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Characterization, selection, and development of an orally dosed drug polymorph from an enantiotropically related system

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ABSTRACT

Solid-state characterization methods are used to study a dimorphic pharmaceutical compound and select a form for development. Polymorph screening found that {4-(4-chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl} acetic acid can crystallize into two non-solvated polymorphs designated Forms 1 and 2. Physical methods including vibrational spectroscopy, X-ray powder diffraction, solid-state NMR (SSNMR), thermal analysis, and gravimetric water vapor sorption are used to fully characterize the two polymorphs. Temperature-dependent competitive ripening experiments and solubility measurements indicated that the polymorphs in this system exhibit enantiotropy with a thermodynamic transition temperature of $35 \pm 3\,^{\circ}C$. This complicates the selection of a polymorph to progress in drug development. Both forms had undesirable qualities; however, a particular drawback of Form 1 was found in its tendency to convert to Form 2 upon milling. Combining this effect and the desired formulation approach with physical property results led to a rationale for the choice of Form 2 for further development. Because this form is thermodynamically metastable at room temperature, analytical approaches were developed to ensure its exclusive presence, including a quantitative infrared spectroscopic method for drug substance and ^{13}C and ^{19}F solid-state NMR limit tests for the undesired form in drug product at drug loads of 8.3% (w/w).

1. Introduction

The solid-state properties of pharmaceuticals constitute a significant area of research, given that the preferred and most cost-effective means of drug delivery is the oral solid dosage form. Many challenges confront the successful development of a pharmaceutically active molecule. One important step is finding a suitable solid crystalline form of the drug for progression through development. Since amorphous solids have a higher relative free energy compared to their crystalline counterparts, which can lead to instability, crystalline solids are often the most practical choice for development (Byrn et al., 1999). However, finding a suitable solid crystalline form or salt can be difficult. The ability of solids to crystallize into different structures, known as polymorphism, complicates the situation. Physical and mechanical properties such as density, solubility, compression behavior, hygroscopicity, and melting point may vary significantly between polymorphs and can lead in some cases to extreme effects upon *in vivo* activity (e.g. chloramphenicol palmitate, where only modification II is pharmacologically active) (Byrn et al., 1999; Aguiar et al., 1967). The process of finding a suitable polymorph for development is further complicated by crystallization of the drug with water and solvents to form hydrates and solvates. Attempts are also made to synthesize different salts with appropriate acids or bases; the resulting products can again be crystalline or amorphous and can also exhibit polymorphism. Besides the need to develop a robust manufacturing process for clinical supplies, the need for solid-state form control is also a requirement of various regulatory agencies. An emphasis is placed on the control measures used to verify the correct form has been produced for clinical trial supplies as well as the effects of polymorphism on bioavailability, manufacturability and product stability (ICH, 1999; Raw et al., 2004).

Understanding the thermodynamic relationship between polymorphs and kinetic barriers to conversion is a critical step in selecting the appropriate form for development. From this information, the potential for one form to convert to another form can be evaluated. To achieve this, it is generally necessary to determine the relative stability order of the polymorphs and classify the thermodynamic relationship between pairs of polymorphs as being enantiotropic or monotropic. The theoretical

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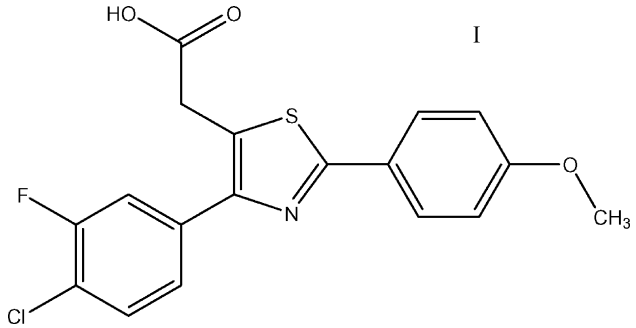
differences between enantiotropy and monotropy were originally studied using pressure–temperature diagrams (Bernstein, 2002; Ostwald, 1885). In the case of enantiotropy, the pressure curves intersect below the melting points of the polymorphs, while for a monotropic system, the intersection occurs after the melting point. Energy–temperature (ET) diagrams are another common method for describing monotropy and enantiotropy (Burger and Ramberger, 1979; Yu, 1995) and for showing which form is most thermodynamically stable at a given temperature; the intersection of the free energies in an enantiotropic system is known as the thermodynamic transition temperature (T_t). Enantiotropic systems cause special concern in pharmaceutical development, especially when T_t is near ambient temperature.

The present work exemplifies the impact on drug development of enantiotropy in a dimorphic system with a T_t near ambient temperature. The molecule, {4-(4-chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl} acetic acid (**I**), is shown below.



I

This molecule is being developed for the treatment of overactive bladder, to open so-called "BK" (big potassium) channels and help regulate bladder activity by limiting the excitability of smooth muscle cells, as an alternative to other drugs with undesirable side effects (Andersson, 2000; Hongu et al., 2002).

In the present work, a multi-disciplinary approach is applied to the characterization of **I** that explores the challenges encountered during drug development with respect to choice of polymorphic form. A comprehensive screening process to discover possible crystalline salts and forms of **I** resulted in two anhydrous free acid polymorphic forms from two different solvent systems. Previous work on this system has explored the structural aspects of the two polymorphs of **I** via their crystal structures (Vogt et al., 2008). Compound **I** was determined to have a pK_a of 3.66; however, no developable salts were discovered during screening. Characterization of the two polymorphs was accomplished using powder X-ray diffraction (PXRD), optical and electron microscopy, solid-state NMR (SSNMR), infrared spectroscopy (IR), Raman spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and gravimetric water vapor sorption (GVS). Solubility measurements, DSC, and competitive ripening experiments are used to establish the ET diagram for the forms. The value of T_t was found to be near ambient temperature, complicating the choice of a polymorph for large-scale development. A rationale based on an understanding of the solid-state properties of the polymorphs and the desired formulation approach (including milling) was used to make a selection. Because the polymorph that was selected is less stable at room temperature, careful attention was paid to analytical methods for detecting the undesired form in drug substance and drug product. A multivariate IR spectroscopic method for polymorph quantification in drug substance is discussed along with SSNMR limit-test methods for detecting the undesired polymorph in drug product; these can be applied at various stages in the manufacturing process and also during stability testing.

2. Materials and methods

2.1. Preparation of materials

Compound **I** was synthetically prepared using previously reported methods (Hongu et al., 2002). The two forms of **I** were discovered by extensive form screening experiments in many solvent systems using different crystallization methods including temperature cycling, slow evaporation, and slurrying. Form 1 is prepared by recrystallization from *tert*-butylmethyl ether (TBME) and cyclohexane, and Form 2 can be produced by recrystallization from acetonitrile. Specifically, Form 1 may be prepared using the following procedure. A 1-L flask is charged with 20 g of **I**, to which 200 mL of TBME and 70 mL of cyclohexane is added. The slurry is heated to reflux until dissolution occurs, and the solution is then cooled to 50 °C. Seed crystals of Form 1 may be added as a suspension in cyclohexane, followed by further cooling to 0 °C over 60 min. Finally, 200 mL of cyclohexane is added and the suspension is stirred for 2 h at 0–5 °C, after which yellow crystals are isolated in about 80% yield. Form 2 may be prepared by first charging a flask with 3 g of Form 1 and 36 mL of acetonitrile. The suspension is heated to 75 °C to cause dissolution, then cooled to 63 °C and seeded with Form 2 crystals suspended in acetonitrile. Additional stirring is performed at 63 °C for 1 h, after which the material is cooled to 0 °C over 2 h. Form 2 crystals are obtained in about 85% yield. Although seeding is used to ensure reproducibility in these procedures, Forms 1 and 2 may still be obtained (albeit less reliably) without this step.

Oral formulations of Forms 1 and 2 containing 8.3% (w/w) of drug substance, with 61% lactose monohydrate, 24% microcrystalline cellulose, 3% croscarmellose sodium, and 1% magnesium stearate in were prepared using a multi-step dry-blending approach. The desired formulation approach was to mill **I** with lactose monohydrate to reduce particle size prior and to improve flow properties; of a variety of other formulations that were attempted, this was found to be the most suitable for formulation of **I**. However, as discussed in detail in the following sections, the sensitivity of Form 1 to form conversion during milling precluded the use of this step in formulations containing Form 1. Formulations containing different ratios of Forms 1 and 2 were also prepared by blending. The highest strength dosage form of interest here was a 307.5 mg film-coated round tablet containing 300 mg of the aforementioned blend (i.e. 25 mg of **I**) with the additional mass caused by film coating.

2.2. Thermal methods

DSC experiments were carried out using a TA Instruments Q1000 system. About 1–2 mg of sample was used for the measurements. An aluminum pan was used for the analysis and was sealed by applying pressure by hand and pushing each part of the pan together. The temperature was ramped from 25 to 300 °C at 10 °C/min. Nitrogen was used as the purge gas with a flow rate of 50 mL min^{-1}. TGA experiments were conducted using a TA Instruments Q500 system. About 5–10 mg of sample was loaded into a platinum pan for the measurements. The sample temperature was ramped from 25 to 300 °C at 10 °C/min. Nitrogen was used as the purge gas at a flow rate of 20 mL min^{-1}.

2.3. Optical and electron microscopy

Samples were analyzed using a Leica DM LM microscope. The optical microscopy samples were prepared and analyzed according to USP procedures (USP, 2005a). Thermal microscopy measurements were collected using the Leica microscope in tandem with a Linkam THMS600 heating and freezing stage. A 10× long working distance objective was used. The stage was heated at a

rate of 10 °C/min. Images were collected every minute using the Linksys software. Scanning electron microscopy (SEM) images were obtained in backscatter mode using a Hitachi SN3500 variable pressure SEM with a beam accelerating voltage of 15 kV.

2.4. Powder X-ray diffraction

PXRD analysis at ambient conditions was accomplished using a PANalytical X'Pert Pro diffractometer equipped with an X'Celerator Real Time Multi-Strip (RTMS) detector. Samples were gently flattened onto a zero-background flat-plate silicon holder. A continuous 2θ scan range of 2–35° was used with a Cu Kα radiation source and a generator power of 40 kV and 40 mA. A 2θ step size of 0.0167°/step with a step time of 10.16 s was used. Samples were rotated at 25 rpm and all experiments were performed at room temperature.

A Bruker D8 AXS X-ray diffractometer was used for variable humidity and variable temperature PXRD experiments. Approximately 30 mg of sample was gently flattened on a stainless steel sample holder, which is placed inside of an Anton Paar TTK450 variable temperature and humidity stage. The sample was scanned without spinning from 2° to 35° 2θ with the generator power set at 40 kV and 40 mA. A Cu Kα radiation source was employed. A step time of 2.0 s was used with a step size of 0.05° 2θ per step. The instrument was operated in variable slit mode.

2.5. Vibrational spectroscopy

IR spectra were obtained using a Nicolet 550 Magna-IR Fourier transform (FT) system equipped with a SensIR Durascope DATR (diamond attenuated total reflectance) accessory. Approximately 2 mg of sample was placed on the diamond probe and flattened using a microscope slide. Pressure was applied to the top of the sample using the pressure applicator on the DATR to ensure the sample underneath was in solid contact with the probe. Sixty-four co-added scans were collected at 4 cm^{-1} resolution. Raman spectra were acquired using a Nicolet FT-Raman 960 equipped with an InGaAs detector. The sample was pressed into a small pellet and placed directly into the beam. One hundred twenty-eight co-added scans were collected at 4 cm^{-1} resolution. A 1.064 μm laser was used with a power setting of 0.6 W.

2.6. Solid-state NMR

A Bruker AMX2-360 spectrometer was used for SSNMR experiments at ^{13}C and ^{1}H frequencies of 90.556 and 360.097 MHz, respectively. A Bruker 4-mm double-resonance MAS (magic-angle spinning) probe was employed. Approximately 40 mg of each sample was packed into 4-mm outer diameter magic-angle spinning rotors, sealed with a drive tip, and spun at 8 kHz ± 2 Hz under active control. Cross-polarization from ^{1}H to ^{13}C utilized a 2-ms ^{13}C linear RF power ramp from 40 to 50 kHz to enhance signal-to-noise and improve reproducibility (Metz et al., 1994). Spinning sidebands were suppressed using a five-pulse TOSS (total suppression of sidebands) pulse sequence (Antzutkin, 1999). ^{1}H decoupling was performed at ~80 kHz using the SPINAL-64 decoupling sequence with an optimized pulse width of 12 μs (Fung et al., 2000). Spectra were referenced to tetramethylsilane (TMS) using hexamethylbenzene as a secondary ^{13}C standard (Earl and Vanderhart, 1982). The ^{13}C spectra of drug substance were the result of 3888 averaged scans and used a 10 s relaxation delay (requiring 10 h) while the spectra of drug product resulted from 7776 scans (requiring 20 h).

^{19}F SSNMR experiments were performed on a Bruker Avance 400 spectrometer operating at a ^{1}H frequency of 399.874 MHz. ^{19}F cross-polarization (CP) spectra were obtained at 376.209 MHz using a Bruker 4-mm triple resonance MAS probe with sample spinning at 12.5 kHz. A linear power ramp from 75 to 90 kHz was used on the ^{1}H channel to enhance CP efficiency (Metz et al., 1994). Proton decoupling was performed at an RF power of 105 kHz using the SPINAL-64 pulse sequence with an optimized pulse width of 10 μs (Fung et al., 2000). ^{19}F spectra were referenced to liquid $CFCl_3$ indirectly using published frequency ratios (Harris et al., 2001). The ^{1}H T_1 relaxation times were determined to be 3.7 and 12.0 s for Forms 1 and 2, respectively, and a relaxation delay of 30 s was chosen for the ^{19}F CP-MAS experiments shown here to minimize the effects of differential relaxation upon the quantitative results. The ^{19}F spectra of drug products shown here were the results of 512 averaged scans using the 30-s relaxation delay (requiring approximately 4.2 h).

An exponential window function of 10 Hz was applied to time-domain NMR data prior to Fourier transformation. All SSNMR experiments were performed at 273 K to minimize the effects of frictional heating from spinning upon the samples.

2.7. Gravimetric vapor sorption

GVS experiments were conducted on a Surface Measurement Systems DVS-1000 instrument. The sample and reference were held at a constant temperature of 25 °C. The instrument was operated in step mode and the relative humidity was increased in 10% RH increments from 40% RH to 90% RH, then decreased from 90% RH to 0% RH, then increased a second time from 0% RH to 90% RH, then decreased from 90% RH to 0% RH (plus an extra step at 75% RH). The mass equilibrium criterion was set at 0.002% change in mass over time (dm/dt). A minimum step time of 10 min and a maximum step time of 240 min were specified.

2.8. Bulk and tapped density

A VanKel tap density tester with a vertical displacement of 3 ± 0.2 mm at a frequency of 250 taps per minute was used for the analysis. A tared 10 mL graduated cylinder was filled so as to minimize the effect on powder properties. The initial volume was recorded and the volume was recorded after each incremental sequence of taps (10, 50, 100, 200, 500, 750, and 1250). Calculations were made according to details in USP 616 (USP, 2005b).

2.9. Solubility measurements

Solubility measurements were conducted in triplicate. Since the aqueous equilibrium solubility of **I** was only 0.07 mg/mL, acetonitrile was used as the solvent. For each temperature, approximately 30 mg of drug substance was added to 1 mL of acetonitrile and shaken using a vibratory shaker in a water bath for 24 h under controlled temperatures. After 24 h of equilibration, samples were centrifuged for 15 min, filtered, and quantitatively diluted by an equal volume of acetonitrile to prevent any precipitation. A liquid chromatography (LC) assay was used for quantitative analysis. An Agilent 1100 LC system equipped with a variable-wavelength UV detector was used. At the time of LC assay of the filtrate, the samples were further diluted by adding 20 μL of supernatant to a volume of 1 mL acetonitrile. The dilution was done in triplicates, i.e. nine samples were used at each temperature point. A 20 μL injection volume was used. The LC method made use of a Phenomenex Luna C18(2) column (50 mm × 4.6 mm, 3 μm particle size) at a temperature of 40 °C. Water and acetonitrile were used as the mobile phases, each modified with 0.1% trifluoroacetic acid. An 8-min gradient run was employed with a mobile phase composition that began at 90% water and ended at 10% water. The peak for **I** was detected at a retention time of 4.0 min via UV absorbance at 256 nm.

2.10. Competitive ripening experiments

For competitive ripening studies (also known as "solution-phase transformation" and "solvent-mediated phase transformation" experiments), samples of ethyl acetate were first brought to temperature equilibrium using a Stem RS-10 cell block. Precise temperature control (to within ±0.1 °C) was maintained throughout the ripening experiments. Each sample was saturated with Form 2 so that solids remained suspended for at least an hour. The samples were then charged with an equal amount of Form 1, giving a mixture of Forms 1 and 2 in each block. The samples were held at their respective temperature for 6 days, and were subsequently removed, dried, and tested for polymorph content by PXRD and DSC.

2.11. Polymorph analytical method development

Drug substance **s**tandards were created with compositions of 10, 25, 50, 75, and 90% of Form 1 mixed with Form 2 by hand mixing. The phase purity of the reference standards of Forms 1 and 2 was verified by SSNMR. Approximately 50 mg of each standard was produced by weighing out each component and physically mixing in a vial for 1 min using a capillary tube. Sample analysis by IR was completed as described above. Mixing homogeneity was verified by replicate analysis of the reference samples. The software program used for the IR method development validation was OMNIC TQ Analyst Version 8.0 (Thermo Electron Corporation, Waltham, MA, USA). Calibration standard spectra were loaded into the program along with their respective actual concentrations. Three spectra were collected for each standard. Two spectra were selected randomly as calibration standards while the last one was used to validate the model.

For the limit-test analysis of drug product, drug substance standards containing 5, 10 and 20% of Form 1 were carried through the formulation process using the same conditions and excipients selected for the drug product. Milling with lactose monohydrate was avoided when Form 1 was present in the sample as this caused conversion to Form 2 as discussed in detail below. Each blend contained 8.3% (w/w) drug substance (either as Forms 1 and 2), which when tableted became 8.1% (w/w) because of the addition of a film coating step. Blends were analyzed using SSNMR without any further sample preparation by packing the material directly into MAS rotors, while tablets were punched with a cork-boring tool sized to match the inner diameter of the rotor into sections that were then gently pushed into the rotor.

3. Results

3.1. Thermal analysis and optical microscopy

Thermal analysis was conducted on the two polymorphs to determine whether the forms existed as hydrates, solvates or anhydrous crystal forms, in addition to determining melting point information. The DSC thermograms for the two polymorphs are shown in Fig. 1. The TGA data (not shown) indicated that both modifications are anhydrous, with little to no volatile content observed for either form. Form 2 shows a single sharp melt at 162 °C with $\Delta H = 83.7$ J/g. Form 1 shows a melt onset at 155 °C, followed by a sharp exotherm and another endotherm that onsets at 162 °C. The sharp exotherm is consistent with a recrystallization event. The thermal profile of Form 1 thus suggests that Form 1 melts, recrystallizes as Form 2, and melts as Form 2. Different heating rates were explored during DSC studies of Form 1; however, the value of ΔH for this form could not be accurately measured by DSC because of overlap between the thermal events.

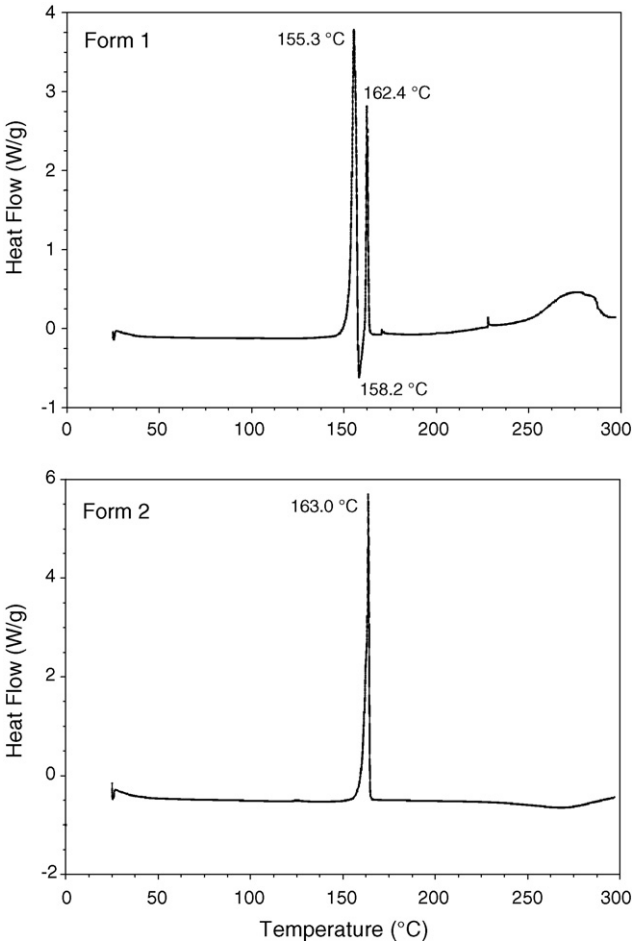


Fig. 1. DSC traces for Forms 1 and 2 obtained at a heating rate of 10 °C/min.

To further study the observations in the DSC trace, additional optical thermal microscopy experiments were performed on Form 1. The optical images of Form 1 gave indications of birefringence and the particles were crystalline by USP criteria (USP, 2005a). The relevant micrographs are shown in Fig. 2. A slight change in birefringence is observed in the image collected at 156 °C, which continues though the image collected at 160 °C. This change is indicative of the Form 1 material beginning to melt. As observed in the image collected at 162 °C, the Form 1 material has completely melted and recrystallization to Form 2 has begun, in agreement with the DSC thermogram for Form 1. The combined thermal and microscopic data indicates that Form 1 is converting to a higher melting form (assumed to be Form 2 from its melting point and habit) at 156 °C and above.

3.2. Electron microscopy

SEM micrographs of the two polymorphs obtained in backscatter electron mode are shown in Fig. 3. The images shown are considered to be representative of the material as surveyed over a number of images. The particle morphology of Form 1 shown in Fig. 3 is acicular in nature; however, Form 1 has also been produced having lath and plate-like particle shapes. Form 2 is consistently produced as needles. Form 2 shows some evidence of minor aggregation in its SEM images that could affect the ability to develop a particle size method if this property was later found to have a critical effect on the tablet performance.

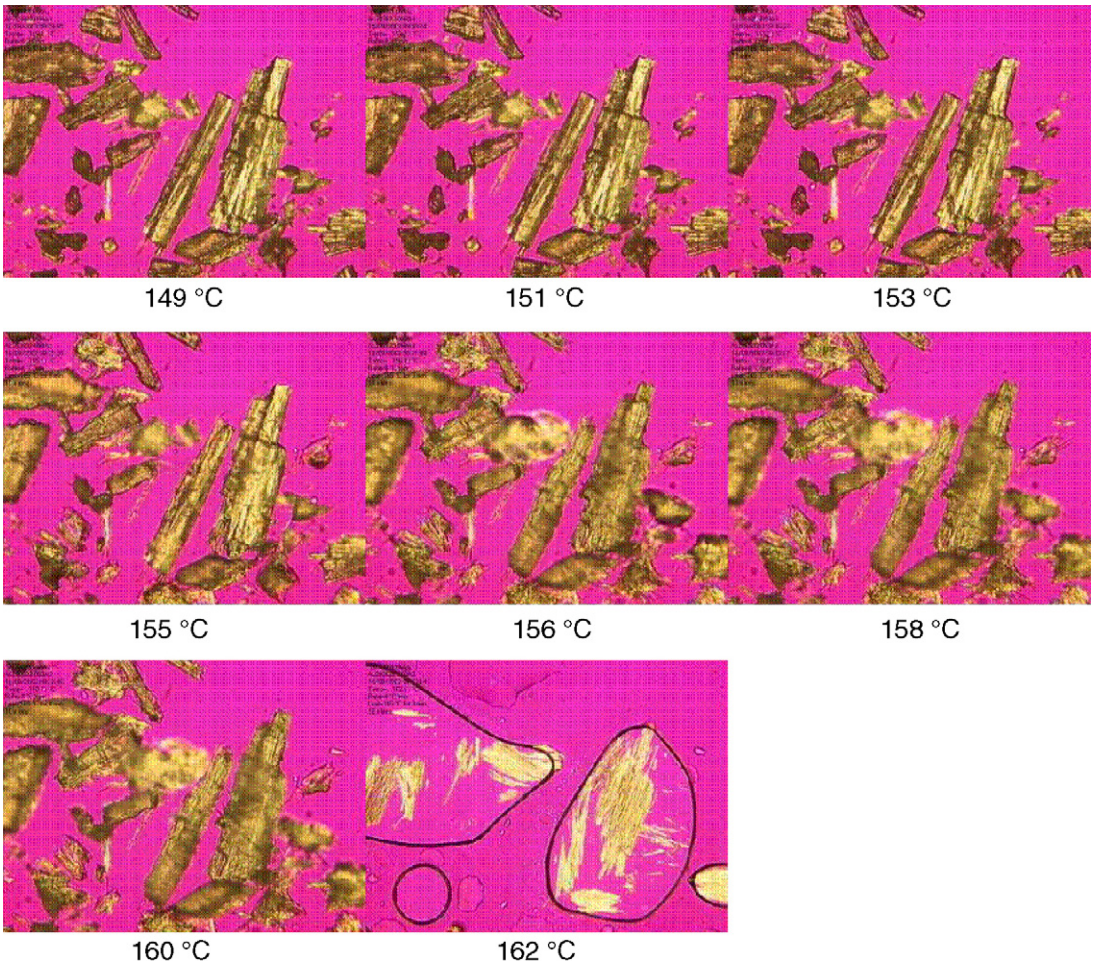


Fig. 2. Selected optical micrographs of Form 1 taken as it is heated through the temperatures indicative of a DSC phase transition. In the image obtained at 162 °C, the appearance of small Form 2 needles is clearly observed.

3.3. Powder X-ray diffraction

The PXRD patterns of Forms 1 and 2, shown in Fig. 4, confirm the crystallinity of these polymorphs as the diffraction patterns show sharp peaks with high intensity. No indication of amorphous material was detected by PXRD. The diffraction patterns for the two modifications have significantly different peak positions and intensities, confirming they are different crystal forms. Form 1 is characterized by strong reflections at 10.03°, 17.14°, 17.79°, 25.66° and 26.09° 2θ. Form 2 is characterized by strong reflections at 10.42°, 19.18°, 19.92° and 28.11° 2θ. It was determined that both forms showed significant evidence of preferred orientation effects in their flat-plate powder patterns, even after grinding, which in this case limits the utility of PXRD in quantitative or semi-quantitative analysis of polymorph content in either drug substance or final product. This is expected given the habit observed by SEM. (The patterns shown in Fig. 4 were obtained from samples that had not been subjected to grinding.) Furthermore, Form 1 partially converts to Form 2 upon extended grinding, as discussed further in the following sections, which potentially limits the use of PXRD in polymorph content quantification. It was found that with approximately 30 s of hand grinding in a mortar and pestle, Form 1 was not observed to convert to Form 2 by PXRD while some preferred orientation effects were removed; however, additional grinding led to the presence of Form 2. Because of the uncertainty involved in this process, PXRD was not considered the best approach for polymorph quantification in drug substance mixtures especially given the alternatives discussed in the following sections.

PXRD was used to study the effects on the drug substance of milling with lactose monohydrate, which was a desired formulation approach for both forms given their poor flow characteristics. As shown in Fig. 4, Form 1 was observed to convert almost completely to Form 2 when mechanically milled with lactose monohydrate (peaks denoted by arrows). A reference PXRD pattern of lactose monohydrate is shown for comparison. Form 2 did not exhibit any conversion (data not shown) and was stable under milling conditions. The sensitivity of Form 1 to milling with lactose is a major drawback to the potential development of this polymorph and factors critically into the form selection decision. Form 2 was not observed to convert to Form 1 by PXRD when mechanically milled with lactose monohydrate.

3.4. Vibrational spectroscopy

Further characterization of the crystal forms was conducted using vibrational spectroscopy methods. The IR spectra of Forms 1 and 2 are shown in Fig. 5. Although not as distinctive as the PXRD patterns for discrimination of the polymorphs of **I**, several differences in the IR spectra can be observed, especially in the region between 1800 and 600 cm^{-1}. Selected band assignments for the IR spectrum of Form 2 are given in Table 1. The most distinctive IR bands for Form 1 are located at 1606, 1395, 1194, and 1117 cm^{-1}.

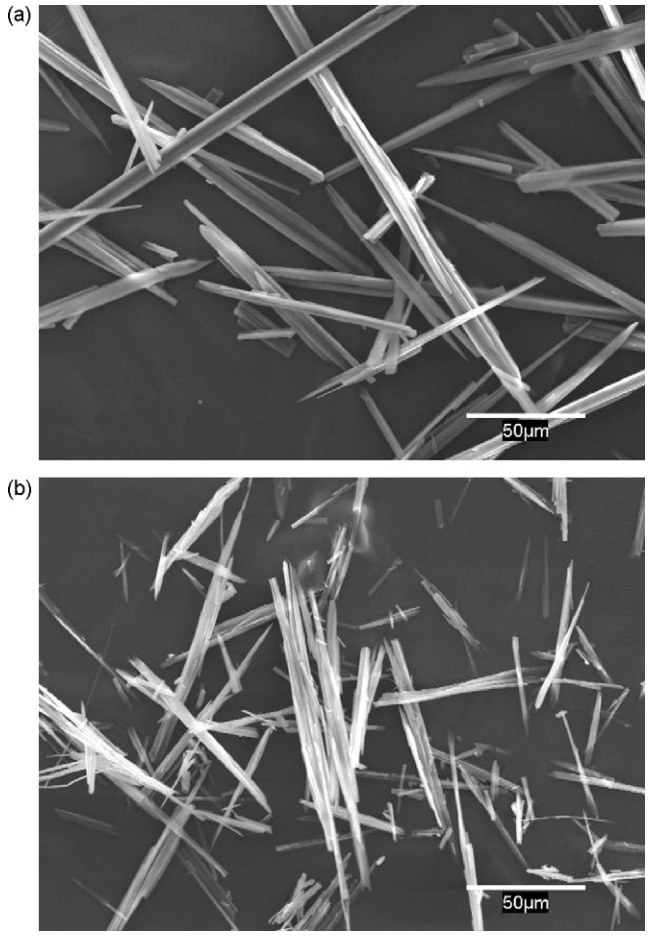


Fig. 3. Scanning electron micrographs of two typical batches of Forms 1 and 2, obtained in backscatter mode with a 15 kV beam accelerating voltage. Form 1 is shown in (a) and Form 2 is shown in (b).

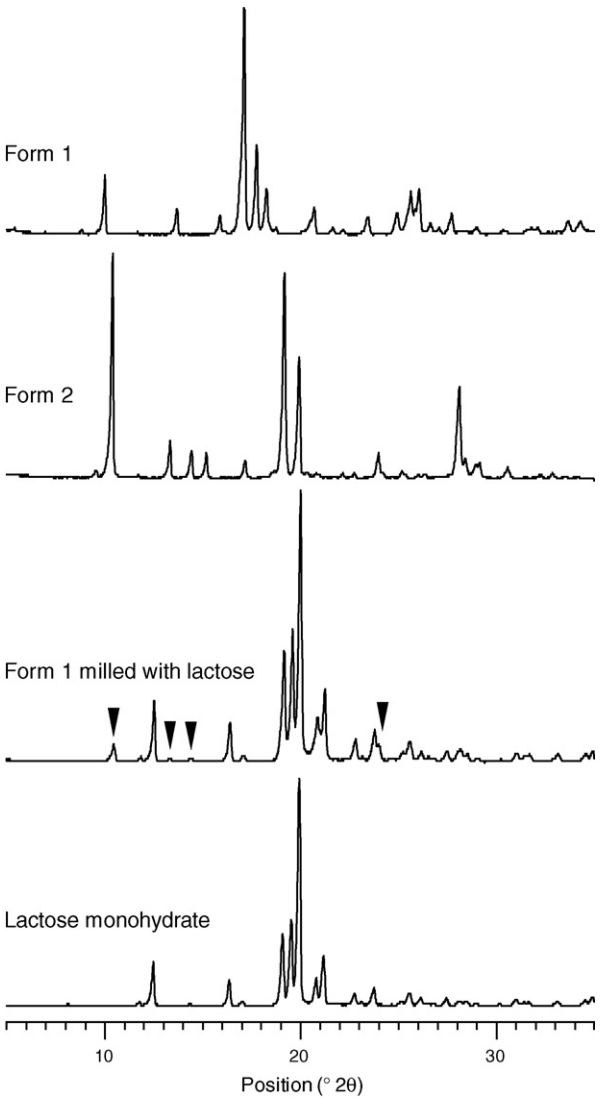


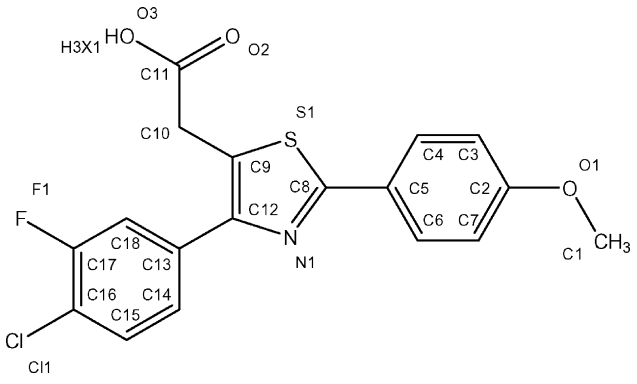
Fig. 4. PXRD patterns for Forms 1 and 2 obtained at ambient conditions using a flat-plate sample holder. A PXRD pattern for a sample of Form 1 milled with lactose monohydrate shows that this sample has converted to Form 2 by the milling process as indicated by the arrows (see text). The PXRD pattern of lactose monohydrate is also shown for comparison.

Form 2 shows characteristic IR bands at 1611, 1100, and 874 cm^{-1}. A number of other distinctive features of the spectra are apparent; however, many bands are overlapped and cannot be used for direct identification without application of second-derivative processing or multivariate methods.

The FT-Raman spectra of Forms 1 and 2 are depicted in Fig. 6. The strongest band in the Raman spectra of both polymorphs is at 1606 cm^{-1} and corresponds to Raman-active aromatic ring stretching modes. In the FT-Raman spectra, Form 1 shows a characteristic split peak with maxima at 1464 and 1457 cm^{-1}. Other diagnostic peaks for Form 1 appear at frequencies of 1538, 1175, 1060, and 794 cm^{-1}. Characteristic peaks for Form 2 appear at Raman frequencies of 1169, 1055, 787, and 687 cm^{-1}. As with the IR data, the Raman spectra are generally overlapped, and are not as distinctive as the PXRD patterns for the polymorphs of **I**. No evidence of laser damage was observed during Raman experiments on these polymorphs.

3.5. Solid-state NMR

Samples of Forms 1 and 2 were analyzed using ^{13}C CP-TOSS experiments at a MAS rate of 8 kHz and a temperature of 0 °C. The spectra are shown in Fig. 7, with assignments taken from previous work and made in reference the following numbering scheme for **I** (Vogt et al., 2008):



The spectra of Forms 1 and 2 indicate that both polymorphs are pure phases with no other detectable forms or included solvents (including amorphous content), as all peaks are assignable to specific carbons in the molecule and no extraneous, small or

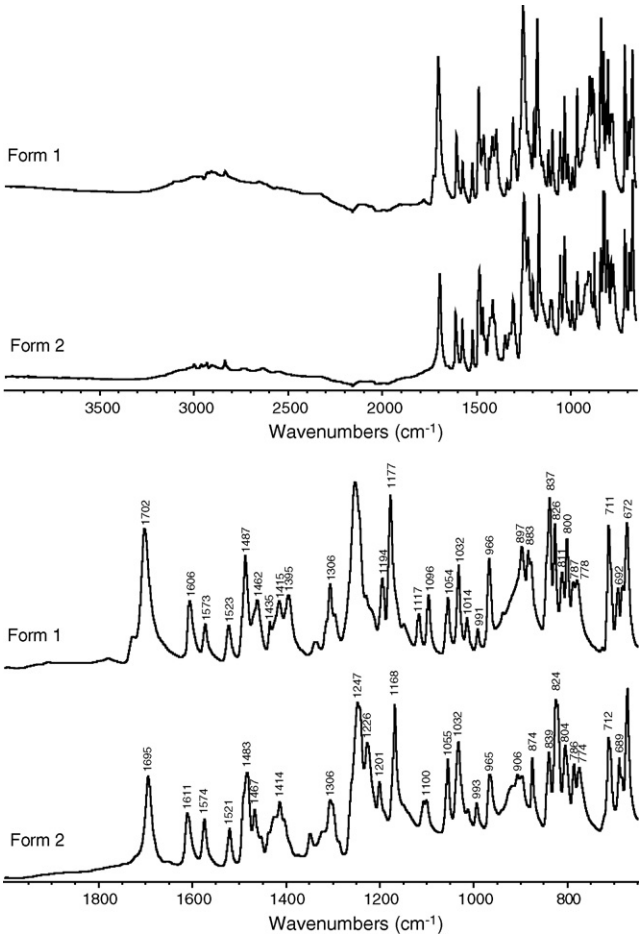


Fig. 5. IR spectra of Forms 1 and 2. The full spectra are shown at the top, with expansions showing the differences in the region 2000–650 cm^{-1} at the bottom.

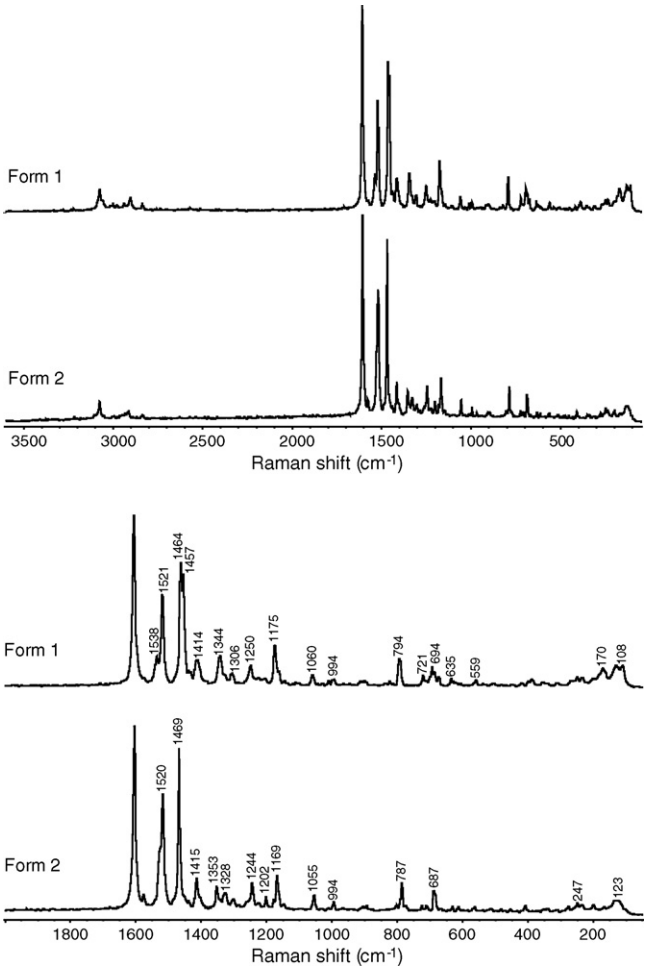


Fig. 6. FT-Raman spectra of Forms 1 and 2. The full spectra are shown at the top, with expansions showing the differences in the region 2000–50 cm^{-1} at the bottom.

broad unidentified peaks characteristic of phase impurities are seen. There was no evidence for phase changes caused by the effects of MAS, as determined by acquiring several data sets over a 24-h period. The ^{13}C CP-TOSS spectra in Fig. 7 are clearly distinctive for both forms. For example, resonances at 175.31, 165.26, 148.6, and 54.63 ppm are all clearly resolved peaks indicative of Form 1. The peaks for Form 1 are split into as many as four components (for sites that are not J-coupled to the ^{19}F nucleus), indicating that there are up to four independent molecules in the Form 1 asymmetric unit cell, which is consistent with indications of splitting seen in the vibrational spectra. The peaks at these locations in Form 2 are not split, indicating that this form contains one molecule in its asymmetric unit cell. Form 2 shows characteristic resonances at frequencies of 178.93 and 55.89 ppm that are useful for identification of this phase. The assignments of the ^{13}C SSNMR spectra in relation to the solid-state structure of these forms are discussed in more detail elsewhere as part of the determination of the crystal structures of Forms 1 and 2 (Vogt et al., 2008).

The ^{13}C CP-TOSS spectra of formulations containing 8.3% (w/w) of each of the polymorphs are also shown in Fig. 7. (Note that the Form 1 blend was not milled with lactose monohydrate.) Many of the distinctive peaks for each polymorph are well-resolved from the excipient signals and are useful for determining the polymorph content in formulations as discussed in detail below. The reduced sensitivity for the drug substance in the formulation is apparent; each of these spectra required approximately 20 h of acquisition time to produce the data shown while the spectra of the drug substance alone each required approximately 10 h. The ^{13}C CP-TOSS spectra showed no evidence that the formulation process had caused polymorphic transformations in either of these formulations (discussed in more detail below).

Table 1
Selected IR band assignments for Form 2

IR frequency (cm^{-1})	Assignment
1695	C=O stretch (carboxylic acid)
1611, 1574	C=N, C=C stretches
1247, 1226	C–O stretch
1201, 1168	Aryl-Cl and aryl-F stretches
1032	Aryl-S stretches (thiazole)
874, 838, 819, 804, 785	Out-of-plane aromatic C–H deformations

The assignments also apply to similar bands observed in Form 1.

3.6. Solubility and competitive ripening experiments

Following the characterization of the two polymorphs, an attempt was made to understand the thermodynamic relationships between Forms 1 and 2. DSC data often contains the information needed to determine thermodynamic stability. However, the thermal data collected for Form 1 show a lack of sufficient peak separation between the melt of Form 1 and the recrystallization exotherm to Form 2. The enthalpies determined from these thermal events are not accurate enough for detailed evaluation. As

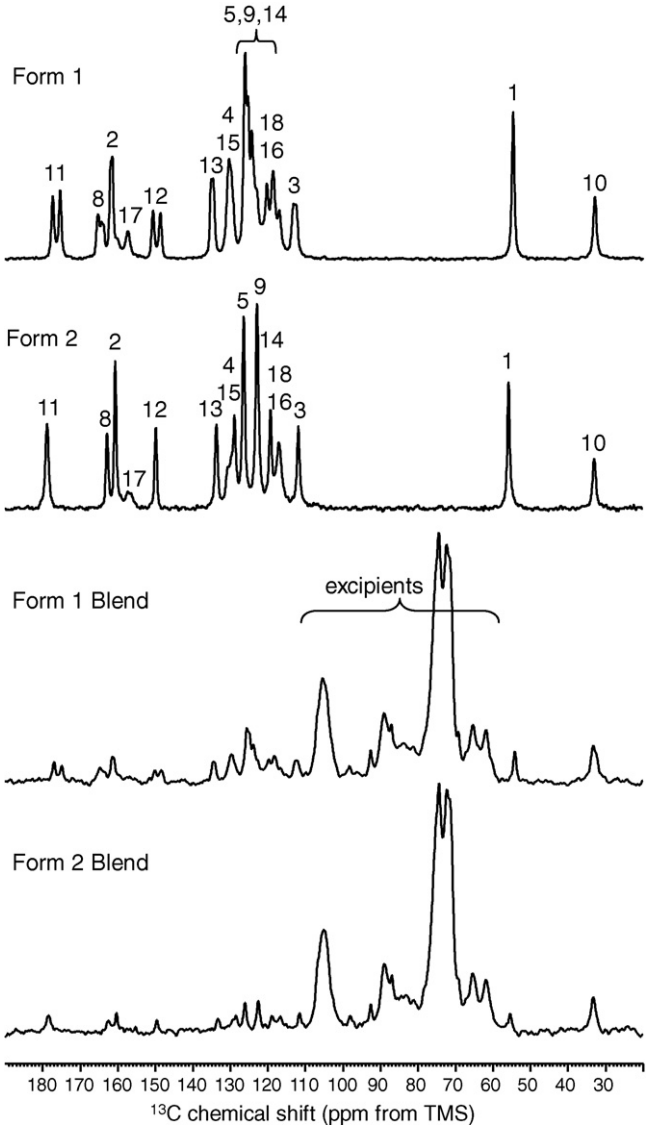


Fig. 7. ^{13}C SSNMR spectra at 90.556 MHz of Forms 1 and 2 as pure drug substance and in a formulation blend containing 8.3% (w/w) of **I**, obtained with a CP-TOSS pulse sequence at a spinning rate of 8 kHz. Assignments are from Vogt et al. (2008) (see text).

such, it is difficult to determine the key thermodynamic values and also to determine if the heat of fusion rule is obeyed for this system (Burger and Ramberger, 1979). It was also difficult to assess whether the IR rule is obeyed by this system, as the OH stretching vibrations were too broad for an effective comparison (Burger and Ramberger, 1979). To determine the relative stability of the polymorphs, temperature-dependent solubility data were collected in acetonitrile. The solubility data obtained at 8.9 °C yielded values of 10.6 and 13.3 mg/mL for Forms 1 and 2, respectively, indicating Form 1 was less soluble at this temperature. At a higher temperature of 43.1 °C, respective solubility values of 20.0 and 16.0 mg/mL for Forms 1 and 2 were obtained, indicating a reversal in the solubility relationship. Forms 1 and 2 show similar solubility, especially in the 10–40 °C region, and as a result, solubility measurements are not readily applied for determining T_t (such minor solubility differences are a common occurrence in polymorphic systems) (Pudipeddi and Serajuddin, 2005). The solubility results do show that T_t is in the vicinity of 10–40 °C, and when combined with

Table 2
Results of competitive ripening experiments at different temperatures on the polymorphs of **I**

Ripening temperature (°C)	Polymorph (from PXRD and DSC analysis)
23.2	1
29.8	1
32.0	1
36.5	2
41.3	2
48.0	2

the DSC results, provides evidence that the polymorphs have an enantiotropic relationship. Competitive ripening experiments at different temperatures within this range were used to obtain a better estimate of the T_t of Forms 1 and 2.

The competitive ripening experiment is based on the observation that the stable form will grow at the expense of other forms in a suspension containing multiple polymorphs (Haleblian and McCrone, 1969). These competitive ripening experiments were performed using ethyl acetate as the solvent, chosen to maximize solubility. Samples of ethyl acetate were brought to temperature equilibrium at temperatures in the range of 10–40 °C. Each sample was saturated with Form 2 until the solid was suspended for at least an hour and was then charged with Form 1, giving a 1:1 mixture of both polymorphs. Samples were removed after 6 days of ripening and tested for form by DSC and PXRD. The results of both techniques were fully consistent, and are listed in Table 2. Below 32 °C, Form 1 was obtained. Above 37 °C, only Form 2 is found. The value of T_t was interpolated from this data and is reported as 35 ± 3 °C. A schematic energy–temperature diagram for the Forms 1 and 2 dimorphic system was constructed from the data, and is shown in Fig. 8.

The T_t for the Forms 1 and 2 dimorphic system occurs in the temperature range in which pharmaceuticals are typically processed (e.g. crystallization, drying, granulation, tableting, and film coating). This makes it more difficult to choose the form to progress in pharmaceutical development. Assuming that the processing to final product can be controlled to produce the desired form, stability can also be an issue. Although Form 1 has the advantage of thermodynamic stability at typical shelf storage conditions (below 32 °C), there is always a thermodynamically driven risk for conversion to Form 2 above 37 °C at accelerated stability testing conditions (such as 40 °C and 75% RH, or 50 °C). Similar issues arise if Form 2

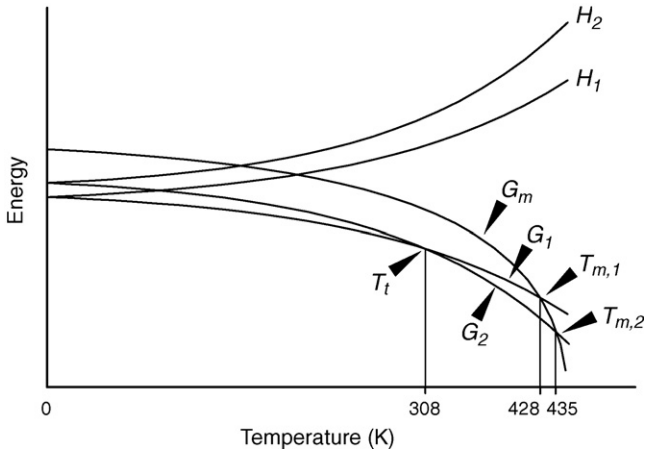


Fig. 8. Approximate energy–temperature diagram illustrating the enantiotropic relationship between Forms 1 and 2. The thermodynamic transition temperature (T_t) and the melting points of Form 1 ($T_{m,1}$) and Form 2 ($T_{m,2}$) are schematically shown.

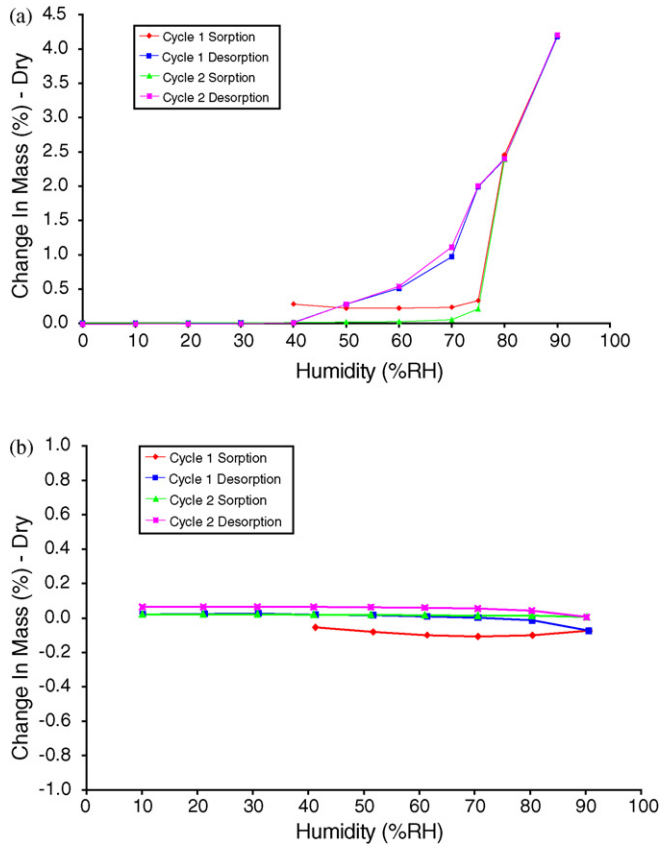


Fig. 9. GVS isotherms obtained for Forms 1 and 2. In (a), the isotherm of Form 1 shows a significant uptake of water greater than 50% RH. The isotherm for Form 2 shown in (b) indicates a negligible water uptake over the 0–90% RH range, which is within the precision limits of the instrument.

is chosen; that is, Form 2 may convert to Form 1 during room temperature stability testing conditions (typically 25 °C/60% RH). This dilemma has been reported occasionally in the literature for other pharmaceutical compounds; for example, polymorphs of piroxicam pivalate have a T_t of approximately 32 °C (Giordano et al., 1998).

3.7. Hygroscopicity

In order to further assess the potential for either of these forms to be progressed in development, GVS experiments were performed to determine how the material would behave at different relative humidity conditions. Fig. 9 shows the moisture sorption profiles for both polymorphs. At 75% RH there appears to be a rapid increase in the amount of water sorption for Form 1, reaching more than 4% (w/w) by 90% RH. This is followed by somewhat slower desorption kinetics. This behavior could indicate formation of a hydrate. Variable humidity PXRD studies were therefore conducted on Form 1 to assess whether a hydration-state change with an accompanying form change had occurred. No form change at 75% RH was observed by PXRD despite the large increase in water content at this humidity. Form 1 is thus classified as a slightly hygroscopic or hygroscopic solid depending on the classification system employed (Callahan et al., 1982; EP, 1999; Byrn et al., 1999). Although Form 1 shows significant moisture sorption, the moisture sorption profile of Form 2 (also shown in Fig. 9) shows no indication of significant water uptake in the relative humidity range of 0% RH to 90% RH. There is no evidence of significant hysteresis in the isotherm. Form 2 is thus classified as non-hygroscopic.

3.8. Flow properties

Particles with an acicular morphology often have poor flow properties due to their high aspect ratio. Poor flow is unfavorable for the secondary manufacturing of the drug product. The flow properties as well as compressibility can be estimated using bulk and tap density measurements. The bulk density was calculated to be 0.07 g/cm^2 and the tap density was calculated as 0.15 g/cm^2 for Form 2. The Hausner ratio for Form 2 was calculated to be 2.1, and the form was therefore deemed to have very poor flow properties (USP, 2005b). Bulk and tap density measurements were not performed on Form 1 because of limitations in the available material; however, based on its similar morphology and the results of small-scale processing experiments, the flow properties were observed to be similar to those of Form 2.

4. Discussion

4.1. Choice of polymorph for development

An understanding of the thermodynamic relationship of Forms 1 and 2 is necessary to progress the development of **I**. A practical way to represent this relationship is to use a schematic ET diagram for a given pair of polymorphs (Burger and Ramberger, 1979; Yu, 1995). This diagram relates the isobars of enthalpy H, free energy G, and liquid melts for two polymorphs using the Gibbs–Helmholtz equation ($G = H - TS$, where T is the temperature and S is the entropy). The free energies of polymorphs can be related either monotropically or enantiotropically. In the latter case, plotting the free energies as a function of temperature for both polymorphs will yield an intersection point below the melting points. This relationship is schematically plotted for Forms 1 and 2 using the experimentally measured T_t and melting points in Fig. 8, showing that Form 1 is thermodynamically stable form below the transition temperature T_t whereas Form 2 is stable above that temperature. Appropriately collected thermal data from differential scanning calorimetry or other complimentary techniques is often sufficient to construct an ET diagram like that shown in Fig. 8 (Yu, 1995). However, as in the present case, if the melting points of two modifications are similar and do not provide adequate separation to integrate the peaks, there may be insufficient information to derive an ET diagram. Solubility measurements and competitive ripening experiments are then applied to determine transition temperatures as in the present case.

Thermodynamic stability is an important criterion for polymorph selection, but there are other criteria of equal importance, such as physical and chemical stability, flow properties, hygroscopicity, and ease of processing. For all of these criteria, Form 2 was superior or equal to Form 1. The most significant factor in the development choice was the poor physical stability of Form 1 during mechanical milling. Mechanical milling with lactose monohydrate led to conversion of Form 1 to 2. Form 2, on the other hand, showed no tendency for conversion under milling conditions. This was critical property, since milling with lactose was a desirable design element of the formulation and was needed to both reduce particle size and to improve powder flow during blending (given the poor flow properties of both polymorphs) to improve the content uniformity of the final tablets. Furthermore, there was no indication of form conversion from Form 2 to 1 under either typical stability conditions (25 °C/60% RH) or accelerated stability conditions (40 °C/75% RH) for one year. The fact that no solid-state form conversion was observed from Form 2 to 1 suggests that there is a relatively high kinetic barrier to this conversion even at temperatures for which Form 1 is the thermodynamically stable form. The

superior hygroscopicity properties of Form 2 compared with those of Form 1 was another important factor in the polymorph selection decision. The moisture sorption behavior of Form 1 at greater than 50% RH indicates that it has a relatively high affinity for water uptake, which can lead to tablet cracking and to possible changes in drug release rates. For these reasons, Form 2 was chosen for development despite the fact that it is not the thermodynamically stable form at ambient conditions.

4.2. Quantitative polymorph determination in drug substance

Given the choice of polymorph, methods were required to determine the phase purity of drug substance during stability studies and for release of clinical batches. Control of polymorphism in drug substance and drug product can be a key regulatory requirement if polymorphs are discovered during development, particularly if a form that is less thermodynamically stable at room temperature is chosen. Although the solubility of Forms 1 and 2 suggests that they will perform similarly *in vivo*, lack of control over the polymorph ratio or lack of proper analytical testing could necessitate an additional clinical equivalence study. In addition, manufacturing, processing, and formulation concerns as well as potential chemical stability differences also require the development of a method for verifying crystal form in the drug substance. It is desirable that the method chosen for polymorphic content in drug substance also be quantitative in case numerical specifications must be set.

In the case of compound **I**, IR spectroscopy was selected as the technique for polymorphic quantitative analysis. IR is a preferred analytical technique for several reasons. The method for confirming the desired polymorph can become a regulatory specification, and the drug substance method may need to be transferred to various manufacturing sites and other organizations. Since IR spectroscopy is widely used as a chemical identification test, many pharmaceutical manufacturing sites have this spectroscopic capability. In addition, fast, robust and easy-to-use quantitative analysis software is widely available from several instrument manufacturers. The software allows for the generation of a quantitative method in a relatively short period of time. In addition to these advantages, quantitative polymorph method development using DATR-IR spectroscopy also benefits from rapid, simple sample preparation, and minimal instrument-to-instrument variability.

The fingerprint region of the Forms 1 and 2 IR spectra did not offer any distinguishing baseline-resolved peaks that could easily be used for quantitative analysis at lower levels (e.g. 5%, w/w of an undesired form). Without a baseline-resolved peak that could be used for quantitation, a simple univariate absorption model is not effective. While second-derivative processing of the IR spectra could be applied to develop a limit test, a fully quantitative method was preferred. The unresolved and overlapping bands make this situation a good candidate for using a PLS (partial least squares) model (Workman and Springsteen, 1998). To conduct this analysis, the TQ Analyst software was used to define spectral regions to use in the model. The refined regions were chosen as 1450–1420, 1360–1330, 1240–1215, 1190–1155, and 820–775 cm^{-1} using the software by analysis of the spectra of pure Forms 1 and 2 as these provided the most discriminating features. The IR spectra obtained for standards prepared in triplicate containing 10, 25, 50, 75, and 90% of Form 1 mixed with Form 2 were analyzed without being subjected to further processing. A PLS calibration model was created using the selected spectral regions, with two of the samples at each point used for calibration and one used for validation. The PLS model provided a reliable fit to the calibration data, with a correlation coefficient (R^2) of 0.991 combined with a low root mean square error of calibration (RMSEC) value of 4.88%. In addition, a predicted residual error sum of squares (PRESS) plot was constructed and indicated that two factors were sufficient to calculate the model. These two factors had root mean square error of cross validation (RMSECV) values of 42.2% and 10.9%, respectively. A loadings plot verified that about 99% of the useful data was in the first two factors. The root mean standard error of prediction (RMSEP) was 5.14%.

The DATR-IR PLS method was used to test drug substance stability and to analyze lab and plant batches of drug substance and determine the polymorphic content of samples to within 5% (w/w) with a lower quantification limit of 10% (w/w), as assessed from replicate quantitative analyses using 10% standards. Attempts to lower the limit of quantification for the DATR-IR PLS method led to unreliable results. Lower limits of quantification and detection for Forms 1 and 2 were also obtained for selected samples during stability testing and process development by use of PXRD and SSNMR analyses; once confidence and understanding was gained using these less efficient methods, the DATR-IR PLS method was then used for routine analysis of batches.

4.3. Polymorph limit-test analysis in drug product

Polymorphic conversion of Form 2 to 1 might also occur during drug product formulation or stability testing, such as during wet granulation or upon extended exposure at elevated temperature (40 °C) and humidity (75% RH). As a result, there is a need to detect polymorphic form in the final drug product, which in the present case is a solid tablet. Although the preferred regulatory strategy would be to test drug substance at the time of manufacture and during stability testing using the efficient multivariate IR method described above, analysis of the tablet itself can provide valuable supporting stability data to ensure the polymorph in the product is also intact. In the present work, this was pursued using the highest strength dosage form of interest, which was a round film-coated 307.5 mg tablet containing 8.1% (w/w) of drug substance, with lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate as previously described with the addition of a film coating. IR and Raman spectroscopy both lacked the sensitivity and resolution needed to detect the undesired Form 1 at a reasonable level in the highest dosage strength, even with the use of multivariate methods. PXRD was found to be susceptible to overlap of the most sensitive and distinctive Form 1 peaks with strong excipient reflections although Form 2 was well-resolved; had detection of Form 2 in a Form 1 formulation been the target, PXRD would have been more straightforward approach at least for the highest dosage strengths.

Fortunately, ^{13}C SSNMR was found to be readily applicable to the detection of small amounts of Form 1 in Form 2 at the highest dosage strength, as it has sufficient resolution between the drug and excipient signals as well as sufficient sensitivity. The results of ^{13}C SSNMR analysis of several mixtures of Forms 1 and 2 are shown in Fig. 10. Each spectrum required an acquisition period of 20 h (approximately 4000 scans). The methoxy peaks at 55–56 ppm were chosen for analysis because of their strong intensity and because they are sufficiently resolved from both excipient peaks and other drug peaks. Using these signals, the spectra in Fig. 10 establish a limit of detection for Form 1 in Form 2 of 10% (w/w). The absolute detection limit for Form 1 given the 8.3% (w/w) drug loading is thus 0.8% (w/w). The results in Fig. 10, which are shown for 8.3% (w/w) API samples, are easily extended to the finished tablet, which can be punched using a steel cork-boring tool into small plugs and then pushed directly out of the tool into a MAS rotor using a metal rod. Using the ^{13}C SSNMR limit test, no conversion of Form 2 into Form 1 was observed in exposed tablets at accelerated stability storage conditions of 40 °C/75% RH for one year. Other recent studies using ^{13}C SSNMR have also demonstrated detection limits for various ingredients in formulated products in the 1–5% (w/w)

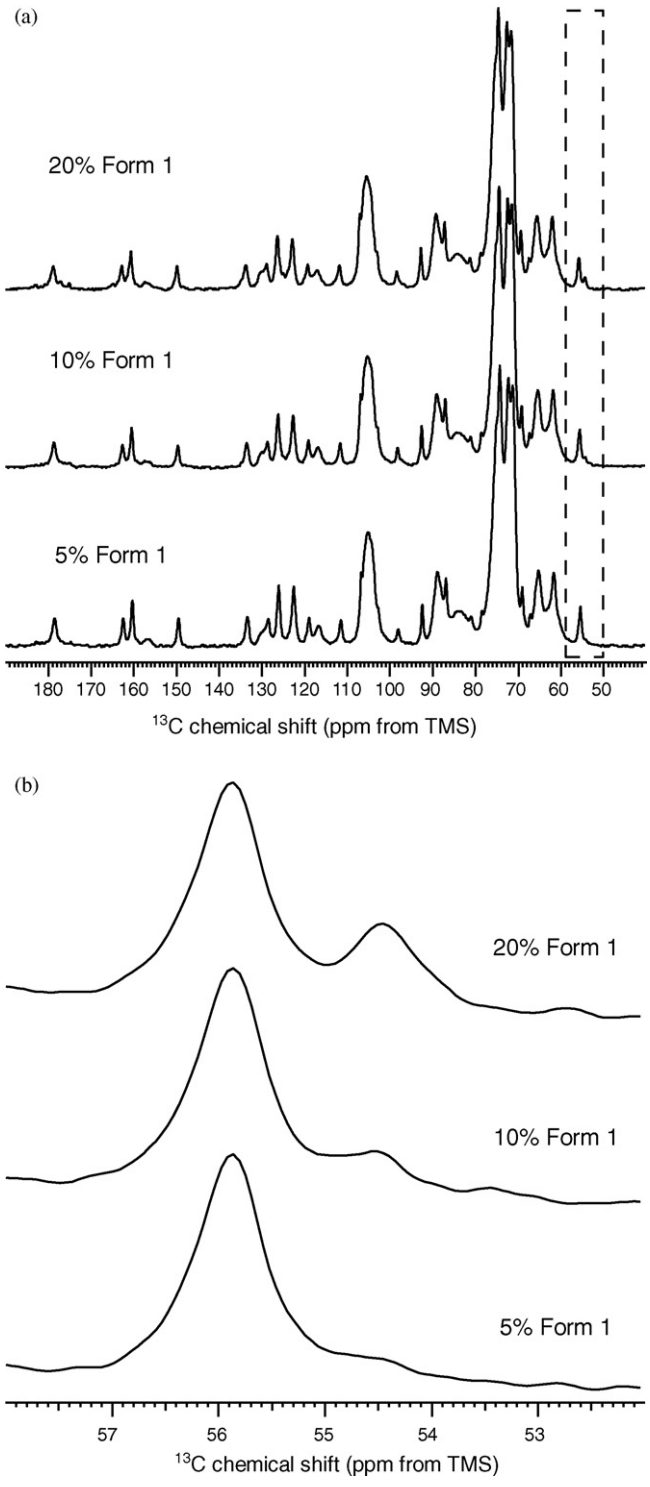


Fig. 10. ^{13}C SSNMR spectra of blends containing 8.1% (w/w) of **I** with different ratios of Forms 1 and 2. Spectra were obtained at an MAS spinning rate of 8 kHz. In (a), the full spectra are plotted and the prominent excipient peaks are noticeable. The region selected for analysis is denoted by a dashed rectangle. In (b), the detection limit for Form 1 in Form 2 is assessed at 10% (w/w) of the undesired form (0.8%, w/w total) in this formulation using this NMR method applied to the methoxyl carbon signal.

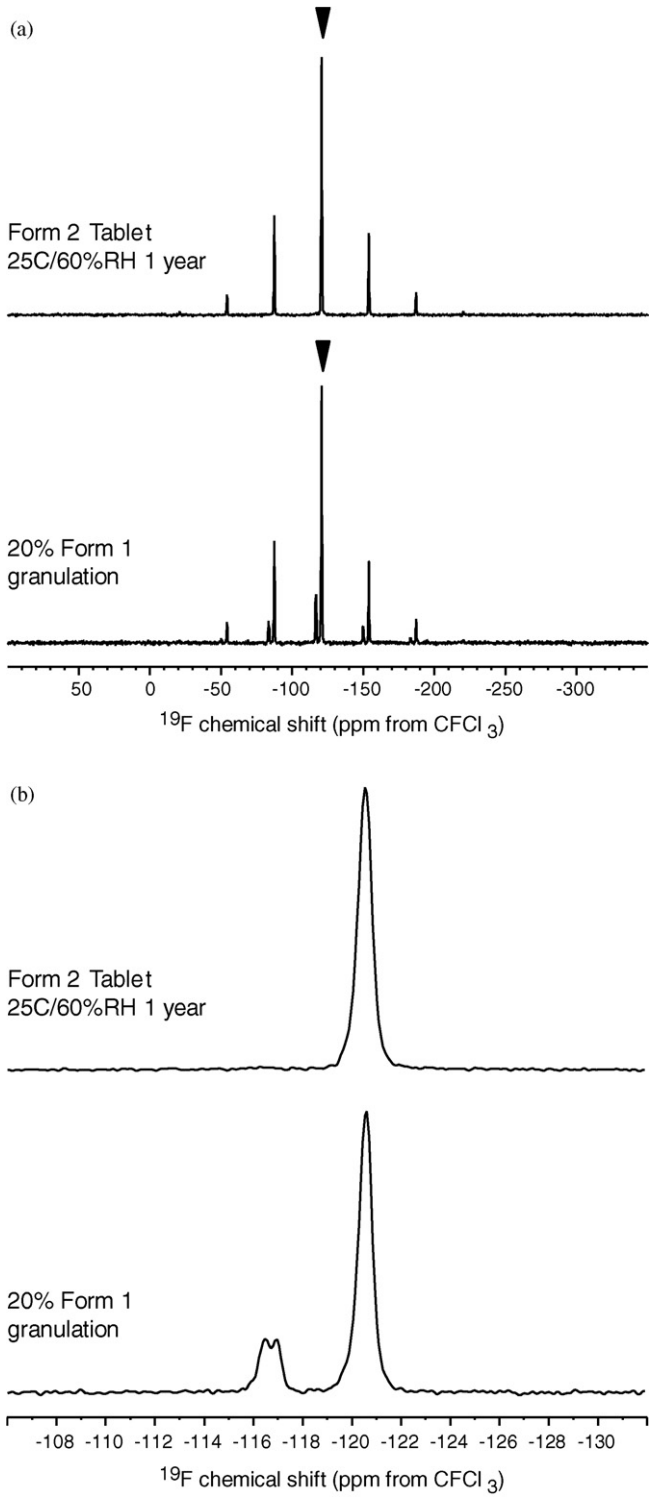


Fig. 11. (a) ^{19}F CP-MAS SSNMR spectrum of a punched tablet with a drug load of 8.1% (w/w), stored at 25 °C/60% RH for one year, and shown in comparison to the spectrum of the 20% Form 1 in Form 2 granulation. Spectra were obtained at a spinning rate of 12.5 kHz. The detection limit for Form 1 in Form 2 is estimated at 1.5% (w/w) of the undesired form (0.12%, w/w total) based on the signal-to-noise ratio of the Form 1 centerband (206:1) in the tablet and the relative signal intensities of the two polymorphs (see text). The centerbands are marked with arrows; all other peaks are spinning sidebands. (b) Expansion of the centerbands of the spectra in (a).

range (Harris et al., 2005). Despite the need for longer experiments often requiring 10–20 h, a limit-test analysis of this sort on a small number of samples can be very valuable supporting data in demonstrating polymorphic stability over long time periods at elevated temperature and humidity. An advantage of SSNMR is the relative ease of sample preparation using tablet punches, as the other commonly used techniques used for drug product analysis either require extensive tablet grinding (PXRD), or can only sample the surface (IR and Raman). Furthermore, the high resolution afforded by SSNMR data means that multivariate approaches and development of training sets are rarely needed for a reliable analysis, thus simplifying the method development.

A more efficient ^{19}F SSNMR method allows for a much faster and more sensitive polymorph check in tablets. The ^{19}F nucleus has an NMR receptivity that is several orders of magnitude greater than that of ^{13}C (Harris et al., 2001), which reduces the long analysis times needed for the ^{13}C SSNMR data discussed above, or alternatively allows access to lower strength dosage forms (e.g. tablets with <1% drug content). Because ^{19}F SSNMR is only applicable to fluorine-containing drugs and because the experiments require more specialized hardware, the ability to transfer the method to other sites or organizations is currently more limited than with ^{13}C SSNMR despite the increasingly widespread use of ^{19}F SSNMR in polymorphism and other solid form science studies (Carss et al., 1995; Wenslow, 2002). The ^{19}F NMR spectra of pure crystalline Forms 1 and 2 have been presented elsewhere; the spectra obtained showed sufficient resolution between the forms so that a polymorphic limit test based on ^{19}F NMR could be readily utilized in the case of compound **I** (Vogt et al., 2008). This is illustrated in Fig. 11, where the formulation containing 20% Form 1 in Form 2 from Fig. 10 is reanalyzed by the ^{19}F CP-MAS experiment; there are no excipient signals and the polymorphs are clearly resolved. The relative area of the signals is evaluated at 1:3.8 (Form 1:2); the expected ratio is 1:4 based on the mass of Forms 1 and 2 added to the mixture. From the high signal-to-noise ratio in the ^{19}F spectra it can be seen that the achievable detection limit of ^{19}F SSNMR for Form 1 is so low that homogeneous physical blends cannot be prepared to directly determine a detection limit; instead this must be estimated from signal-to-noise ratios. Since only a minor ~5% correction for the relative response of the two forms would be required, this is neglected in the present case. From the signal-to-noise ratio obtained for the centerband peak of the one year at 25 °C/60% RH tablet spectrum given as an example in Fig. 11 (206:1), the limit of detection for Form 1 is estimated at 1.5% (w/w) for an absolute detection limit of 0.12% (w/w) Form 1 for the total tablet. This estimated limit is derived based on a minimum signal-to-noise requirement of 3:1 to report the presence of Form 1. Thus, for the example tablet in Fig. 11, the absence of Form 1 at this level can be confirmed after storage at 25 °C/60% RH for one year. This detection limit is obtained using a 4.2-h acquisition period, which can be extended if needed to analyze lower dosage strength tablets. For example, increasing the number of scans fourfold (to a ~21 h experiment) improves the sensitivity to the level that 307.5 mg tablets containing 0.3% (w/w) (1 mg) of drug substance can be analyzed to detect the presence of Form 1 at levels of approximately 18%, for an absolute detection limit of 0.06% (w/w) Form 1 in the tablet. Various combinations of acquisition time and detection limit can thus be estimated and applied as limit-tests in stability studies. The ^{19}F SSNMR method was used to analyze a variety of Form 1 and 2 formulated samples and also helped confirm the selection of Form 2 for development.

5. Conclusions

The process of drug development requires a fundamental understanding of polymorphism, as the physical properties of the drug substance and product can ultimately affect product performance. For the dimorphic system studied in the present work, after identification of the crystal forms, their relative thermodynamic stabilities were determined and their potential for interconversion was evaluated. In the case of compound **I**, the choice of form for development is complicated by an enantiotropic relationship with a T_t of 35 ± 3 °C, which is in the middle of the region at which processing and storage occur. A complete evaluation of both crystal forms allowed for a decision to be taken to select Form 2 over Form 1 for progression due to physical stability. Although less thermodynamically stable at room temperature, Form 2 showed good physical stability as the crystal form and other properties were unaffected after one year at real time and accelerated stability conditions. Form 1 showed excessive moisture uptake at higher relative humidities and, most importantly, converted to Form 2 during milling with lactose monohydrate (which was a desired formulation to improve flow properties and reduce particle size). Form 2 was progressed at risk, even though the potential for the form to recrystallize to Form 1 at ambient conditions existed. Once Form 2 was chosen for progression, this risk was mitigated by development of methods to detect and quantitatively analyze Form 1 in drug substance and drug product during manufacture and stability testing. The different analytical techniques available for polymorph analysis in drug substance and drug product were evaluated with respect to their relative merits. Vibrational spectroscopy was used in tandem with multivariate analysis to provide a fast, sensitive and robust method for polymorph quantification in drug substance. ^{13}C SSNMR provided a limit test capable of detecting 0.8% (w/w) of the undesired Form 1 polymorph in an 8.1% (w/w) tablet formulation of Form 2, and ^{19}F SSNMR achieved a detection limit estimated at 0.12% (w/w) of the undesired polymorph in the same tablet using a faster experiment.

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