cell parameters are $a = 10.069(5)$ Å, $b = 45.831(10)$ Å, and $c = 9.822(5)$ Å and the unit cell volume is 4533(3) $Å^3$ with $Z = 8$. The structure of TXL, obtained by single-crystal X-ray diffraction, is shown in Fig. 3. Selected bond lengths and angles are given in Table 2. TXL possesses an asymmetric carbon (C16); however, the present non-centrosymmetric crystal structure *Iba*2 indicates, due to its glide planes, that both enantiomers must be present as a racemic compound.

The TXL molecule consists of three groups, the thiophene-2-carboxamide group, the ethyl benzoate group, and the aminopropyl group (or 2-hydroxy-3-(tert-butylamino)propoxy group)(see also Fig. 1). The thiophene ring is planar, with a maximum deviation from the plane of −0.013(5) Å for atom C2. The benzene ring is planar, with a maximum deviation of −0.007(3) Å for atom C6. The angle between the planes of the thiophene ring and the benzene ring is 55.5(2)°; the two rings and the ester moiety deviate from coplanarity to diminish steric repulsion. For (*R*)-TXL, the torsion angles C6–N1–C5–C4 and C8–C12–O3–C13 are (+)anti-periplanar and C9–O4–C15–C16 is (−)anti-periplanar.

One (*R*)-molecule is connected by hydrogen bonding to three (*S*)-molecules and vice versa as shown in Fig. 4. Along the *c* axis, interchanging (*R*)- and (*S*)-enantiomers form infinite hydrogen bond chains N–H···O with graph set assignment **C**(4) (cf. inset bottom Fig. 4) (Etter, 1990; Etter et al., 1990). The main axis of the molecules alternatingly makes a positive or a negative angle of about 45° with the *b* axis along the **C**(4) chain in the *c* direction (Fig. 5). Thus, if all (*R*)-enantiomers make an angle with the *b* axis of +45°, the (*S*)-enantiomers on the same **C**(4) chain will make an angle of −45°. Following the molecular axis upward, an opposite enantiomer is bound by two hydrogen bonds O–H···N (graph set assignment $\mathbf{R}_2^2(10)$) to the first enantiomer (cf. inset top Fig. 4) in alignment with the molecular axis. Next to the infinite **C**(4) chain along *c*, hydrogen bonded TXL molecules form infinite zigzag chains along the *a* axis. Two separate zigzag chains are interlocked with a small undulation along *c* (cf. Fig. 5), but they are not interconnected by hydrogen bonds. N–H···O and O–H···N hydrogen bonds (Table 3) form 2*D*-networks of thick sheets containing the two interlocked zigzag chains (cf. Supplementary materials Fig. S1). It can be seen in Table 3 that the strongest interactions are the

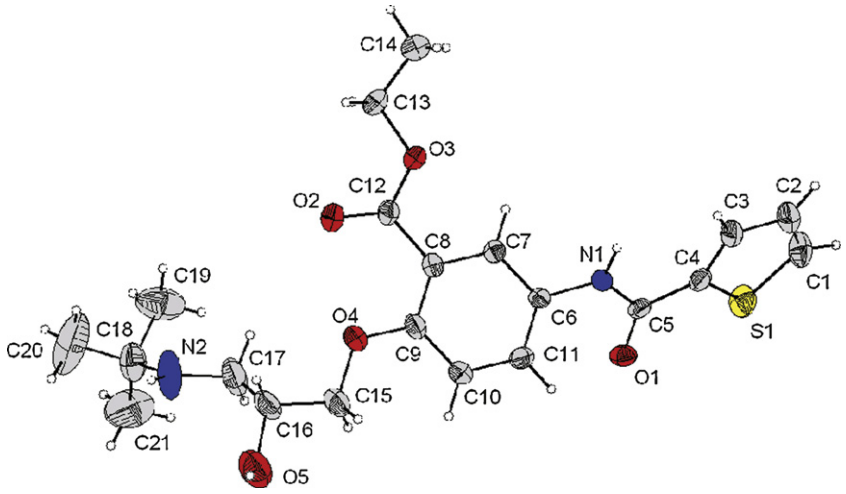

Fig. 3. Molecular structure of (*R*)-tienoxolol with 30% anisotropic displacement representation showing non-H atoms labeling.

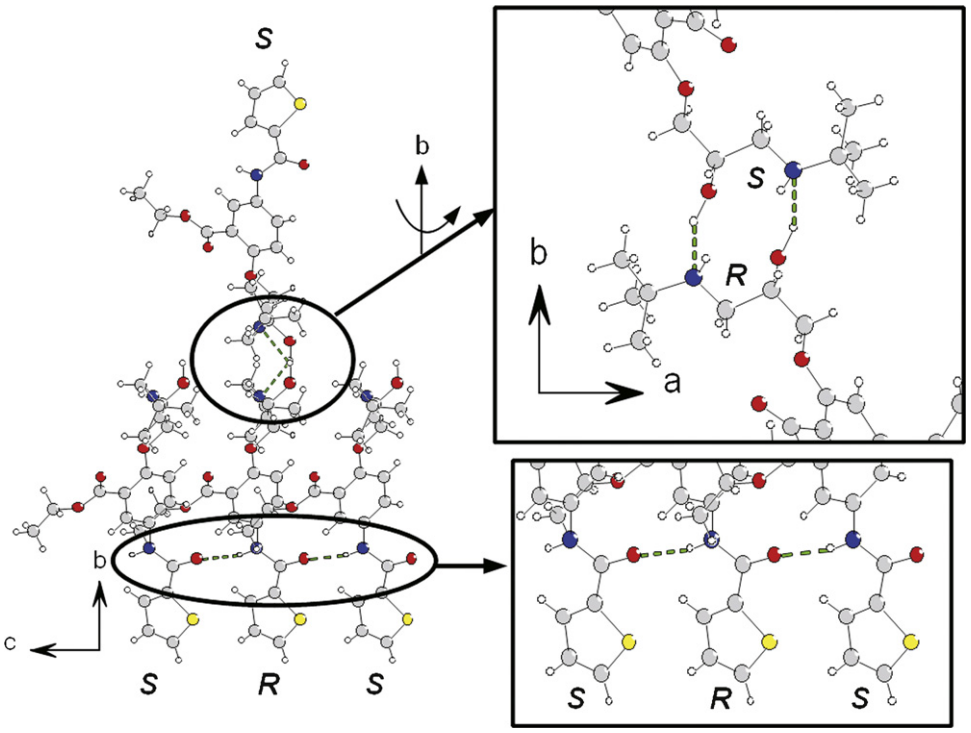

Fig. 4. Hydrogen-bond network of tienoxolol. Left hand side: (*R*)-tienoxolol connected to three (*S*)-molecules by hydrogen bonding. Right-hand side bottom: close-up of hydrogen bonding (N–H···O) chain, graph set **C**(4). Right-hand side top: close-up of hydrogen bonding (O–H···N) ring, graph set $\mathbf{R}_2^2$ (10).

N1–H1A···O1 hydrogen bonds of the **C**(4) infinite chains along the *c* axis. There are no hydrogen bonds along *a*.

3.2. Aging and purity

The X-ray powder diffraction pattern of the 25-year-old sample unambiguously demonstrates that there are no unindexed Bragg peaks for TXL (Fig. 6); thus any impurities in the sample are most likely non-crystalline.

To quantify the purity of the sample, which had been sealed in a vial by a plastic stopper for 25 years, DSC runs were conducted at 10 K min^{-1} and liquidus-like endothermic peaks were recorded (cf. Fig. 7). The resulting curves can be compared to those obtained shortly after the synthesis (Fig. 7). By applying the Schröder equation, a rough estimate of the quantity of impurity can be obtained:

$$\ln(x) = \frac{\Delta H}{R(1/T_{fus} - 1/T_x)}$$

x is the mole fraction of TXL, ΔH is the enthalpy of fusion of pure TXL, R the gas constant, T_{fus} the melting point of pure TXL and T_x the melting temperature of the sample with a mole fraction x of TXL. For T_{fus} and ΔH, the values 403.25 K and 41.68 kJ mol^{-1} were taken, measured shortly after synthesis.

With T_{max} of the melting peak in the aged sample found at 397.9 K, the purity was calculated to be about 85%. This means that approximately 15% degraded over 25 years. Since the specimens were polycrystalline sea urchins kept as such with no crushing, it is assumed that impurities formed first at the surface of the crystals, which means that the purity of a crystal depends on its size. This is

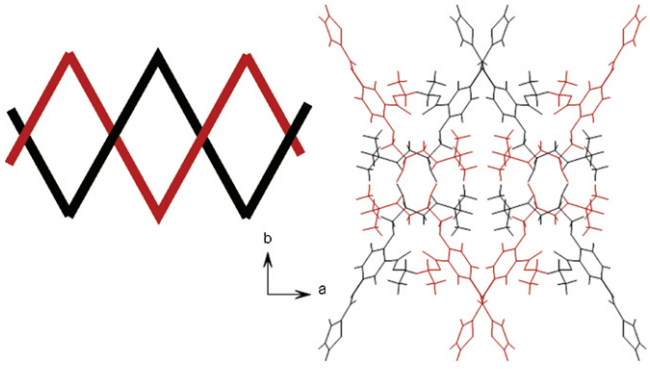

Fig. 5. Zigzag chain along the *a* axis. Left: schematic view of the arrangement of the main molecular axis. Right: same arrangement showing the individual molecules. In addition to the double zigzag (black and red) along *a*, there is also a smaller undulation along *c*: the black zigzag first passes before the red one and then behind (cf. schematic view left). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

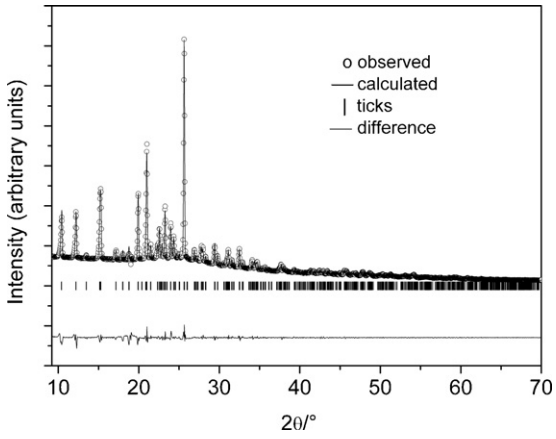

Fig. 6. Room-temperature X-ray powder diffraction pattern of 25-year-old tienoxolol (Co Kα_1 radiation; λ = 1.78897 Å). Profile matching with constant scale factor (i.e. Le Bail fit).

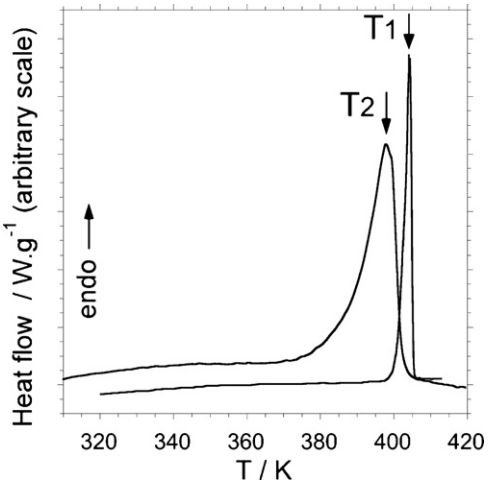

Fig. 7. Two DSC curves of the melting transition of tienoxolol. One is obtained shortly after its synthesis (T1) and one after 25 years of storage (T2). The displacement of the peak and its width are representative for the amount of impurity (see text and also Supplementary materials).

particularly well illustrated in the supplementary materials, where a sequence of pictures is shown of melting TXL as a function of temperature (Supplementary materials Fig. S2). A tiny crystal melts well before the larger crystals start to melt; this coincides with the much lower onset temperature of peak T2 (Fig. 7) obtained by DSC as well as with its shape in comparison with the peak of the pure compound (T1, Fig. 7).

The HPLC–UV–MS^2 measurements indicate the presence of one major impurity; the other impurities were present in much lower amounts. The elution area of TXL was 86.5% of the total area and the peak of the major impurity equaled 12.66%. The latter peak was characterized by *m/z* 393 with a difference of 29 *u* most likely due to the absence of the ethyl ester on the benzoate group as suggested by the HPLC–tandem mass spectrometry (cf. Supplementary materials Table S1 and Fig. S3). Other much smaller peaks had elution areas of 0.24% or smaller. Thus during 25 years of storage, the main decomposition route is the hydrolysis of the ester, whereas the thiophene and the aminopropanol groups appear to have considerably higher stabilities.

The purity measurements by DSC or by HPLC–UV lead to similar estimates for the purity of TXL. Therefore, if the temperature of fusion of a drug molecule is known, DSC can quickly give a quantitative measure for the purity of the sample based on the position of the peak as illustrated in Fig. 7. Guechot et al. (1988) determined the presence of two impurities in TXL hydrochloride, a positional isomer involving the thiophene group and 2-(3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy)-5-[(2-thienylcarbonyl)amino]benzoic acid (392.14 g mol^{-1}). The positional isomer was removed in a purification step after synthesis and can never be a product of decomposition. The benzoic acid on the other hand is still the major impurity after 25 years, at least in the case of the basic form of TXL.

4. Concluding remarks

The find of naturally aged samples of TXL provided a perfect opportunity for purity and persistence studies on aged TXL even if aging studies in its strictest sense have to be performed following regulatory procedures. In addition, the crystal structure, which had not been obtained before, was solved. TXL, synthesized by a non-stereospecific reaction, crystallizes as a racemic compound. The impurities in TXL are amorphous as determined by X-ray diffraction and the purity after 25 years is about 86.5% as determined by HPLC–MS. Although the results obtained by HPLC–MS are qualitatively and quantitatively more precise, the purity estimate obtained by DSC is fast and it only needs advance information about the shape of the melting peak of the pure compound. Visual inspection of the melting peaks will immediately lead to a conclusion about the presence of impurities due to aging and the application of the Schröder equation will provide a rough quantitative result. It is also important to choose the samples wisely, because small crystallites, due to their large surface area, will probably have a different purity profile than large crystals.

The main impurity of TXL is the acid formed by hydrolysis of the ester. It is well known that esters are sensitive to the presence of water; thus TXL should be stored in the absence of humidity.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.10.025.

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