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Quantification of crystallinity in blends of lyophilized and crystalline MK-0591 using X-ray powder diffraction

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Abstract

X-ray powder diffraction was used to determine the percent crystallinity in blends of crystalline and X-ray amorphous MK-0591, a potent indirect leukotriene biosynthesis inhibitor. A linear calibration curve was obtained. Preliminary studies using powder blends of MK-0591 with pregelatinized starch, microcrystalline cellulose, and magnesium stearate demonstrated the feasibility of using this method to determine (or quantify) the percent crystallinity of MK-0591 in tablet formulations.

Keywords: L-686,708; MK-0591; Leukotriene biosynthesis inhibitor; X-ray powder diffraction; Crystallinity, quantitative determination

1. Introduction

MK-0591, the sodium salt of 3-[1-[(4-chlorophenyl)methyl]-3-9*t*-butylthio]-5-((2-quinoly)methoxy)-1H-indole-2]-α,α-dimethylpropanoic acid, is a potent and selective indirect leukotriene biosynthesis inhibitor (Prasit et al., 1991, 1993). It acts by binding to the 5-lipoxygenase activating protein (FLAP) and thereby inhibits the translocation and activation of 5-lipoxygenase (Evans et al., 1991). In the solid state it exists in two principal forms: random (amorphous) or highly ordered (crystalline). The crystalline form is non-bioavailable, presumably due to its low aqueous solubility (5 μg/ml) compared to the greater bioavailability of the more water soluble (60 mg/ml, obtained after agitation in the absence of light at room temperature for 24 h) X-ray amorphous form.

Because of its greater bioavailability, X-ray amorphous MK-0591 was recommended as a drug candidate for safety assessment studies to ensure that sufficiently high systemic levels would be achieved. X-ray amorphous MK-0591, prepared either by spray-drying or by lyophilization, is thermodynamically metastable. Prior to the initiation of safety assessment studies, a method of evaluating the physical stability of X-ray amorphous batches was required. Differential scanning calorimetry (DSC) was used to follow the physical stability of samples placed on accelerated solid-state stability (Clas et al., 1995). Crystallization

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did not occur in lyophilized MK-0591 samples after 6 months at 60° C in the absence of moisture. This is in large part due to its high glass transition temperature (T_g). A T_g of approx. 125° C (midpoint, 10° C/min) was measured by DSC. Materials below T_g are rigid and brittle, and only rotational or vibrational short-range motions are possible. As the material approaches T_g, larger scale motions are possible and there is onset of segmental molecular mobility (Eisenberg, 1984; Billmeyer). The molecules then have sufficient mobility to reorganize and crystallize.

However, in the case of X-ray amorphous MK-0591 where the T_g is greater than 50° C above room temperature, there is insufficient molecular mobility for the molecules to reorganize into a crystalline lattice at room temperature in the absence of moisture. While DSC proved to be a rapid easy method for determination of crystallinity, it was of interest to develop an alternative method which could be used on drug/excipient mixtures, and ultimately on formulated tablet. Ideally, the method should be sensitive to low concentrations of crystalline material (approx. 5% or less) and should be relatively easy to use.

Quantitative X-ray powder diffraction (XRPD) has been used for many years to determine the crystallinity of materials (Klug and Alexander, 1954; Popović et al., 1983; Suryanarayanan and Mitchell, 1985; Jenkins and De Vries). For example, the effect of grinding on the percent crystallinity of cefixime trihydrate and ampicillin trihydrate was studied by X-ray powder diffraction to evaluate the solid-state stability of the compounds (Takahashi et al., 1984; Kitamura et al., 1989). A rapid quantitative XRPD method was developed to evaluate lyophilized batches of imipenem doped with X-ray amorphous cilastatin sodium (Ryan, 1986). Linear calibration curves have been obtained to quantify the relative amounts of anhydrous carbamazepine in blends of anhydrous carbamazepine and carbamazepine dihydrate (Suryanarayanan, 1989). This was extended to formulations of carbamazepine with microcrystalline cellulose, starch, stearic acid and silicon dioxide where intact tablets could be studied by XRPD (Suryanarayanan and Herman, 1991). In another example, the percent crystallinity of indomethacin was determined by measuring the intensity of the indomethacin form I crystalline reflection at $2\theta = 11.6°$ (Imaizuni et al., 1980).

In this work, quantitative X-ray powder diffraction was used to derive a standard curve of the percent crystalline content in lyophilized MK-0591 blends doped with crystalline MK-0591. The standard curve was verified with additional blends of crystalline MK-0591 in lyophilized X-ray amorphous MK-0591, as well as with added excipients, such as pregelatinized starch, microcrystalline cellulose and magnesium stearate.

2. Materials and methods

2.1. Materials

X-ray amorphous MK-0591 was obtained by lyophilization and has been described previously (Down and Hutchinson, 1993). For the purpose of this study, X-ray amorphous MK-0591 was assumed to be 100% amorphous. Crystalline MK-0591 was obtained by crystallization from ethanol (Prasit et al., 1991, 1993) and assumed to be 100% crystalline. Both were ground by hand in a mortar and pestle for 1 min each. The internal standard, lithium fluoride (99.99% pure, Aldrich), was used as received. All preceding materials were kept in a desiccator. Pregelatinized starch (Starch 1500, Colorcon), microcrystalline cellulose (Avicel PH101 FMC Canada), and magnesium stearate (Witco Canada) were used as received.

2.1.1. Blends of crystalline and X-ray amorphous MK-0591

Ground crystalline and X-ray amorphous MK-0591 (400 mg, total blend weight) were weighed into 30 cm^3 HDPE bottles. To this was added LiF (20 mg) such that a total of 420 mg of powder was prepared. Different ratios of crystalline (10–90%) to X-ray amorphous MK-0591 were prepared to form the standard curve. Mixing was carried out alternatively by manual shaking (approx. 1 min) and by shaking on a Thermolyne Maxi Mix 1 type 16700 mixer (approx. 1 min) for a total of 8 min. Before withdrawing samples, the bottles were

shaken, following the same sequence, for an additional 2 min to reverse the effects of possible powder packing and to better ensure uniformity.

2.1.2. Excipient blends of crystalline and X-ray amorphous MK-0591

Drug/excipient mixtures were prepared in the following fashion. All blends contained 25% drug and 75% excipients. The drug portion consisted of blends of crystalline and X-ray amorphous MK-0591(50% and 90% crystalline MK-0591) to which was added LiF (5% of total mixture weight), while the excipient portion was comprised of 35 parts pregelatinized starch mixed with 35 parts microcrystalline cellulose and 1 part of magnesium stearate.

2.2. Methods

2.2.1. Thermal analysis

A Seiko robotic differential scanning calorimeter (RDC-220) was used to verify the homogeneity of crystalline and X-ray amorphous MK-0591 blends. Details of the experimental method are described elsewhere (Clas et al., 1995). In previous work, two crystalline polymorphic forms of MK-0591 were identified (Varsolona and McCauley, personal communication). Form I was identified as the ethanol-crystallized form and is the form used in these studies.

2.2.2. X-ray powder diffraction

X-ray powder diffractograms were obtained using a Philips PW1840 X-ray powder diffractometer. All samples were exposed to CuKα radiation (40 kV, 30 mA) through a nickel filter. The samples were analyzed between 10 and 70° 2θ with a step width of 0.04 and step time of 2.00 s. The X-ray powder diffractometer was standardized daily with a silicon disk (silicon: 97.5% pure). Two separate samples were prepared from each of the blends. Each sample was analyzed for three consecutive runs, a total of six runs per blend. The six runs were then averaged and smoothed to produce one standard point per blend. In the case of the excipient blends, three samples were analyzed in triplicate, a total of nine runs. The three runs for each sample were averaged and smoothed. Thus, three points were obtained for each blend. The resulting three diffractograms were subtracted from a diffractogram of a 75% excipient/25% LiF blend. LiF was used as an inert packing material and did not contribute to the peak intensities in the region of interest.

To ensure consistent packing and reduce orientation effects, the standard powder holder was backfilled to the same packing depth against frosted glass as recommended by Klug and Alexander (1954; p. 300). The sample holder (Philips PSW1172/01) of dimensions 20×15 mm and a depth of 1.9 mm was filled to a depth of approx. 0.7 mm. The amount of solid added was weighed and a 1.2 mm glass slide cut to fit in the sample holder was pressed into place and the metal backing was then clipped on. The glass slide in the holder does not interfere with the diffraction pattern of the sample. It allows a smaller sample size to be used, thus, minimizing drug use. The holder was turned over to reveal the top face covered by the frosted glass slide and the slide carefully removed to reveal a flat, evenly packed and largely randomly oriented sample layer flush with the holder's top surface.

2.2.3. Calculations

Peak table files containing integrated peak intensity data were produced from the PC-APD diffractograms (Philips version 2.0) on an IBM computer (PC/2, model 50Z) using Spectra Calc (version 3.0, Galactic Industries Corp.). The three runs were averaged into a single diffractogram. This diffractogram was then smoothed with Savitsky-Golay smoothing (seven point, Spectra Calc). Integrated peak intensities (peak areas), were calculated. Six lines were selected for integration (at 2θ values of 16.3, 18.9, 19.9, 23.8, 24.8, and 27.0) and their integrated intensities summed. The total peak areas were then plotted as a function of percent crystallinity.

3. Results and discussion

Preferred orientation may distort the overall diffraction pattern by magnifying diffraction of

X-rays at certain angles, and weakening it at others. To reduce preferred orientation effects for quantitative analysis using X-ray powder diffractometry, it is recommended that the particle size be between 1 and 50 μm (Jenkins and De Vries). Suryanarayanan used samples pressed into tablets which had preferred orientation, however, by using the same preparation techniques and pressures, the preferred orientation was reproducible and consistent (Imaizuni et al., 1980). Unground crystalline MK-0591 is comprised of acicular crystals which are of the order of 100 μm in length. Repeated X-ray analysis on different samples clearly showed different intensities of the crystalline reflections due to orientation effects. Grinding the crystalline samples for 1–5 min gave reproducible crystalline patterns. Thus, orientation effects were minimized. Grinding was kept to 1 min since no additional benefits were obtained after grinding for 5 min. Grinding of the X-ray amorphous samples from 1 to 5 min did not result in any observable changes by X-ray powder diffraction. Orientation effects can be introduced also when packing the sample holder. However, this was minimized by using a ground glass slide to backfill the holder. The X-ray powder diffraction patterns of duplicate samples were similar within experimental error. Thus, it was felt that orientation effects were not significant.

The blends of crystalline MK-0591 in lyophilized X-ray amorphous MK-0591 were verified by DSC for their crystalline content before and after X-ray powder diffraction. No changes in crystallinity were observed. Homogeneity of the blends was verified by dividing the X-ray samples into four quadrants and determining the

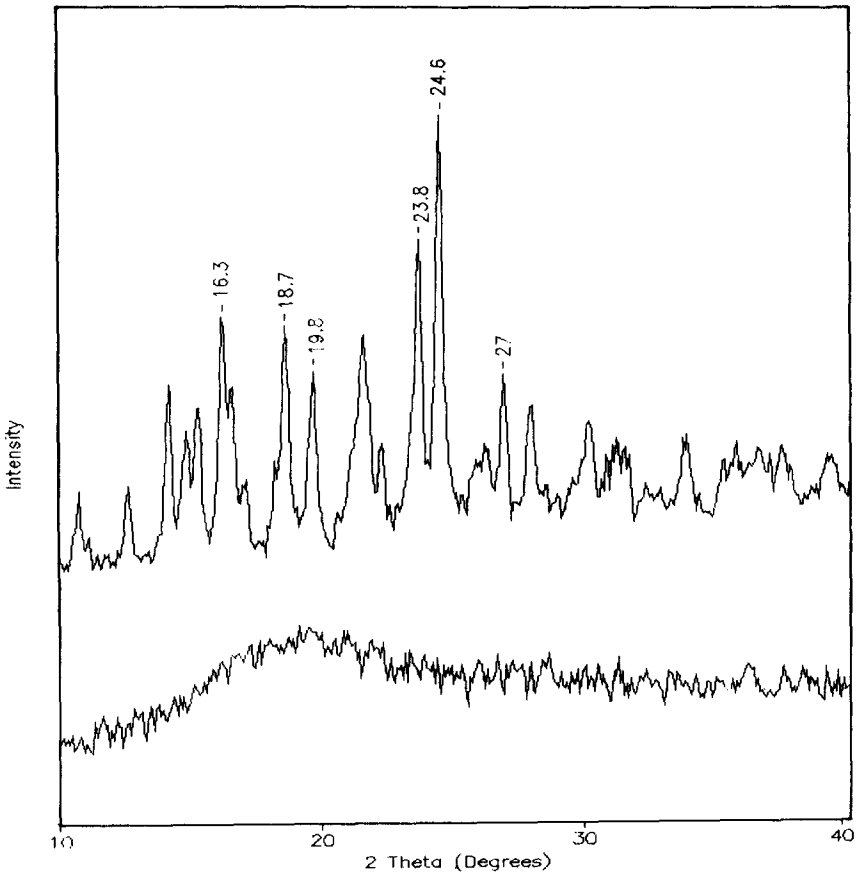

Fig. 1. X-ray diffraction pattern of amorphous MK-0591(lower diffractogram) and crystalline MK-0591 (upper diffractogram).

percent crystallinity of each quadrant by DSC. The crystallinity of the blends was similar within experimental error.

The internal standard (LiF) was used to determine if there were variations in peak position and intensity during the experiments. In all cases, the intensity of the LiF standard and its peak position remained constant. Thus, the integrated peak intensities were used to calculate the percent crystallinity. The XRPD pattern of lyophilized MK-0591 can be seen in Fig. 1. The integrated peak intensities of six crystalline reflections were chosen in the crystalline MK-0591 diffraction pattern (Fig. 1). These reflections were selected because of their definition and high intensities. Even as low as 10% crystalline content, the six crystalline reflections are clearly visible superposed on the amorphous halo. In addition, the crystalline reflections remain narrow and clearly defined, largely unaltered by the halo beneath them. The position of the crystalline reflections remained the same within experimental error, independent of the amorphous halo. The intensity of the crystalline peaks decreased with decreasing crystalline content, while the amorphous halo increased with increasing amorphous content (Fig. 2). The integrated peak intensities of the six crystalline reflections in the blends were summed and plotted as a function of the crystalline content. As previously mentioned, two assumptions were made: (1) that the X-ray amorphous material was 100% amorphous and (2) that the crystalline material was 100% crystalline. A linear calibration curve of the integrated intensity (y) as a function of the % crystalline content (x) of $y = 45.2 \times + 53.3$ and a correlation coefficient of 0.9985 was obtained. To ensure reproducibility with decreasing X-ray beam intensities as a function of instrument drift, the crystalline peak reflections were standardized with the crystalline reflections of the same peaks in the 100% crystalline sample (Fig. 3). A correlation coefficient of 0.9991 with a linear equation of the relative intensity (y) as a function of the % crystallinity (x) of $y = 0.00986 \times + 0.00595$ was derived. A 95% confidence range of -0.0127 to 0.0246 for the intercept and 0.0096 to 0.0101 for the slope were obtained. Verification of the calibration

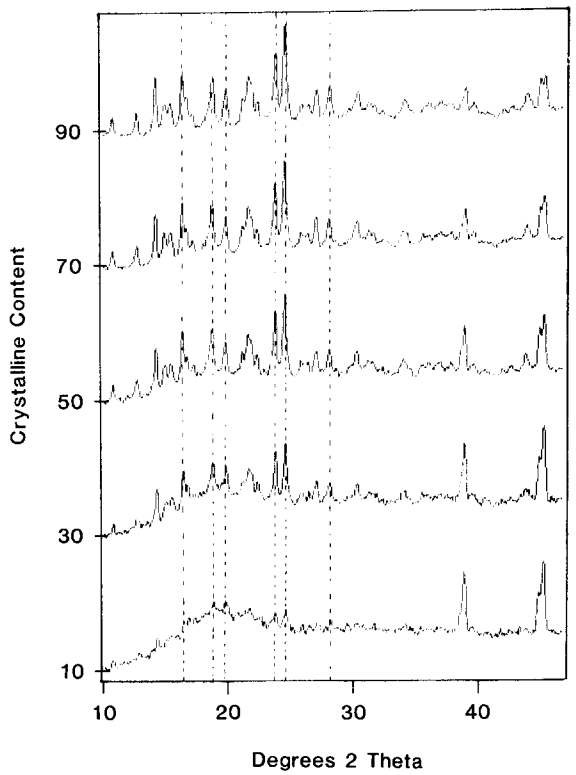

Fig. 2. X-ray diffraction patterns of blends of X-ray amorphous MK-0591 and crystalline MK-0591.

curve was carried out by preparing additional blends which fell on the standard curve as seen in Fig. 3. Powder blends of crystalline and X-ray amorphous MK-0591, pregelatinized starch, microcrystalline cellulose and magnesium stearate were tested containing 12.5 and 22.5% crystalline MK-0591 (Fig. 4). As can be seen in Fig. 4, the powder blends correlate well with the calibration curve suggesting that this method may be useful to evaluate solid formulations with MK-0591.

Detection limits with the present system are approx. 5–10% of the MK-0591 crystalline phase. Longer diffraction times would be required to improve the detection limit. Pikal et al. found that X-ray powder diffraction studies were not as precise as solution calorimetry to detect the presence of a second phase for blends of amorphous and crystalline cephalosporins. Depending on the detector, the sample, and method used, levels as low as 0.5% of a crystal form of 1,2-dihydro-6-

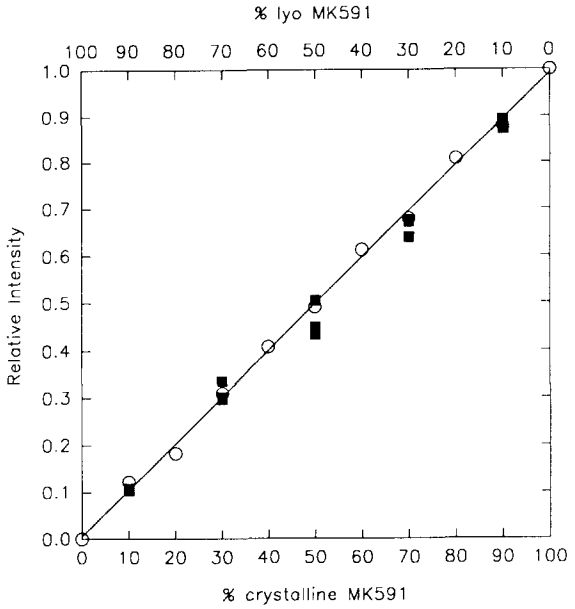

Fig. 3. Peak intensities of crystalline MK-0591 divided by the peak intensities of 100% crystalline MK-0591 (relative intensities) in blends of crystalline and X-ray amorphous MK-0591 as a function of percent crystalline MK-0591. Reproducibility of the relative peak intensities of crystalline MK-0591 as a function of percent crystalline MK-0591 in blends (■).

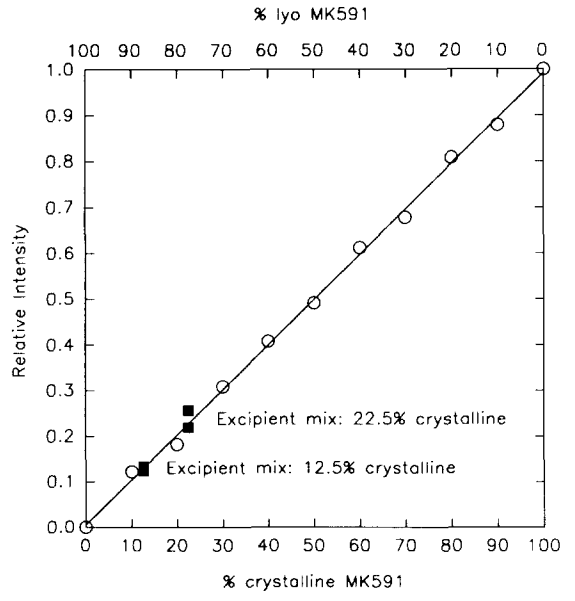

Fig. 4. Relative peak intensities of crystalline MK-0591 in excipient mixes containing 12.5% and 22.5% crystalline MK-0591 (■) as a function of percent crystalline MK-0591.

neopentyl-2-oxonicotinic acid have been detected (Chao and Vail, 1987). Suryanarayanan (1990) was able to detect levels as low as 2% of α-carbamazepine in a mixture of α- and β-carbamazepine. Similar studies using DSC on MK-0591 suggest that DSC is a more sensitive technique, able to detect crystalline levels as low as 5% or less (Clas et al., 1995). However under certain conditions, X-ray powder diffraction may be prove to be a better method. In both cases, the choice of the method of detection depends on the type of excipients present and the intensity of the signal.

4. Conclusions

Quantitative X-ray powder diffraction is a useful method to determine the percent crystallinity in powder blends of crystalline and lyophilized MK-0591. A linear calibration curve of $y = 0.00986 \times + 0.00595$ with a correlation coefficient of 0.9991 was obtained. Preliminary studies using powder blends of MK-0591, pregelatinized starch, microcrystalline cellulose and magnesium stearate demonstrated the feasibility of using this method, possibly on compressed tablet formulations.

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