International Journal of Pharmaceutics 527 (2017) 42–51

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Characterization of new crystalline forms of hydroxyprogesterone caproate

Jeffrey Caplette[a], Timothy Frigo[a], Michael Jozwiakowski[a,*], Helene Shea[a], Mahmoud Mirmehrabi[b], Peter Müller[c]

[a] AMAG Pharmaceuticals, Inc., 1100 Winter St, Waltham, MA, 02451, USA
[b] Solid State Pharma Inc., 1344 Summer St, Halifax, Nova Scotia B3H 0A8, Canada
[c] The Structure Factory, LLC., 7 Larkin Rd, Medford, MA, 02155, USA

ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form 6 May 2017
Accepted 13 May 2017
Available online 15 May 2017

Keywords:
Polymorphism
Hydroxyprogesterone caproate
17-alpha-hydroxyprogesterone caproate
17P
17-OHPC
Makena

ABSTRACT

A systematic polymorph screening process was conducted on the steroid hydroxyprogesterone caproate, which had only one previously described orthorhombic crystalline form (A), in order to fully elucidate its solid state properties. Cooling, anti-solvent and evaporative techniques largely reproduced the same polymorph, but slurries in various solvents over two days produced a new triclinic form (B). Experiments at different temperatures in ethyl acetate or isopropyl alcohol confirmed this was an enantiotropic system with a transition temperature of approximately 30 °C. DSC was used to confirm the transition of Form B to Form A below the melting point. Form B was the thermodynamically stable form at room temperature, and 8% less soluble in a non-aqueous solvent mixture. In viscous solvents used commercially to dissolve the oil-soluble steroid for injection, solutions near the solubility limit can remain supersaturated after exposure to cooler temperatures for months. In resolving the crystalline structure of Form A, a third conformational polymorph was detected that exists only at −133 to −143 °C; this monoclinic form was designated Form C, and converts back to Form A upon warming to room temperature. These studies have increased the understanding of this drug and how the polymorphs may affect its physical stability in different dosage forms.

1. Introduction

The occurrence of polymorphism in crystallization or post-processing steps can have major legal, medical and economic consequences (Aguiar et al., 1967; Byrn, 1982; Bernstein, 2002; Chemburkar et al., 2000; Rouhi, 2003). The differences in properties of polymorphs due to molecular packing of crystals may include crystal shape (Bernstein et al., 1999), bulk and tap density, compressibility (Sun and Grant, 2001), stability (Chemburkar et al., 2000), solubility (Beckmann et al., 1984), dissolution rate/bioavailability (Aguiar et al., 1967), and melting point. Grant (1999) presented a comprehensive list of the properties that may differ among various polymorphs. Various polymorph screening techniques have been used to elucidate new crystalline structures of drugs and evaluate their relative stability and solubility properties (Huang et al., 2015; Kuang et al., 2016; Tan et al., 2016; Santos et al., 2016). The International Conference on Harmonisation (ICH Q6A, 1999) has proposed decision trees for evaluation of the effect of polymorphs on the performance of the drug product and setting appropriate specifications.

Purposeful manipulation of the solid state has often been used by formulation scientists to increase aqueous solubility and enhance bioavailability of drugs that are administered as solid or suspension dosage forms (Myrdal and Jozwiakowski, 2008). Polymorphism has been shown to be prevalent in steroids and their synthetic derivatives (Higuchi et al., 1963; Sarkar and Rohnai, 2014; Kassuha et al., 2015; Frelek et al., 2015). This can have therapeutic implications due to the pharmacological activity and low aqueous solubility of these hydrophobic molecules.

Hydroxyprogesterone caproate (HPC, Fig. 1), a progestin with a hexanoic ester on the 17 position of the steroid backbone, has been utilized therapeutically as a long-acting intramuscular agent in

* Corresponding author.
E-mail addresses: jcaplette@amagpharma.com (J. Caplette), tfrigo@amagpharma.com (T. Frigo), mjozwiakowski@amagpharma.com (M. Jozwiakowski), hshea@amagpharma.com (H. Shea), Mahmoud@solidstatepharma.com (M. Mirmehrabi), pmueller@structurefactory.com (P. Müller).

http://dx.doi.org/10.1016/j.ijpharm.2017.05.031

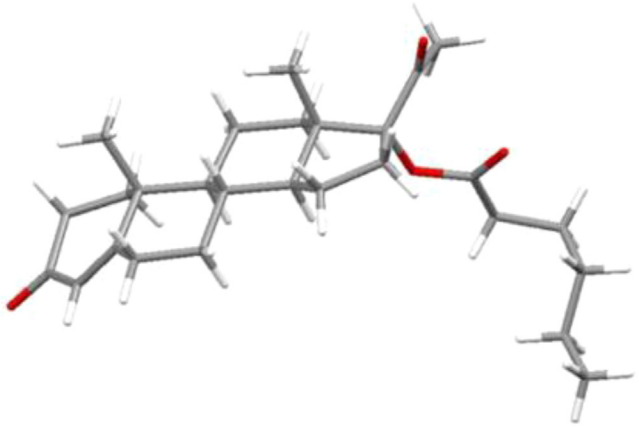

Fig. 1. Depiction of Chemical Structure of Hydroxyprogesterone Caproate (red indicates position of oxygen atoms).
Note: Intended for color in both Web and print version

women's health since the 1950s. Meis et al. (2003) demonstrated that weekly intramuscular oil-based formulations of HPC could be used to reduce the risk of recurrent preterm birth. This led to FDA approval of Makena® (Hydroxyprogesterone Caproate Injection) in the United States in 2011, for women pregnant with a singleton baby who have a history of singleton spontaneous preterm birth (<37 weeks gestation), which is a known risk factor for the current pregnancy being preterm.

Food and Drug Administration regulations (FDA, 2007) generally only require control of the crystalline form of the drug when solid or suspension dosage forms are developed, and Makena® was approved as castor oil/benzyl benzoate solution for intramuscular injection. However, knowledge of the potential for polymorphic forms is still relevant to ensure control of the dissolution step in formulation, to understand the potential for precipitation of less soluble forms during storage of solution dosage forms, and to consider future formulations of suspension dosage forms (higher concentrations or aqueous-based vehicles) with adequate physical stability. There are a number of factors influencing polymorphism of a pharmaceutical solid. Some of these factors are type of solvent, supersaturation level, crystallization temperature, rate of cooling, impurities, additives, and epitaxial growth. The type of solvent is the most prominent factor in polymorphic selectivity. This effect arises from the solvent-solute interaction at the molecular level. In addition, depending on thermodynamic stability, the crystallization temperature could favor one polymorph over the other (Mirmehrabi and Rohani, 2005). The screening protocols were developed based on previously published techniques (Mirmehrabi et al., 2004; Mirmehrabi, 2005).

Despite the fact that hydroxyprogesterone caproate has been used for over 50 years in many different countries, the only known crystalline form had been an orthorhombic polymorph described in the literature by Krstanovic et al. (1989). Periodic reports of precipitation occurring for oil-based commercial injectable solutions stored below the recommended storage condition (room temperature), and the lack of literature on the physical properties of this older drug pointed out the need for further research on the solid state. The objective of the present work was to conduct a thorough screening for other crystalline polymorphs or solvated forms of HPC and characterize the physical properties and relative stability of any newly discovered solid state forms.

2. Materials and methods

2.1. Materials

American Chemical Society (ACS) grade solvents were purchased from Sigma-Aldrich (Milwaukee, WI), including methanol, ethanol, isopropyl alcohol, acetone, methyl, *t*-butyl ether, ethyl acetate, acetonitrile, cyclohexane, dichloroethane, toluene, tetrahydrofuran and acetic acid. HPC was obtained from Aspen (Oos, Netherlands). This solid, which we designated as Form A, was the only previously known solid form. The Ward's Science crystallographic templates were purchased from VWR International (Mississauga, ON). These templates were Fluorite, Garnet, Pyrite, Apophyllite, Dolomite, Corundum, Tourmaline, Topaz, Celestine, Staurolite, Diopside, and Amazonite.

2.2. Crystallization techniques and polymorph screening

2.2.1. Solution-mediated transformation

Slurry experiments were conducted to promote solution–mediated transformation in various solvents and temperatures. Excess solid was slurried in various solvents and at different temperatures. After the specified mixing time, which was at least 2 days, the solids were filtered and analyzed using a Powder X-ray Diffractometer. In general, about 1 mL of solvent was used for slurry and stirred using the magnetic stir bar throughout the slurry time.

2.2.2. Evaporative crystallization

For evaporative crystallization, the saturated or near-saturated solutions were left at 50 °C in an open cap vial to slowly evaporate

Table 1
Crystalline form obtained by evaporative crystallization of HPC.

Solvent	Evaporation at 50 °C		Evaporation at 23 °C	
	Concentration HPC, mg/mL	Resulting Pattern	Concentration HPC, mg/mL	Resulting Pattern
Methanol	142	A	21	A
Ethanol	118	A	18	A
Isopropyl Alcohol	91	A	19	A
Acetone	789	A	19	A
Methyl-t-butyl Ether	166	A	17	A
Ethyl Acetate	419	A	19	A
Acetonitrile	620	A	18	A
Cyclohexane	20	A	7	A
Dichloromethane	650	A	19	A
Toluene	460	A	23	A
Tetrahydrofuran	620	A	24	A
Acetic acid	259	A	23	A

Table 2
Crystalline form obtained by cooling crystallization of HPC [Slow = 60 °C to 25 °C over 1 h, Rapid = 60 °C to ~0 °C in an ice bath].

Solvent	Cooling method	XRD results
Heptane	Slow	A
Cyclohexane	Slow	A
Isopropyl Alcohol (IPA)	Slow	A + some B
IPA:heptane (1:2)	Slow	A + some B
Methyl-t-butyl Ether:Heptane (1:2)	Slow	A + some B
Cyclohexane:heptane (1:1)	Slow	A
Methanol:water (8:2)	Slow	A
Heptane	Rapid	A
Cyclohexane	Rapid	A
Isopropyl Alcohol	Rapid	A
Methyl-t-Butyl Ether:Heptane (1:2)	Rapid	A + some B
Cyclohexane:heptane (1:1)	Rapid	A
Methanol:water (8:2)	Rapid	A

Table 3
Powder X-Ray Diffraction (XRD) of the two days slurry experiments.

Solvent	T, °C	Starting Pattern	Resulting Pattern
Methanol	25	A	B
Ethanol	25	A	B
Isopropyl Alcohol	25	A	B
Acetone	25	A	B
Methyl-t-butyl Ether	25	A	A
Ethyl Acetate	25	A	B
Cyclohexane	25	A	A
Heptane	25	A	A
Methanol-water (1:1)	25	A	A
Isopropyl Acetate	25	A	B

overnight, and then the resulting solid was left under vacuum and at 50 °C. The saturated solution was prepared by slurrying the solid in the solvents followed by centrifugation to separate the solid.

2.2.3. Anti-solvent crystallization

Two types of anti-solvent crystallization were performed, namely direct and reverse addition. In direct addition method, the anti-solvent (water or heptane) was added to saturated solution of Form A dissolved in a solvent. In reverse addition method, the saturated solution of Form A dissolved in a solvent was added to the anti-solvent. The amount of solvent was added based on the solubility data in each solvent systems. The anti-solvent volume was about half the volume of the solvent when water was used and was about the same volume as the solvent when heptane was used.

2.2.4. Cooling crystallization

Cooling crystallization was done by dissolving Form A at 60 °C followed by cooling to a lower temperature. Two cooling rates were used; a constant rate of 35 °C/h and rapid cooling to 0 °C by placing the vials containing hot dissolved solution in an ice bath.

2.2.5. Crystallization from melt

For crystallization from melt, the HPC solid was melted in 4 mL vials, and heated to 130 °C using a hot plate. Then the vials were placed at four different temperatures (55 °C, 25 °C, 0–5 °C and −20 °C) for 2 days.

2.2.6. Amorphous form generation

One of the techniques in polymorph screening is slurrying the amorphous solid in various solvent systems in order to possibly generate metastable polymorphs. Amorphous form generation was attempted in this work by multiple consecutive milling of the API. Even though a very low crystallinity solid was achieved, a pure amorphous form could not be obtained during the course of these experiments. Therefore, the low crystalline solid was not further used for screening.

2.2.7. Epitaxial growth crystallization

HPC was dissolved in isopropyl acetate at an undersaturated concentration of about 20 mg/mL at room temperature. The solution was then dispensed into 12 vials with 5 mL in each vial. Twelve different crystallographic templates were added to the vials as irregular granules of about 1 cm diameter and the solvent was allowed to evaporate slowly on the surface of template.

2.3. Characterization methods for polymorph properties

X-ray powder diffraction patterns (XRD) were obtained using a Bruker D8 Advance X-Ray Diffractometer equipped with a Cu Kα radiation source (λ = 1.54060°A) in locked/coupled mode. A 9-position sample changer and LYNXEYE high speed detector were used. Samples were placed on zero-background, silicon plate holders. The step was 0.05° and count times were 1.3 s per step.

Differential Scanning Calorimeter (DSC) data were collected using a TA Instruments Q1000 DSC equipped with an auto-sampler. Samples (~2–5 mg) were placed in hermetic alodined aluminum sample pans and scanned from 30 to 300 °C at a rate of 10 °C/min

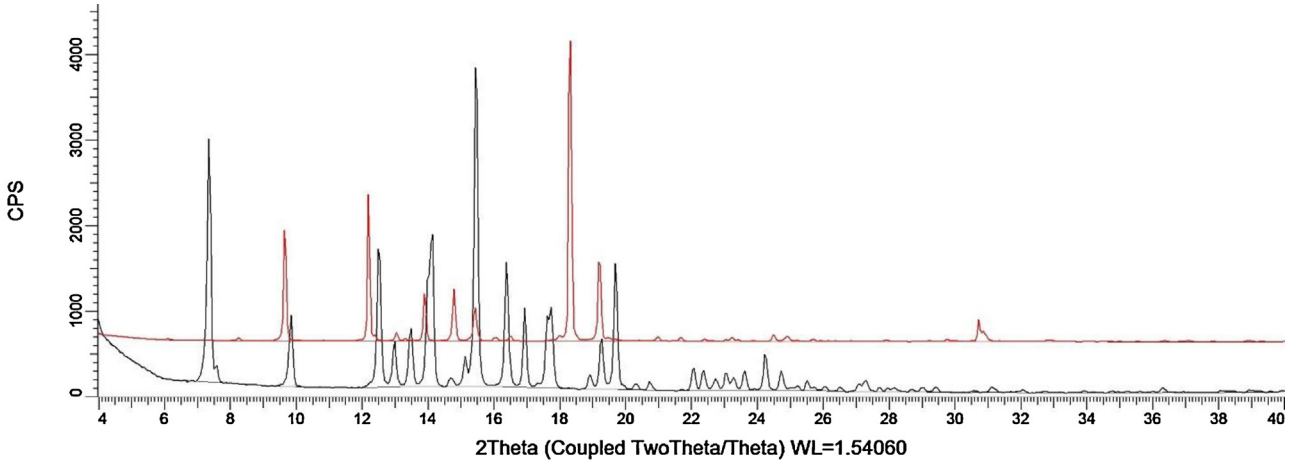

Fig. 2. Comparison of Powder X-Ray Diffraction Patterns of Form A (bottom, black) and Form B (top, red).
Note: Intended for color in both Web and print versions

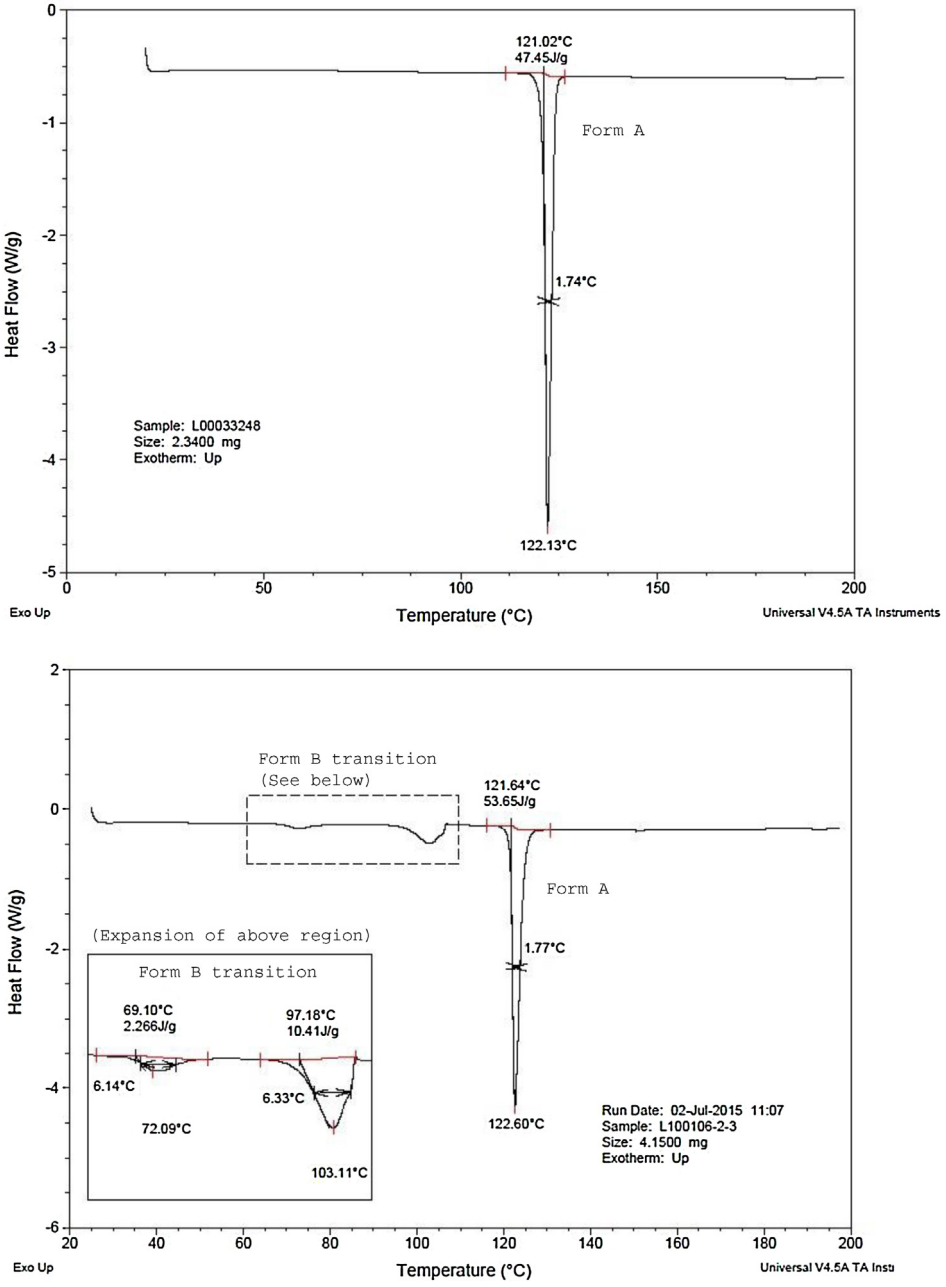

Fig. 3. DSC of Form A (top) and Form B (bottom).

under a nitrogen purge of 50 mL/min. Then the pan was cooled to 25 °C at a rate of 20 °C/min.

A Hitachi HPLC equipped with DAD detector was used for solubility and purity tests. The HPLC column was C18 5 μ 100 A, 4.6 mm × 250 mm was used. The concentration was monitored at a wavelength of 242 nm. HPLC grade methanol and distilled water were used to prepare a mobile phase of methanol:water (9:1). The column temperature was set at 30 °C and the flowrate was 0.9 mL/min. For microcapsule analysis, the provided gradient test method was used.

Hot stage microscopy with a Linkam thermal stage was used to visualize the form conversion events.

Dynamic Vapor Sorption (DVS) was done using DVS Intrinsic 1. The sample (typically 25 mg) is loaded into a sample pan and suspended from a microbalance. Nitrogen gas bubbled through distilled water provides the desired relative humidity. The chamber initially equilibrated at 50% RH followed by desorption to 2%, then sorption cycle to 95%, then desorption cycle to 2% and finally sorption cycle to 50%.

2.4. Single crystal structure determination

2.4.1. Sample preparation

Single crystals were grown in ethyl acetate by slow evaporation at temperatures that each polymorph was thermodynamically stable. To generate single crystals of Form A, the slow evaporation was done at about 40 °C. To generate Form B, the slow evaporation was done at about 22 °C. The specimens chosen for data collection were a cut into shape with a razor blade and had the dimensions $0.31 \times 0.43 \times 0.58\ mm^3$ (Form A) and $0.23 \times 0.45 \times 0.55\ mm^3$ (Form

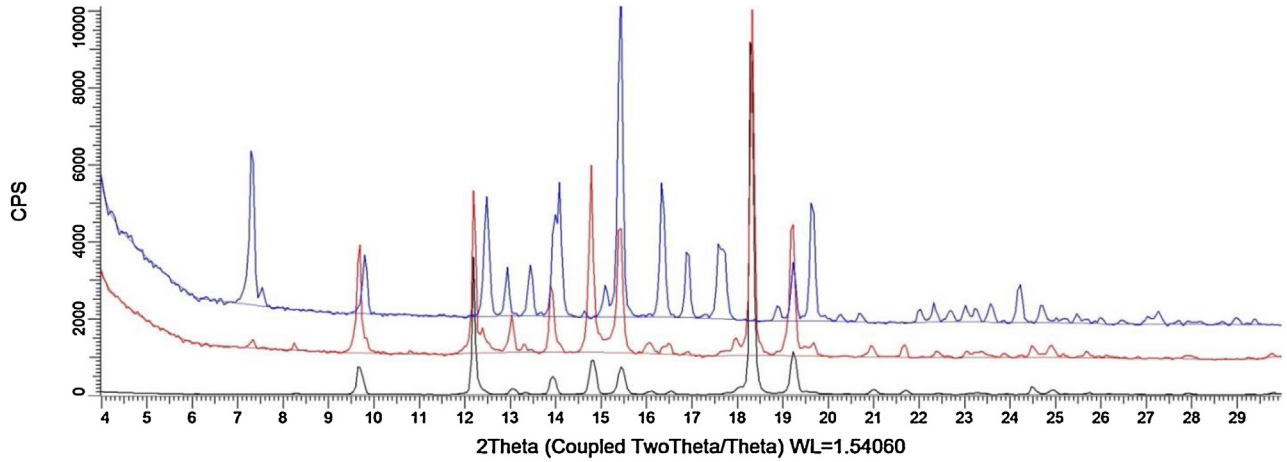

Fig. 4. Powder XRD of Form B (bottom); Thermally-treated Form B Heated to 90 °C showing traces of Form A (middle); and Thermally-treated Form B Heated to 110 °C (Form A pattern).
Note: Intended for color in both Web and print versions

B). The crystals were mounted on a MiTeGen™ mount using mineral oil (STP Oil Treatment). The first diffraction patterns at temperatures above −133 °C showed the crystals to be of high quality without traces of nonmerohedrally twinning. When the crystals were flash frozen to −173 °C on the diffractometer using an Oxford Cryosystems low-temperature device (which is standard procedure for low temperature data collection), they cracked completely and the diffraction pattern did not look clean enough for structure determination. When cooled more slowly (a cooling rate of 120 K per hour), the analyzed crystal cracked only into three domains, which could be treated adequately as a non-merohedral twin.

2.4.2. Data collection and data reduction

Diffraction data (φ- and ω-scans) were collected at −133 °C and −173 °C on a Bruker-AXS X8 Kappa diffractometer coupled to a Bruker APEX2 CCD detector using Cu $K\alpha$ radiation (λ = 1.54178 Å) from an IμS microsource. In addition, a re-determination of the high temperature phase from a crystal of the second sample was performed prior to cooling it down.

All structures were solved with direct methods using the program SHELXT (Sheldrick, 2015a) and refined against F^2 on all data with SHELXL (Sheldrick, 2015b) using established refinement techniques (Müller, 2009). All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in geometrically calculated positions and refined using a riding model while constraining their U_{iso} to 1.2 times the U_{eq} of the atoms to which they bind (1.5 times for methyl groups). The disordered *n*-pentyl ester moiety of one of the two crystallographically independent molecules in the structure of Form B was refined with the help of similarity restraints on 1–2 and 1–3 distances and displacement parameters as well as advanced rigid bond restraints for anisotropic displacement parameters. Similar ADP as well as advanced right-bond restraints were applied to all atoms in the structure of the low-temperature Form C in order to counteract correlation effects caused by the three-fold non-merohedral twinning.

2.5. Determination of physical stability and solubility determination

Solution-mediated transformation is a powerful technique for determining the thermodynamic stability of polymorphs as a function of temperature. In this study, two solvents, ethyl acetate and isopropanol, were used to study the competitive slurry of the two forms at six different temperatures, 0–5 °C, 25 °C, 27 °C, 30 °C, 35 °C and 55 °C. The solvent was initially saturated with HPC at the specified temperature. Form A was used for the initial saturation. In the saturated solution both Form A and B were added, approximately 30 mg of each, and left stirring for two days until complete conversion was achieved. The excess solid was monitored for polymorphic form by powder XRD.

For solubility determination, about 1.2 g of Form HPC (Form A or Form B) was added to 4 mL vials and ∼3 mL of the prepared solutions (equilibrated at 20 °C) were added to each of the respective vials. Upon the addition of the solvent (73.1% benzyl benzoate, 24.2% castor oil, 2.7% benzyl alcohol by weight), the vials were immediately transferred to a temperature-controlled jacketed block attached to a refrigerated bath circulator. Stir bars were added to each of the vials and the vials were stirred for 48 h. The vials were periodically monitored and additional HPC added if necessary, until the system resulted in a slurry. The stirring was turned off at the end of each day and the suspended solids in the vials were allowed to settle down overnight. After overnight settling, the clear supernatant was sampled for concentration analysis by HPLC. HPLC analysis was done using an isocratic reversed-phase method using a C18 column and ultraviolet detection, as previously described (Chollet and Jozwiakowski, 2012). A sample of the solid was also withdrawn for powder XRD analysis.

3. Results and discussion

3.1. Polymorphic form produced by different crystallization procedures

A systematic polymorph screening process was performed on HPC, a compound that had been exhibiting only one orthorhombic polymorph for many years (designated here as Form A). X-ray powder diffraction patterns on these samples resulted in the discovery of a new triclinic polymorph, which we designated as Form B.

Table 1 shows that the evaporative crystallization in a wide range of solvents and concentrations and at two different temperatures, exclusively resulted in Form A. Similarly, when HPC was precipitated through evaporative crystallization in the presence of crystallographical templates, it resulted in Form A in all cases. The same trend was observed in the anti-solvent

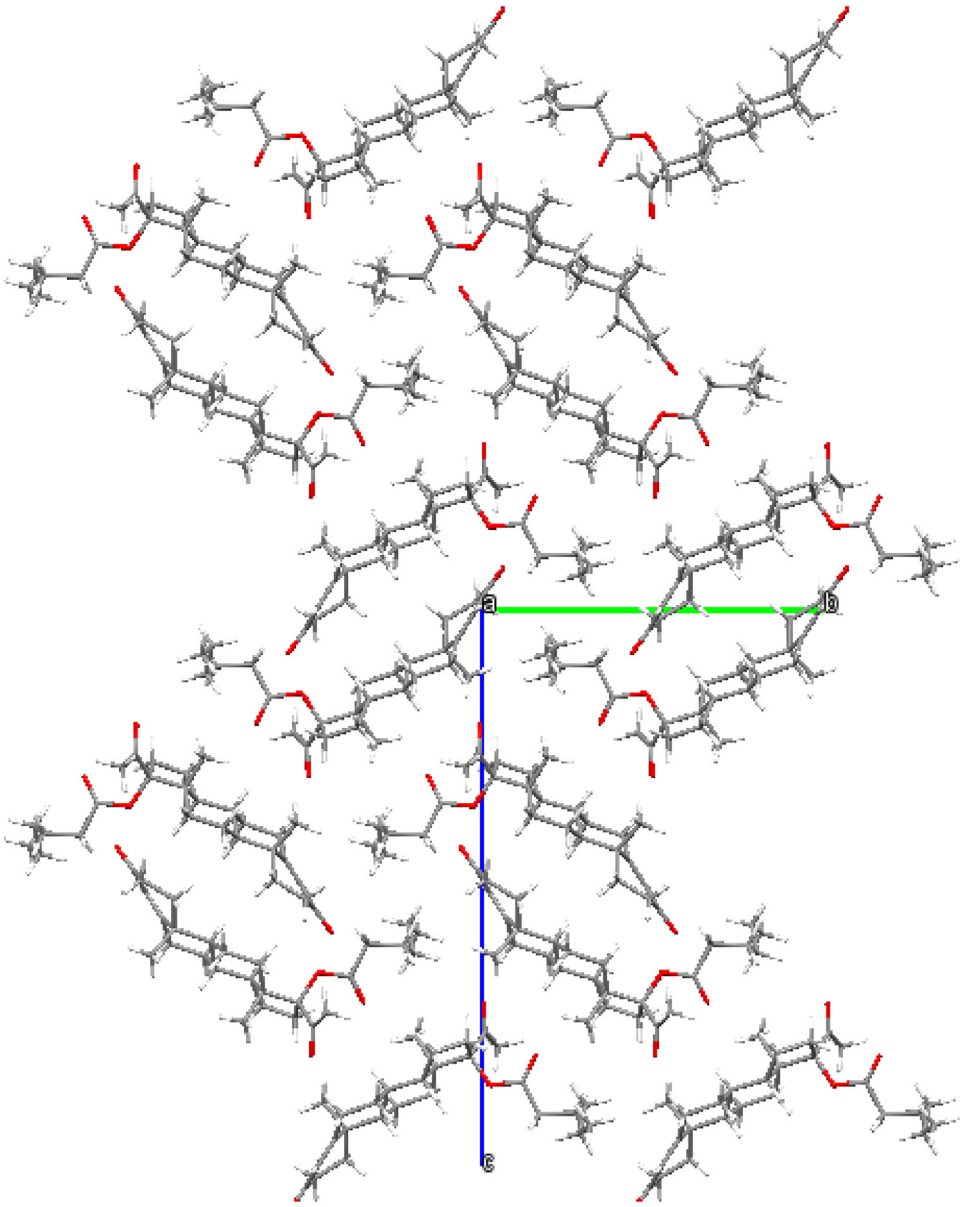

Fig. 5. Crystal packing diagram for orthorhombic Form A viewed along an axis, lines indicate unit cell axes.
NOTE: Intended for color in both Web and print versions

Table 4
Crystal lattice parameters for the three polymorphic forms of HPC.

Empirical Formula	$C_{27}H_{40}O_4$		
Formula Weight	428.59		
Wavelength	1.54178 Å		
Polymorph	Form A	Form B	Form C
Crystal System	orthorhombic	triclinic	monoclinic
Space Group	$P2_12_12_1$	P1	$P2_1$
Unit Cell Dimensions	a = 7.4417 Å	a = 7.5958 Å	a = 7.4390 Å
	b = 14.0792 Å	b = 11.0914 Å	b = 22.6449 Å
	c = 22.8711 Å	c = 14.7532 Å	c = 14.0722 Å
	α = 90°	α = 93.8615°	α = 90°
	β = 90°	β = 104.489°	β = 93.6888°
	γ = 90°	γ = 102.1789°	γ = 90°
Volume	2396.28 Å^3	1166.80 Å^3	2365.63 Å^3
Z	4	2	4
Density (calculated)	1.188 mg/m^3	1.220 mg/m^3	1.203 mg/m^3
Resolution indices (all data)*	R1 = 0.0313	R1 = 0.0320	R1 = 0.0342
	*w*R2 = 0.0800	*w*R2 = 0.0859	*w*R2 = 0.0920

crystallization regime, where HPC was dissolved in organic solvents such as methanol, isopropyl alcohol and ethyl acetate, and then an anti-solvent such as heptane or water was added to the solution. Form A was predominant in all of these anti-solvent experiments, when performed at room temperature. In all of the experiments crystallizing from the melt (starting at 130 °C and cooling at 55 °C, 25 °C, 0–5 °C and −20 °C), the solid form also resulted in Form A.

Subsequent experiments found that new XRD patterns (representing Form B) could be produced when certain solvent and temperature combinations were utilized, indicating that generation of another crystalline form (B) possibly requires more time to convert upon cooling and a medium such as solvent. The results from cooling crystallization in various solvent systems resulted in a mixture of Forms A and B, but still the majority of cases resulted in Form A and no pure Form B was observed (Table 2). As with most crystalline polymorphs, the metastable and stable forms do not readily convert in the dry solid state, but transformation can be

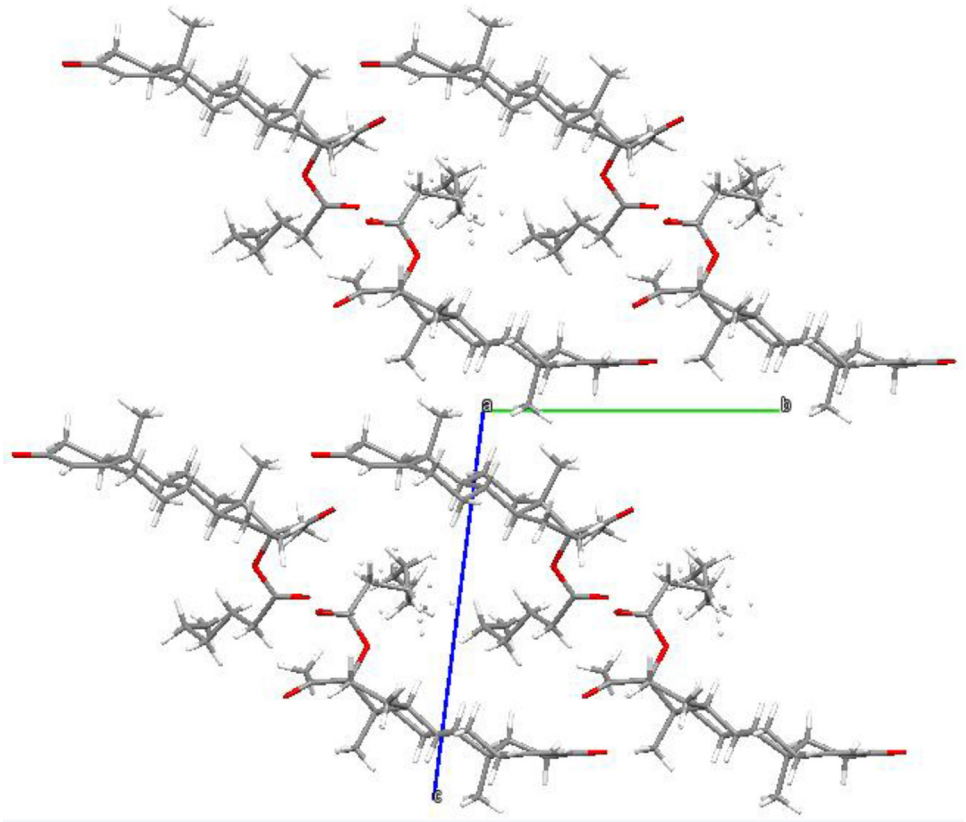

Fig. 6. Crystal packing diagram for triclinic Form B viewed along an axis, lines indicate unit cell axes.
Note: Intended for color in both Web and print versions

induced by solution-mediated mechanisms under some conditions used in polymorph screening experiments.

Per Table 3, in many solvents Form A converted to pure Form B at 25 °C after two days in a slurry, but the opposite transition was never seen, indicating that Form B is the more thermodynamically stable polymorph at room temperature. This discovery was significant, since only Form A was previously observed and the thermodynamically more stable Form B was previously unknown. It is speculated that since commercial processes of HPC synthesis and purification usually involve heating followed by cooling crystallization, initial nucleation and precipitation occurs above room temperature, and the process results in Form A. The yield is harvested by filtration prior to conversion to Form B at lower temperatures, since our experiments have shown this can take several days to convert. Form B was previously unknown because the crystalline form does not readily convert in the solid state and only solution dosage forms had been developed for this drug. This example illustrates that formulation scientists should not be complacent when working with older compounds, which may not have been thoroughly investigated with respect to their potential crystalline forms, as new polymorphs can be discovered during late stage development.

Grinding of Form A was undertaken both dry (mortar and pestle) and with several solvents (heptane, cyclohexane, isopropyl alcohol, dimethyl sulfoxide) to attempt to convert it to Form B. The grinding did not cause this phase transformation, but reduced the crystallinity (as assessed by XRD) to 80–90%. Dry grinding of Form B for 5 h resulted in formation of a solid with approximately 90% amorphous character and only 10% crystallinity by XRD; the pure amorphous form could not be produced by this method.

3.2. Characterization of polymorph Form A and Form B

3.2.1. Powder XRD analysis

The powder XRD patterns of Forms A and B were distinctly different. Fig. 2 shows the powder XRD patterns of these two forms. Specifically, Form A has a peak at 2θ of 7.3° which is absent in Form B, while Form B has a characteristic peak at 2θ of 18.3° which is missing in Form A. Thus the two forms can be readily distinguished by XRD patterns, and once pure Form B was produced using slurry methods described in Table 3, the two forms could be fully characterized.

3.2.2. Thermal analysis by DSC

DSC of Form A only showed one endothermic peak at onset of about 121 °C, which corresponds to melting of Hydoxyprogesterone Caproate USP and was confirmed by observation under hot stage microscopy (Fig. 3, top). Form B, however, showed a small endothermic peak at about 69 °C (2.27 J/g) and a more substantial endothermic peak at 97 °C (10.41 J/g) before the melting event of Form A at approximately 122 °C (53.65 J/g). This transition of Form B was also observed by hot stage microscopy, and XRD was performed on samples heated above these temperatures and cooled before melting. Fig. 3 (bottom) also illustrates the DSC thermogram of Form B. Since Form B showed extra peaks before the melting event, additonal thermal treatment experiments were performed to understand the nature of these events (Fig. 4). Form B was heated to 90 °C, cooled to room temperature and analyzed by XRD which showed traces of Form A starting to appear. The test was repeated using Form B but this time the temperature was increased to 110 °C, which is beyond the transition endothermic

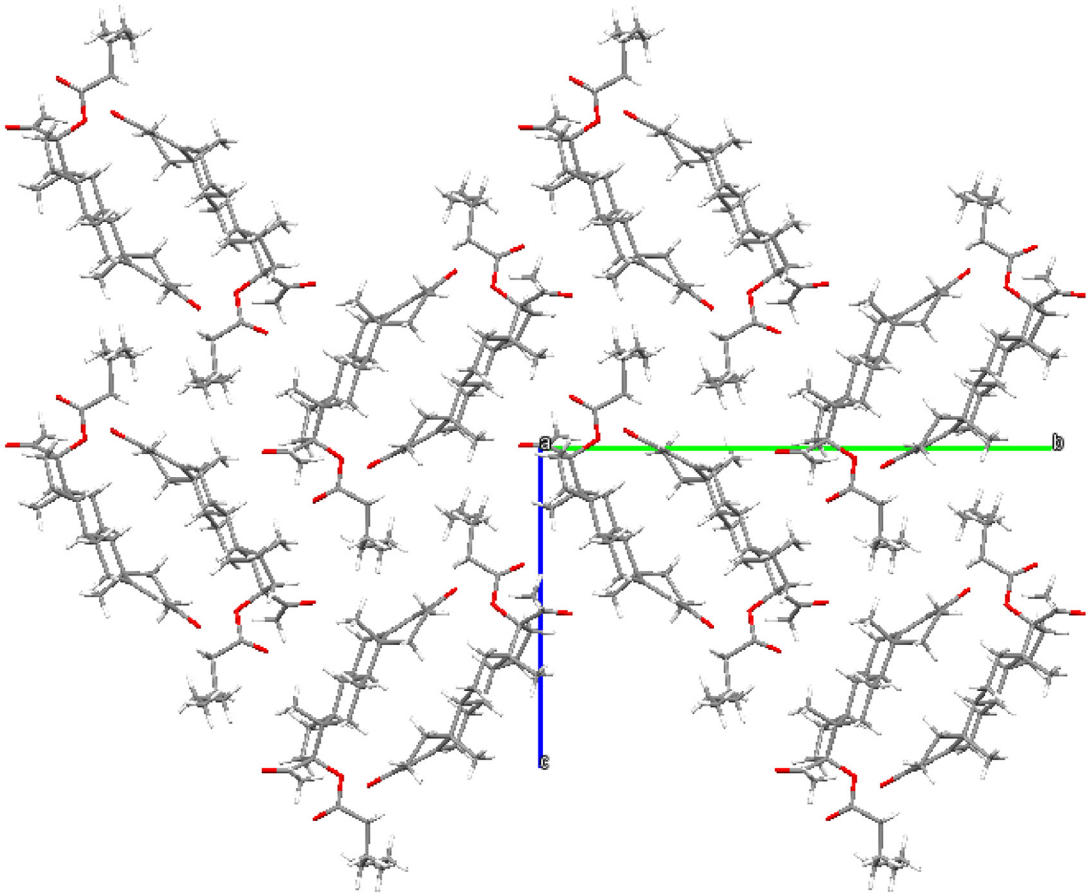

Fig. 7. Crystal packing diagram for monoclinic Form C viewed along an axis, lines indicate unit cell axes.
Note: Intended for color in both Web and print versions

peak and below the melting peak. The solid was cooled to room temperature and analyzed by XRD which showed pure Form A. Considering the DSC, hot melt, and XRD data, the endothermic peak at about 97 °C is confirmed as where Form B converts to Form A in the solid state. Similar experiments conducted after heating the sample of Form B beyond the 69 °C endothermic peak and cooling showed no changes in the initial XRD pattern. It could not be definitively determined what is responsible for this small endothermic peak, but is speculated that it may represent a minor change in structure at elevated temperatures in the crystal lattice order.

Table 5
Competitive Slurry of Forms A and B by Temperature.

Solvent	T, °C	Starting	1 day	2 days
Ethyl acetate	0–5	A + B	B	B
Isopropyl Alcohol	0–5	A + B	A + B	B
Ethyl acetate	25	A	–	B
Isopropyl Alcohol	25	A	–	B
Ethyl acetate	27	A + B	B	–
Isopropyl Alcohol	27	A + B	A + B	B
Ethyl acetate	30	A + B	B	–
Isopropyl Alcohol	30	A + B	A + B	–
Ethyl acetate	35	A + B	A	–
Isopropyl Alcohol	35	A + B	A	–
Ethyl acetate	55	A + B	A	–
Isopropyl Alcohol	55	A + B	A	–

3.2.3. Microscopy

Hot stage microscopy showed that Form B melts at about 97 °C and recrystallizes into a new solid (Form A), which then melts near 120 °C, consistent with the results from DSC where Form B was heated to the transition temperature followed by cooling to room temperature and analysis by XRD showing the complete conversion to A.

3.2.4. Hygroscopicity

None of the polymorphic forms were hygroscopic. The solid also kept its physical form stability during dynamic vapor sorption testing (DVS). Therefore, the XRD pattern before and after the DVS analysis remained the same for either form.

3.3. Single crystal X-ray structural determination and discovery of form C

Single crystals of Form A were generated using an anti-solvent process using dichloromethane as the solvent and heptane as the anti-solvent. Single crystals of Form B were successfully generated by creating a saturated solution of HPC in acetone at room temperature, then adding a few particles of Form B as seed crystals and leaving the saturated solution at 0–5 °C undisturbed for one week. It then resulted in relatively larger crystals. The liquid was decanted and a few bigger crystals were separated.

The crystal structure of Form A was determined at −133 °C in the orthorhombic chiral space group $P2_12_12_1$ with one molecule of HPC and no solvent in the asymmetric unit (Fig. 5). The crystal

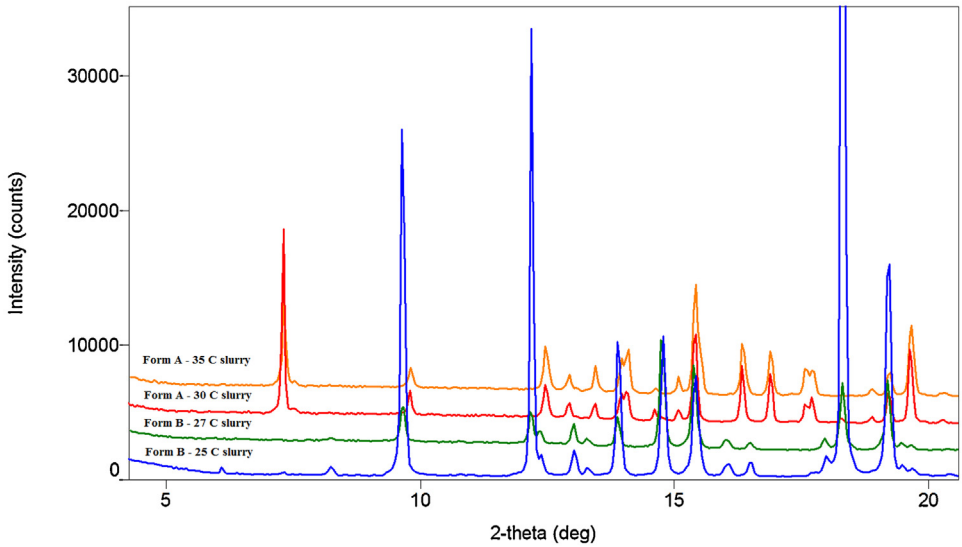

Fig. 8. Powder x-ray diffraction patterns for HPC in isopropyl alcohol after one-two days at 25 °C (bottom, blue), 27 °C (second from bottom, green), 30 °C (second from top, red) and 35 °C (top, yellow), illustrating the transition between Form A and Form B.
Note: Intended for color in both Web and print versions

lattice parameters for Form A are given in Table 4. This orthorhombic crystalline form corresponds closely in dimensions to the crystalline form previously described by Krstanovic et al. (1989). The calculated pattern of Form A conformed to the actual powder pattern.

Form B crystallizes in the triclinic chiral space group *P*1 with two molecules of HPC and no solvent in the asymmetric unit (Fig. 6). The data contained significant anomalous signals and the absolute configuration could be determined based on anomalous diffraction: The six chiral atoms had the configuration C8: R, C9: S, C10: R, C13: S, C14: S, C17: R. The crystal lattice parameters for Form B are also given in Table 4; this describes the new crystalline form generated during the polymorph screening in the present work. The calculated pattern of Form B conformed to the actual powder pattern.

Cooling Form A to lower temperatures resulted in a transformation to a new conformational polymorph between −143 °C to −133 °C. This polymorph was designated as Form C, which was determined at −173 °C in the monoclinic chiral space group *P*21 with two molecules of HPC and no solvent in the asymmetric unit (Fig. 7). The phase transition was reversible, however upon cooling below the transition temperature, the crystals crack into multiple domains. This was treated as three-fold non-merohedral twin during structure determination. The data contain significant anomalous signals and the absolute configuration could be determined based on anomalous diffraction: The six chiral atoms had the configuration C8: R, C9: S, C10: R, C13: S, C14: S, C17: R. The crystal lattice parameters for Form C, the lower temperature conformational polymorph discovered when refining the structure of Form A, are given in Table 4. Form C is not of practical significance for pharmaceutical formulation, since it only exists at very low temperatures and changes conformation to Form A upon heating to relevant formulation temperatures.

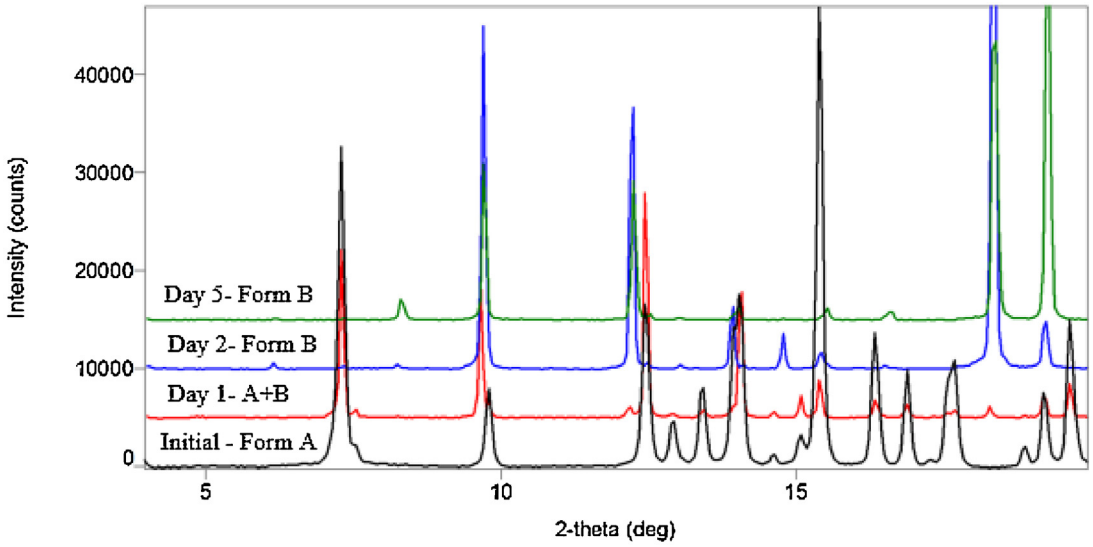

Fig. 9. Conversion of Form A to Form B during the solubility experiments conducted at 20 °C in a solvent mixture of 73.1% benzyl benzoate, 24.2% castor oil and 2.7% benzyl alcohol by weight.
Note: Intended for color in both Web and print versions

3.4. Relative physical stability of Forms A and Form B

As shown in Table 5, all slurries at 27 °C and below converted to Form B regardless of the solvent used, indicating that Form B is the thermodynamically stable polymorph at those temperatures. At 35 °C and higher, Form A was more stable, which is why it is more likely to precipitate in commercial recrystallization processes conducted at higher temperatures. Representative powder XRD patterns for the isopropyl alcohol solvent system after two days at 25 °C (all Form B), 27 °C (all Form B), 30 °C (mostly Form A) and 35 °C (all Form A) are given in Fig. 8. Therefore, these are enantiotropic polymorphs (the transition temperature is below the melting temperature) with a transition temperature of approximately 30 °C.

Although the commercially available crystalline form is the orthorhombic Form A, given this transition temperature, solution formulations near saturation at room temperature may precipitate upon exposure to cooler conditions as triclinic Form B. Temperature fluctuations (Ostwald ripening) and the solubility differences between polymorphic forms are known to cause crystal growth in suspension formulations (Hem, 1986). The only way to ensure a thermodynamically stable formulation of solid or suspended HPC is to utilize Form B – by synthesizing it directly or fully converting the commercial Form A during the process.

3.5. Solubility of Form A and Form B in non-aqueous solvent mixtures

The nature of the excess solid in the HPC slurries equilibrated at 20° in a non-aqueous solvent system is shown in Fig. 9. When starting with Form A, there is some limited conversion to Form B within 1 day, and full conversion to Form B in both 2 day and 5 day samples.

The equilibrium solubility of Form B in the nonaqueous solvent mixture described in the Methods was determined to be 278 mg/mL. When Form A was used as the starting material, an apparent solubility of 301 mg/mL, approximately 8% higher, is obtained in this metastable system. While it is difficult to determine the true equilibrium solubility of a metastable polymorph (Form A at this temperature), it is certainly expected to be higher than the thermodynamically-stable polymorph. Due to the high viscosity of this solvent system, the solution in the first case remains supersaturated with respect to HPC even after conversion of the excess solid to Form B. In fact we have observed that supersaturated formulations of HPC, made by cooling saturated solutions at room temperature and placing in refrigerated conditions, can remain supersaturated without precipitation of Form B for >six months. This may explain why commercial formulations of HPC at 250 mg/mL, close to the solubility limit at the recommended room temperature storage condition, do not readily precipitate when exposed to cooler conditions during shipping or inappropriate storage under refrigeration. Nevertheless, the knowledge of the existence of Form B, and its relative thermodynamic stability and equilibrium solubility, is vital for understanding the required conditions for producing HPC formulations that will maintain quality under long term storage.

4. Conclusions

Additional solid state research has shown that there are 3 crystalline forms of hydroxyprogesterone caproate, only one of which has been previously described, and two of which are relevant for formulation work near room temperature. The two polymorphs which occur near ambient conditions are enantiotropically related, with Form B more thermodynamically stable below 30 °C and Form A more thermodynamically stable above 30 °C. Because the transition temperature is close to room temperature, it has implications on the optimal formulation and storage of dosage forms using this drug that should be understood by formulators working with this drug.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Aguiar, A.J., et al., 1967. Effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate. J. Pharm. Sci. 56, 847–853.
Beckmann, W., Boistelle, R., Sato, K., 1984. Solubility of the A, B, and C polymorphs of stearic acid in decane, methanol, and butanone. J. Chem. Eng. Jpn. 20, 211–214.
Bernstein, J., Davey, R.J., Henck, J.O., 1999. Concomitant polymorphs. Angew. Chem. Int. Ed. 38, 3440–3461.
Bernstein, J., 2002. Polymorphism in Molecular Crystals. Oxford University Press, New York.
Byrn, S.R., 1982. Solid-State Chemistry of Drugs. Academic Press, New York.
Chemburkar, S.R., et al., 2000. Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development. Org. Proc. Res. Dev. 4, 413–417.
Chollet, J.L., Jozwiakowski, M.J., 2012. Quality investigation of hydroxyprogesterone caproate active pharmaceutical ingredient and injection. Drug Dev. Ind. Pharm. 38 (5), 540–549.
Food and Drug Administration, 2007. Guidance for Industry ANDAs: Pharmaceutical Solid Polymorphism. US FDA Center for Drug Evaluation and Research July 2007.
Frelek, J., et al., 2015. Comprehensive spectroscopic characterization of finasteride polymorphic forms. does the form x exist? J. Pharm Sci. 104 (5), 1650–1657.
Grant, D.J.W., 1999. Theory and origin of polymorphism. In: Brittain, H.G. (Ed.), Polymorphism in Pharmaceutical Solids. Marcel Dekker, Inc., New York, pp. 1–33.
Hem, S., et al., 1986. Basic chemical principles related to emulsion and suspension dosage forms, In: Lachman (Ed.), The Theory and Practice of Industrial Pharmacy. 3rd edition Lea and Febiger, Philadelphia, pp. 116–117.
Higuchi, W.I., et al., 1963. Polymorphism and drug availability solubility relationships in the methylprednisolone system. J. Pharm. Sci. 52, 150–153.
Huang, Y., et al., 2015. New polymorphs of 9-nitro-camptothecin prepared using a supercritical anti-solvent process. Int. J. Pharm. 496 (2), 551–560.
International Conference on Harmonisation, 1999. Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances, Q6A.
Kassuha, D.E., et al., 2015. Preparation and characterization of polymorphs of the glucocorticoid deflazacort. Pharm. Dev. Tech. 20 (4), 401–409.
Krstanovic, et al., 1989. Structure of 17α-hydroxyprogesterone caproate. Acta Cryst. C45, 478–480.
Kuang, et al., 2016. Crystal form control and particle size control of RG3487, a nicotinic α7 receptor partial agonist. Int. J. Pharm. 508 (1–2), 109–122.
Müller, P., 2009. Practical suggestions for better crystal structures. Crystallogr. Rev. 15, 57–83.
Meis, P.J., et al., 2003. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N. Engl. J. Med. 348, 2379–2385.
Mirmehrabi, M., Rohani, S., 2005. An approach to solvent screening for crystallization of polymorphic pharmaceuticals and fine chemicals. J. Pharm. Sci. 94 (7), 1560–1576.
Mirmehrabi, M., Rohani, S., Murthy, K.S.K., Radatus, B., 2004. Solubility, dissolution rate and phase transition studies of ranitidine hydrochloride tautomeric forms. Int. J. Pharm. 282 (1), 73–85.
Mirmehrabi, M., 2005. Characterization and Control of Polymorphism in Pharmaceutical Solids. PhD Dissertation. Western University, London, Ontario, Canada.
Myrdal, P.B., Jozwiakowski, M.J., 2008. Alteration of the solid state of the drug substances: polymorphs, solvates and amorphous forms. In: Liu, R. (Ed.), Water-Insoluble Drug Formulation. CRC Press, Boca Raton, pp. 531–566.
Rouhi, A.M., 2003. The right stuff. Chem. Eng. News 24, 32–35.
Santos, O.M., et al., 2016. Structure, solubility and stability of orbifloxacin crystal forms: hemihydrate versus anhydrate. Molecules 21 (3), 328.
Sarkar, A., Rohnai, S., 2014. Investigation on polymorphic behavior of progesterone and stabilization by co-crystallizations: a review. Mini Rev. Med. Chem. 14 (10), 853–861.
Sheldrick, G.M., 2015a. SHELXT – Integrated space-group and crystal-structure determination. Acta Cryst. A71, 3–8.
Sheldrick, G.M., 2015b. Crystal structure refinement with SHELXL. Acta Cryst. C71, 3–8.
Sun, C., Grant, D.J.W., 2001. Influence of crystal structure on the tableting properties of sulfamerazine polymorphs. Pharm. Res. 18, 274–280.
Tan, X., et al., 2016. Morphological and crystalline transitions in monohydrous and anhydrous aripiprazole for a long-Acting injectable suspension. AAPS PharmSciTech epublication.