RESEARCH ARTICLE

Optimization of Sample Preparation Conditions for Detecting Trace Amounts of β-Tegafur in α- and β-Tegafur Mixture

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ABSTRACT: We report a semiquantitative method for determining trace amounts ($<1\%$) of thermodynamically stable forms in polymorphic mixtures, focusing on sample preparation effects on solid phase transitions. Tegafur [5-fluoro-1-(oxolan-2-yl)-1,2,3,4-tetrahydropyrimidine-2,4-dione] was used as a model material in this study. The amounts of the thermodynamically stable β tegafur were increased to levels detectable by powder X-ray diffractometry by grinding the samples in a ball mill in the presence of water. The limit of detection for this method was as low as 0.0005% of β tegafur in α and β tegafur mixtures. The amount of β tegafur after sample preparation was found to be proportional to the initial weight fraction of β tegafur. The sum of Langmuir and Cauchy–Lorentz equations was used to describe the change in conversion degree due to the added water volume, where Langmuir equation described water sorption during the sample preparation and Cauchy–Lorentz equation described the grinding efficiency. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

About two-thirds of organic compounds and about 80% of active pharmaceutical ingredients under certain conditions can exist in more than one polymorphic form. Polymorphism is defined as the ability of a drug compound to crystallize into more than one different crystalline form that differs with molecule packing arrangements and/or conformations within the crystal lattice.[1–4] The often encountered differences in stability, solubility, and bioavailability of active pharmaceutical ingredient polymorphs require a control over solid phase composition of these products. It is a common requirement in Pharmacopeia monographs that active pharmaceutical ingredients in drugs must exist in one fixed crystalline form.

The most thermodynamically stable form is usually chosen for pharmaceutical use, but sometimes a metastable form has better solubility or bioavailability, and is selected for manufacturing.[5] In this case, it becomes very important that the final manufactured product is free from the thermodynamically stable form because even trace amounts can facilitate a phase transition to the unwanted thermodynamically stable form. Therefore, methods for detecting the stable form in trace amounts ($<1\%$) are necessary to ensure kinetic stability of metastable solid pharmaceutical ingredients.

As shown in a previous publication,[6] it is possible to determine trace amounts ($<0.01\%$) of the thermodynamically stable form in mixtures of thermodynamically stable and metastable forms. Our aim for this work was to determine sample processing impact on phase quantification. The amount of the thermodynamically stable form was increased by grinding the sample with water additive in a way similar to literature precedents.[7–11] The increased amount of the stable form could then be determined with X-ray diffractometry, which was selected as the most appropriate method for phase quantification. Also infrared, Fourier transform Raman, solid-state nuclear magnetic resonance spectroscopy, and thermal analysis methods can be used for phase analysis.[1,2]

Accurate measurement of intensity, height, and plotted area of diffraction peaks is most important for obtaining reliable and reproducible results and

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calibration curves.[12] Nature of the samples, instrument and sample preparation parameters, type of sample holders, sample rotation, particle size, powder packing, and dominant orientation are also crucial factors that affect diffraction peak intensities and areas, thus influencing quantification results.[12–14]

Tegafur [5-fluoro-1-(oxolan-2-yl)-1,2,3,4-tetrahydropyrimidine-2,4-dione] was chosen as a model material in this study because it does not form hydrates and solvates that could gradually loose associated solvent during sample preparation, and thus affect phase quantification. Tegafur is an antitumor agent, widely used in the treatment of various malignancies, particularly gastrointestinal and breast cancers.[15] Over years, α, β, γ, δ, and ε forms of tegafur have been reported in pharmaceutical literature,[15–17] but only α and β modifications are used for therapeutic purposes.

MATERIALS AND METHODS

Materials

Commercial samples of α and β tegafur were supplied by JSC *Grindeks* (Riga, Latvia).

Sample Preparation

The entire batches of α- and β-tegafur used in experiments were ground separately for 3 min to ensure required sample homogeneity and avoid preferred crystal orientation effects.

Determination of Optimum Grinding Frequency

Mixtures of 2.0%, 1.0%, 0.50%, 0.10%, 0.050%, 0.010%, 0.0050%, 0.0010%, 0.00050%, and 0.00010% β tegafur in α tegafur (3.0 g each) were prepared from a 2.0% stock mixture that was diluted to the required concentrations. Samples during preparation were homogenized in a Retsch MM300 ball mill (Retsch GmbH, Haan, Germany) for 5 min at 20°C, with 15 Hz shaking frequency. The analytical balance (BOECO, Hamburg, Germany) had an accuracy of ±0.0001 g. The samples of homogenized mixtures (0.50 g) were each treated with 0.07 mL water and ground in the ball mill at 7, 10, and 15 Hz shaking frequencies for 5 min.

Determination of the Optimum Added Water Volume

A mixture of tegafur α and β forms containing 1.0% weight fraction of β form was weighed and homogenized in the ball mill for 5 min at 20°C, with 15 Hz shaking frequency. The 0.50 g samples of homogenized mixture were ground in the ball mill with variable water additive amounts for 5 min at 20°C, with 10 Hz shaking frequency. The added water volume was from 0.02 to 0.20 mL, with 0.01 mL step size. When the amount of water additive exceeded 0.06 mL,

a thick paste formed, and it became necessary to dry the samples for 30 min after grinding.

Sample Preparation for Recrystallization Studies

A sample (0.50 g) of tegafur α and β form mixture, containing 1.0% weight fraction of β form, was ground in the ball mill for 5 min at 20°C with 15 Hz shaking frequency, with 0.20 mL water added just before grinding. The obtained thick paste was pressed into glass sample holder right after grinding, and powder X-ray diffraction (PXRD) pattern was recorded. Consecutive PXRD patterns were recorded every 5 min, until no further phase transition was observed (∼1 h).

Determination of Surface Area

Three samples of tegafur α and β form mixture (0.50 g each), containing 1.0% weight fraction of β form, were ground in the ball mill for 5 min at 20°C, with 10 Hz shaking frequency. The first sample had no added water, the second had 0.06 mL of water, and the third had 0.12 mL of water. Water was added just before grinding. The samples were removed from grinding vessels immediately, and were dried at ambient temperature for 3 h. Surface area determination requires a 1–2 g sample; therefore, three parallel samples for each volume of added water were ground in the ball mill and then combined to obtain the required sample mass.

Surface area was determined by a modified chromatograph "Hrom 3," detecting the amount of argon involved in a monolayer adsorption–desorption process.

PXRD Analysis

Samples were analyzed with a Bruker D8 Advance powder X-ray diffractometer (Bruker AXS, Karlsruhe, Germany), equipped with a PSD LYNXEYE detector. Measurements were performed with CuK radiation (1.54180 Å) at room temperature. The following conditions were used: step-scan mode with a step size of 0.01°; scan speed: 0.2°/min; 2θ range: 9.0°–12.7°; voltage: 40 kV; current: 40 mA; divergence slit: 0.6 mm; scattering slit: 8 mm.

Powder samples were packed into glass holders with a weight capacity of approximately 150 mg and pressed by a clean glass slide to ensure coplanarity of the powder surface with the surface of the holder. The obtained diffractograms were analyzed with DIFFRAC[plus] EVA (ver. 9.0) software (Bruker AXS, Karlsruhe, Germany).

Quantitative Analysis of α and β Polymorph Mixture

Individual diffraction peaks area method was used in tegafur α and β form quantification. The areas of the plotted diffraction peaks were calculated by using the computer program TOPAS 3 (Bruker AXS, Karlsruhe, Germany), and weight fractions of β form were