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Research Paper

Determination of trace amounts of β tegafur in commercial α tegafur by powder X-ray diffractometric analysis

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Abstract

Objectives The main objective of this work was to develop a suitable analytical technique for determining trace amounts of the thermodynamically stable solid form in bulk samples of metastable form, to a sensitivity of 0.005%–1.0%. Tegafur (5-fluoro-1-(tetrahydro-2-furyl)-uracil) α and β crystalline forms were used as a model for this problem.

Methods The trace content of the thermodynamically stable β polymorphic form in tegafur samples was increased by promoting phase transition from the bulk of thermodynamically metastable α form to β form, and achieving sufficient β form content for a quantitative powder X-ray diffractometry (PXRD) analysis. The phase transition was stimulated by adding water to the samples and then grinding in controlled conditions (temperature, time, grinding speed). A calibration line was constructed using the least squares method.

Key findings By using a solvent that does not form hydrates with the analysed polymorphs, it was possible to promote the phase transformation from metastable form to the thermodynamically stable form. After sample preparation, the thermodynamically stable solid form content in the analysed mixture had increased proportionally to the initial weight fraction (0.005%–1.0%) of the stable form seed crystals in the samples, and the coefficient of proportionality was 43.0 ± 0.9, with a standard deviation $S_n = 1.5\%$.

Conclusions A simple, sensitive, semi-quantitative analytical method was developed for the low-level determination of the thermodynamically stable polymorphic form in mixtures of thermodynamically stable and metastable polymorphs.

Keywords drug polymorphism; powder X-ray diffraction; semi-quantitative analysis of trace amounts; tegafur

Introduction

The impact of crystal polymorphic transformations on drug product performance is well recognized in the pharmaceutical industry. Various crystal structures of a given substance often exhibit different physical properties.[1]

To ensure product stability, the polymorph most stable in ambient conditions is normally chosen for development into the final dosage product. Recently, however, metastable forms have been utilized due to enhanced dissolution or bioavailability profiles.[2] Sometimes metastable polymorphic forms may be inadvertently generated due to the stress produced by temperature, mechanical treatment and moisture during processing or storage of the drug product.[3] Contamination by polymorphic impurities can influence both the stability and performance of the final product and during the last decade the requirements for identification, specification and control of active pharmaceutical ingredient polymorphs have become a part of the quality assurance process.[4] Therefore it is necessary to develop quantification methods for measuring low level contamination with undesired crystalline phases.[1] A multitude of analytical techniques is available to quantify crystal forms in mixtures but those methods have not been routinely applied to quantify low amounts of one polymorph in the presence of another.[5] FT-infrared spectroscopy has been used to determine polymorph content down to 1–5%.[6,7] Powder X-ray diffraction (PXRD) has been employed to determine low-level polymorph impurities with the minimum quantifiable level of 1–2.5%.[6,8,9] Solid-state NMR spectroscopy has also been applied to the detection of polymorph traces,[8] though solid-state NMR methods involve significant sample preparation or analysis time.

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Due to the growing quality requirements directed at active pharmaceutical substances there is a demand for a rapid and simple quantification of unwanted solid forms at low levels (<1%).[10]

Advantages like the non-destructive nature, simplicity and ambient temperature measurements of either drug substances or final dosage forms make PXRD the most preferred and extensively used technique for quantification of polymorphic mixtures.[4] In addition, PXRD is one of the most sensitive methods for detection of low-level solid forms, therefore PXRD was chosen as the most appropriate method for phase quantification in this study.

The purpose of this study was to investigate the quantification of low-level polymorphic impurities and to establish an analytical method with a detection limit below 1% for the determination of the thermodynamically stable polymorphic form in mixtures with a metastable polymorphic form. The α and β forms of tegafur (5-fluoro-1-(tetrahydro-2-furyl)-uracil), a cancer chemotherapy drug,[11] were selected as the model system for this study. Our low-level determination technique was based on a new approach to the sample preparation process, which included a solvent-promoted stimulation of phase transition from a thermodynamically metastable form to the stable form. The conditions were selected to increase the stable form content to a level high enough to enable the quantification of mixture with PXRD. Consequently, a new, sensitive semi-quantitative PXRD analytical method was developed for detection of trace amounts (0.005–1.0% weight fraction) of thermodynamically stable polymorphic impurity in a metastable commercial product. Since the ever-growing quality requirements for active pharmaceutical ingredients tend to increase the expense of quality assurance, our contribution of a simple, rapid and low-cost analytical technique will be useful to the pharmaceutical industry as a part of quality control for active pharmaceutical ingredients.

Materials and Methods

Materials

The α and β forms of pharmaceutical-grade tegafur were obtained from JSC Grindeks (Riga, Latvia).

Optimization of sample preparation

A mixture of tegafur α and β forms containing 1.5% weight fraction of the β form was prepared and separated into six samples of 0.50 g. An analytical balance (BOECO, Hamburg, Germany) of $\pm$0.0001 g accuracy was used. Weighted samples were ground at 20°C with a Retsch MM300 shaker (Retsch GmbH, Haan, Germany) at a shaking frequency of 15 Hz for 3, 5 and 7 min with the addition of one or two drops (~0.07 ml or ~0.15 ml, respectively) of water before each grinding operation. Water was added to the samples before grinding to ensure faster phase transition.

Sample preparation

Pure α and β polymorphs of tegafur were ground in a mortar separately for 2 min, to ensure sample homogeneity. The ground β form was weighed and mixed in various ratios (1.0, 0.50. 0.25, 0.10, 0.050, 0.010 and 0.0050% w/w) with the ground α form. The total mass of each mixture was 0.75 g. The prepared samples were homogenized at 20°C by shaking in a Retsch MM300 shaker for 5 min at a shaking frequency of 15 Hz.

In each case 0.50 g of homogenized sample was weighted for wet grinding, but the rest of the mixture was used for the next sample preparation. A drop of water (~0.07 ml) was added to each sample before grinding. The prepared samples were ground for 5 min at 20°C with a Retsch MM300 shaker with shaking frequency 15 Hz.

Two parallel samples were prepared and analysed for each mixing ratio of α and β tegafur.

Powder X-ray diffractometric analysis

Samples were analysed with a powder X-ray diffractometer Bruker D8 Advance (Bruker AXS, Karlsruhe, Germany). The divergence and scattering slits were set at 1.0 mm, and the receiving slit was set at 0.6 mm. Diffraction patterns within the 2θ range of 9° to 13° were recorded at room temperature using CuK_α radiation at 1.54180 Å, with the following measurement conditions: tube voltage 40 kV, tube current 40 mA, step-scan mode with the step size $2\theta = 0.02°$, and the counting time 10 s/step.

Powder samples were packed into glass holders with ~150 mg weight capacity and pressed by a clean glass slide to ensure coplanarity of the powder surface with the surface of the holder. Obtained diffractograms were analysed with DIFFRAC^{plus} EVA (version 9.0) software.

Quantitative analysis of tegafur α and β form mixtures

A full profile analysis was used for quantitative analysis, in which all points of X-ray patterns were used for quantification of tegafur α and β forms. Experimental points were saved as **.raw* file format and then converted to **.uxd* file format, which can be used for quantification. In *MS Excel* worksheet columns were created for 2θ angles (step size 0.02°), intensities of pure tegafur α and β forms (counts/s), intensities of the analysed tegafur α and β form mixture (counts/s), theoretically calculated intensities of the mixture (counts/s) and least square values of differences between theoretically calculated and experimental intensities. Reflex intensities for pure α and β forms and intensities of analysed mixtures were copied from previously prepared **.uxd* files. Theoretical intensities were calculated using Equation 1.

$$I = Q \cdot \left(I_\beta \cdot \omega_\beta + I_\alpha \cdot \left(1 - \omega_\beta\right)\right) \quad (1)$$

where I is the theoretical intensity in the analysed sample (counts/s); I_α, I_β are the intensities of pure tegafur α and β forms, prepared using the same method as the sample (counts/s); ω_β is the weight fraction of tegafur β form in the sample; Q is a normalization coefficient. This coefficient must be close to 1, and it was established to prevent errors related to the sample preparation.

The weight fraction of tegafur β form ω_β was calculated using *MS Excel* add-in *Solver*. The minimum values of least square sums were found by optimizing the normalization coefficient Q and weight fraction ω_β, by using Equation 2.

$$S^2 = \sum_{i=1}^{n} \left[(I_{teor})_i - (I_{\exp})_i \right]^2 \quad (2)$$

Results and Discussion

It has been reported that tegafur β form is stable at temperatures under 34–39°C, while tegafur α form is stable at higher temperatures.[12] The X-ray patterns of tegafur α and β forms in 2θ interval from 9° to 13° are shown in Figure 1.

It is evident that the β form diffraction reflex at $2\theta = 12.2°$ overlapped with the strongest α form reflex, which was twice as intensive as the rest of α form reflexes, but full profile analysis allowed us to use this area for quantification. The homogenous composition of the analysed mixture and the normalization coefficient close to 1 ensured that the reflex intensities of each phase were linearly dependent on phase weight fractions in the sample. Therefore it was possible to use a calibration line.

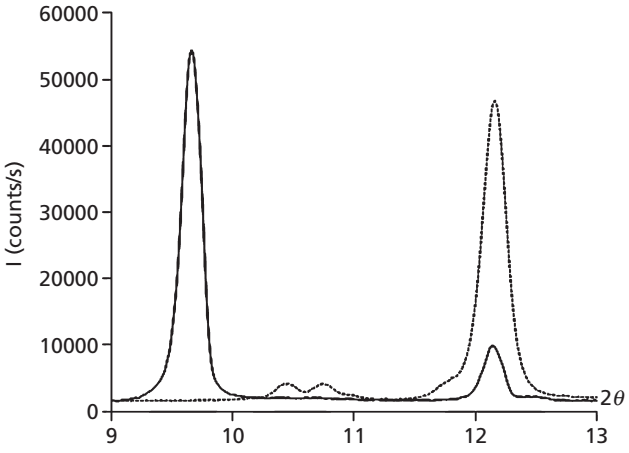

Figure 1 Powder X-ray diffraction patterns of tegafur α (- - -) and β (—) form.

Six experiments were carried out to establish the optimal grinding conditions for reproducible multiplication of the thermodynamically stable phase content in the samples. The samples containing 1.5% weight fraction of β tegafur were treated with a drop (~0.07 ml) or two drops (~0.15 ml) of water and subsequently ground for 3, 5 and 7 min. The aim of these experiments was to investigate sample preparation conditions in which α modification after grinding does not completely transform to the β form, but the sample still contains a reasonably high content of α tegafur. The samples that were ground for 5 and 7 min had practically the same composition, but the samples that were ground for 3 min had a noticeably lower fraction of the β form (Figure 2a). To promote the phase transformation of β tegafur to the α form, one drop (~0.07 ml) or two drops (~0.15 ml) of water was added to the sample before grinding. In all experiments the content of β tegafur in the samples was significantly higher if a single drop of water was added to the sample before grinding (Figure 2b).

Any solvent that does not form solvates or hydrates with analysed polymorphic forms could be used to promote the phase transformation. We chose water (one drop for each 0.50 g sample), followed by grinding for 5 min.

Powder X-ray diffraction patterns of calibration samples after this optimized treatment are shown in Figure 3.

Our developed method was found to be linear in the range of 0.005–1.0% weight fraction of β form in the initial mixture. The relationship between tegafur β form initial and final weight fraction after grinding is shown in Figure 4.

The calibration factor was equivalent to the slope of the linear regression equation. The regression line was described by the function $y = ax$, taking into account the intersection with the origin. If the calibration factor would be calculated from the equation $y = ax + b$, then the approximated line would intersect the y-axis at a non-zero value, meaning positive phase content at zero peak intensity ($I_{peak} \neq 0$, when $\omega = 0$), which is physically impossible. The optimal linear slope was calculated using *MS Excel* function *Linest*. The

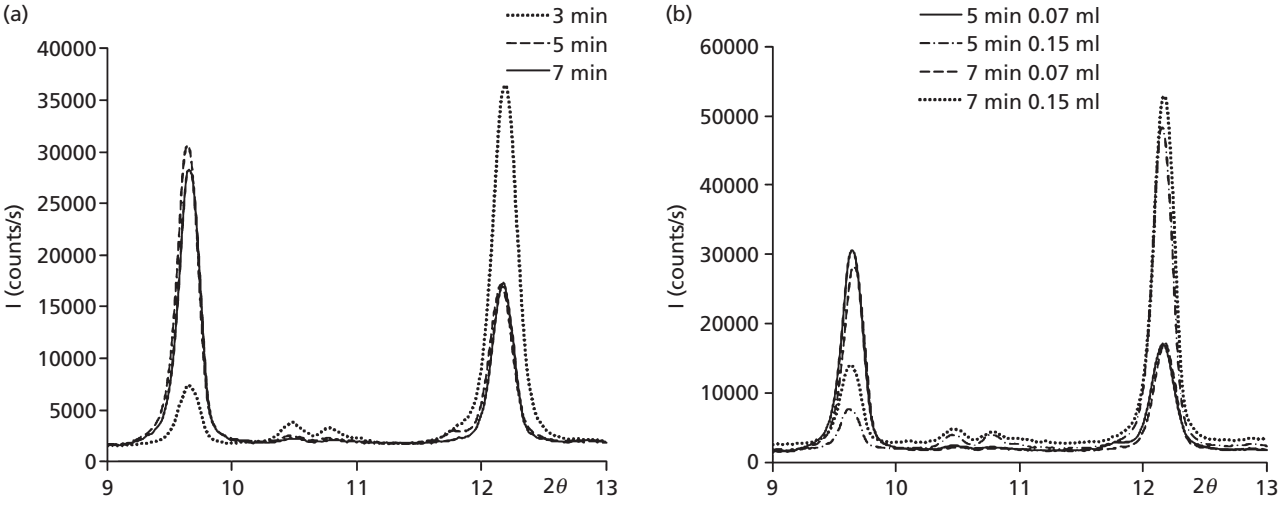

Figure 2 (a) Powder X-ray diffraction patterns of the tegafur α and β form mixture containing 1.5% weight fraction of β tegafur after grinding for 3, 5 and 7 min. (b) Powder X-ray diffraction patterns of the tegafur α and β form mixture containing 1.5% weight fraction of β tegafur after grinding for 5 and 7 min if the phase transition was induced adding a drop (~0.07 ml) or two drops (~0.15 ml) of water.

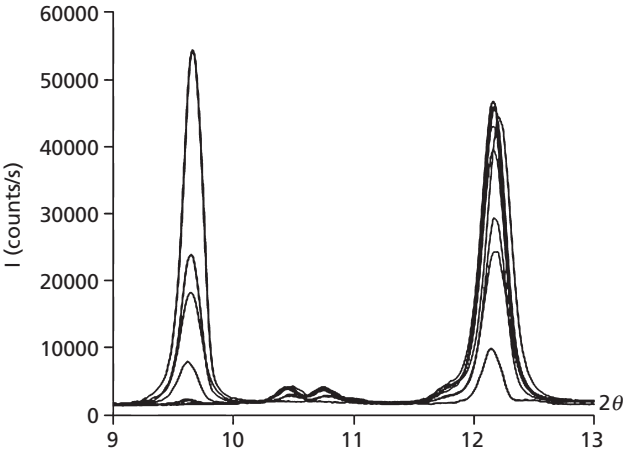

Figure 3 Powder X-ray diffraction patterns of calibration samples after inducing the phase transformation with adding a drop of water to the sample and grinding for 5 min.

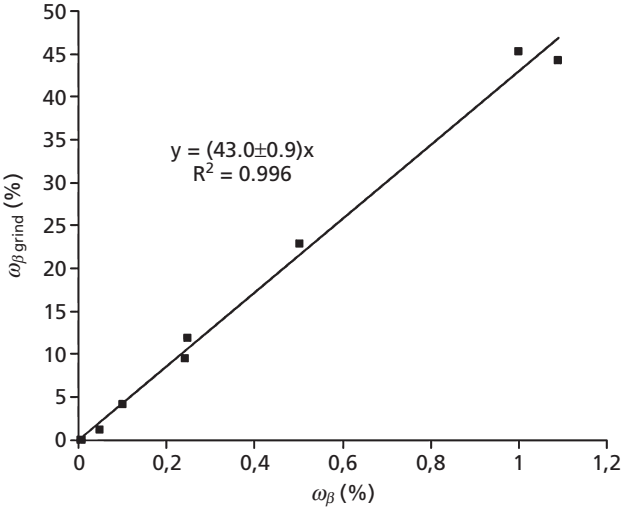

Figure 4 The dependence of tegafur β form weight fraction after grinding upon the initial β form content in the sample.

equation for the calibration curve was $y = (43.0 \pm 0.9)x$, the correlation coefficient $R^2 = 0.996$, and the regression residual mean square error or the standard deviation S_n, which characterized the dispersion between the measured (y_i) and theoretically calculated value (Y_i), was 1.5%.

The detection and quantitation limits were calculated from standard deviation through Equation 3 and Equation 4, respectively, as recommended by the ICH guideline:[13]

$$\text{Limit of detection (LOD)} = 3.3\, S_n/S \quad (3)$$

$$\text{Limit of quantitation (LOQ)} = 10\, S_n/S \quad (4)$$

where S_n is the standard deviation of the response and S is the slope of the calibration curve.

The calculated LOD was 0.12%, and the calculated LOQ was 0.35%. However, taking into account that these LOD and LOQ values were calculated from standard deviation, and the standard deviation was determined as deviation from the linear calibration slope, we could not expect low LOD and LOQ values. The developed quantitation method was based on crystal seeding and solvent-promoted phase transition that led to relatively high statistical deviation. During the development process we determined that the PXRD method allowed detection of the analytical signal if β tegafur weight fraction was as low as 0.005% (i.e. 24 times smaller than the LOD). Despite the large dispersion of experimental data points, the developed sample preparation method allowed rapid and simple determination of trace amounts as low as 0.005%, that so far have been difficult to achieve with any known method of polymorphic form analysis. The essence of this method is in the sample processing phase, while the subsequent quantitative analysis of prepared mixtures could be done not only with PXRD, but any other quantitative technique that allows the construction of a calibration curve.

Through this method it becomes possible to quantify trace amounts of thermodynamically stable polymorphic impurities in bulk drug samples. In addition to active pharmaceutical ingredients, this method is generally able to detect trace amounts of polymorphic impurities in final dosage forms, but as the dosage forms contain excipients affecting the grinding efficiency, it is necessary to construct new calibration plots for each analysed object.

Conclusions

This study demonstrates that very low levels (0.005–1.0%) of unwanted polymorphs in pharmaceuticals can be determined by using a sensitive semi-quantitative method. This trace polymorph analysis is based on a new approach to sample preparation, including solvent-promoted stimulation of phase transition from a thermodynamically metastable form to the stable form. The method was developed for model mixtures of α and β tegafur. The optimal grinding time for tegafur α and β phase analysis at 20°C was 5 min, if the shaking frequency was 15 Hz. The phase transition was facilitated by the addition of a single drop of water to each 0.50 g sample. The content of tegafur β form after the sample preparation was linearly proportional to the initial β form weight fraction in the sample, and the coefficient of proportionality was 43.0 ± 0.9, while the standard deviation S_n was 1.5%. Through this method it becomes possible to quantify trace amounts of stable polymorph impurities in thermodynamically metastable bulk drug samples.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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