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Dealing with the Impact of Ritonavir Polymorphs on the Late Stages of Bulk Drug Process Development

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Abstract:

Ritonavir (Kempf, D. J.; Marsh, K. C., Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X. P.; Wideburg, N. E.; Saldivar, A.; Ruitz, L.; Kati, W. M.; Sham, H. L.; Robins, T.; Stewart, K. D.; Hsu, A.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W. *Proc. Natl. Acad. Sci. U.S.A.* 1995, *92*, 2484) is Abbott's novel protease inhibitor, for human immunodeficiency virus (HIV), the causative organism of acquired immunodeficiency syndrome (AIDS). It is marketed as Norvir. From the discovery of ritonavir until the new drug application (NDA) filing, only one crystalline form was known to exist. Attempts to identify other possible crystal forms were unsuccessful. Two years after the launch of Norvir to the market, some lots of Norvir capsules failed a dissolution specification. Investigation of this phenomena revealed the existence of a crystal form of ritonavir other than the one already known (Form I). This new crystal form was designated as Form II. The two crystal forms are polymorphs and differ substantially in their physical properties such as solubility. In this article, we will discuss the challenges these polymorphs created for the bulk drug substance as well as for the formulation, and how we dealt with these challenges.

Introduction

Ritonavir was discovered at Abbott Laboratories in late 1992. The new drug application (NDA) was filed in December, 1995. Commercial start-up followed in January, 1996, and FDA approval, in March, 1996. The final drug product, Norvir, was introduced to the market as a semisolid capsule formulation and as a liquid formulation. In early 1998, an unexpected occurrence was observed. Many final product lots started failing the dissolution test. A large portion of the drug substance was precipitating out of the final (semisolid) formulated product. Further investigation into this matter revealed the fact that a new, previously unknown, thermodynamically more stable and much less soluble crystalline form had emerged. This new polymorph was referred to as Form II of ritonavir, and the originally known solid form was referred to as Form I. The semisolid formulation consisted of a nearly saturated solution of Form I. Since Form II was much less soluble in the solvents used for formulation, it was very supersaturated with respect to Form II. Soon, Form II started spreading, and all attempts to formulate the semisolid capsules failed. The drug substance was converted to Form II and precipitating out of solution. This put Abbott into a market crisis. The supply of the semisolid capsules was depleting very quickly. Once Form II was found in the formulation, samples were brought into our laboratories to study the properties of this form. Within a few days all of the lots of ritonavir prepared in the lab turned out to be Form II. From that point on all attempts to make Form I failed until a process was developed to control Forms I and II. A team of scientists who had been exposed to Form II visited our manufacturing facility in Italy to investigate if any significant changes had been made to the bulk manufacturing process. Until this time no detectable quantities of Form II had been detected in the bulk drug lots. Whether or not it was coincidence, soon after this visit significant amounts of Form II started showing up in Abbott Italy bulk drug during the manufacturing process. Although the origin of Form II remains highly debatable, the fact was that this issue had to be addressed as soon as possible. A two-prong approach was taken to tackle this problem. First was development of a formulation to accommodate Form II of ritonavir. Second was the establishment of a controlled process to consistently generate Form I bulk drug. It was also feared that any contamination of Form II in the bulk drug will not go completely into solution and hence can later serve as an initiator of Form II crystallization in the formulated drug.

The first synthetic route used for commercial production of ritonavir is referred to as the phase I process.[2,3] Subsequent development with very minor changes lead to the phase II process. In both cases, the drug substance is synthesized by a convergent coupling of three key intermediates in a series of chemical reactions called the assembly steps, as shown in the Scheme 1. In the phase I synthetic process, the three key intermediates are boc-core-succinate, 5-wing, and 2,4-

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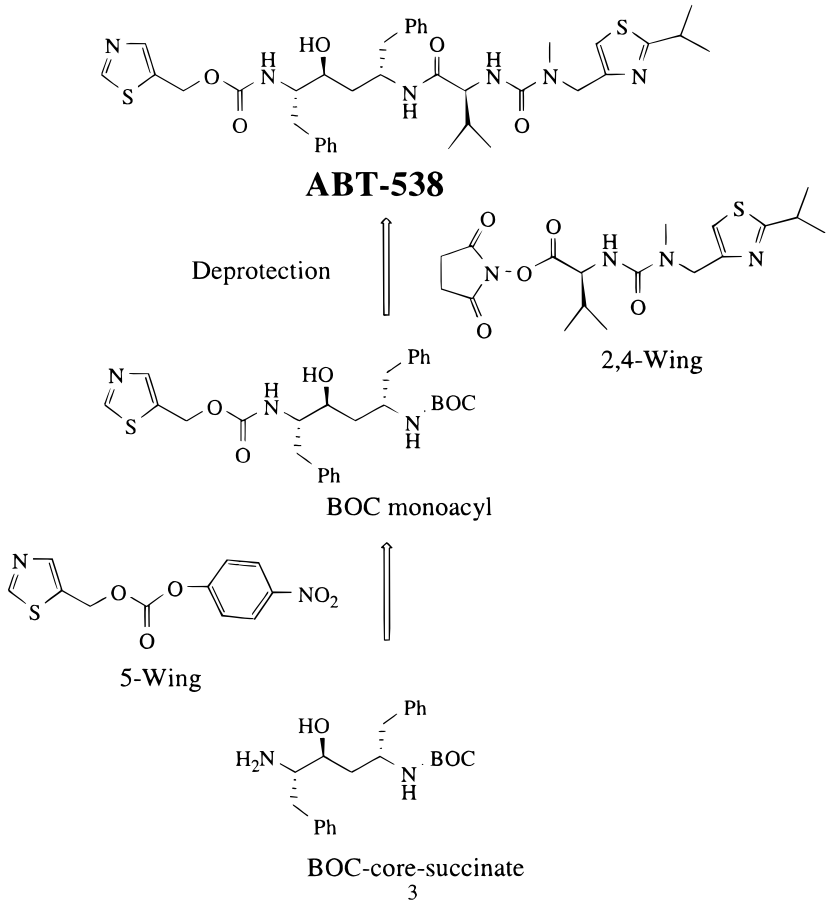
Scheme 1

Synthetic route:



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wing acid. The 2,4-wing acid was converted by in situ reaction to the 2,4-wing active ester, which underwent further assembly reaction to produce ritonavir. The phase II synthetic process differs from the phase I process as it uses 2,4-wing active ester as a starting material for the convergent synthesis of ritonavir.

Polymorphism

Polymorphism is the ability of a solid compound to exist in more than one crystalline form. These crystalline forms, although chemically identical, result from a different ordered arrangement of molecules within the crystalline lattice. Consequently, two polymorphs of the same compound can differ in physical properties that depend on the crystal lattice stability, such as melting point and solubility. The solubilities of different polymorphs of the same compound reflect the differences in free energy between their respective crystalline states (which are different for each polymorph) and the solvated state. Thus, at a given temperature, different polymorphs may have significantly different solubility values. It is widely recognized that once a compound is in solution, any differences in solid-state structure are no longer applicable.[4] This is indicated by the decision process regarding polymorphism in the Proceedings of the Fourth International Conference on Harmonization, Brussels, 1997, "for a drug product that is a solution, there is little scientific rationale for polymorph control".[5] Once the solid has been completely dissolved and there are no undissolved crystals present, the properties of the compound are unaffected by the original crystal form.

Since the discovery of ritonavir Form II, several characterization studies have been conducted. Forms I and II have different crystal habits. When examined using polarized light microscopy, Form I is usually observed as lath crystals or rods, whereas Form II crystals appear as fine needles.

In Form II, all of the strong hydrogen bond donors and acceptors have been satisfied, and the hydrogen bonds are strong. The difference between the solubilities of Forms I and II can be explained in terms of the strength of the hydrogen bonds present within the crystal. Since the strength and completeness of the hydrogen bonding has attained the maximum possible in the Form II lattice, it is not thought possible that another undiscovered polymorph of ritonavir would exist with equivalent or lower solubility than that of Form II.

Additional studies have been carried out to investigate the crystallization behavior using different solvent systems

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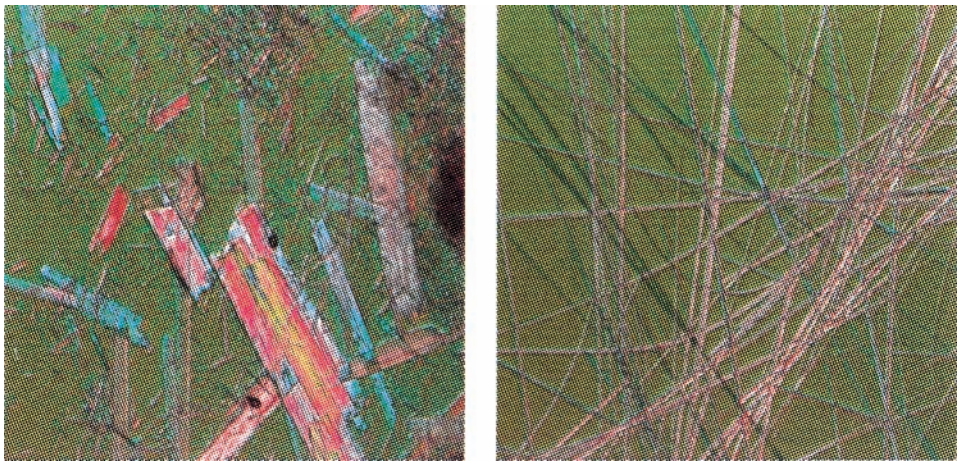

Figure 1. **Video micrograph of crystal Form I (left) and Form II (right).**

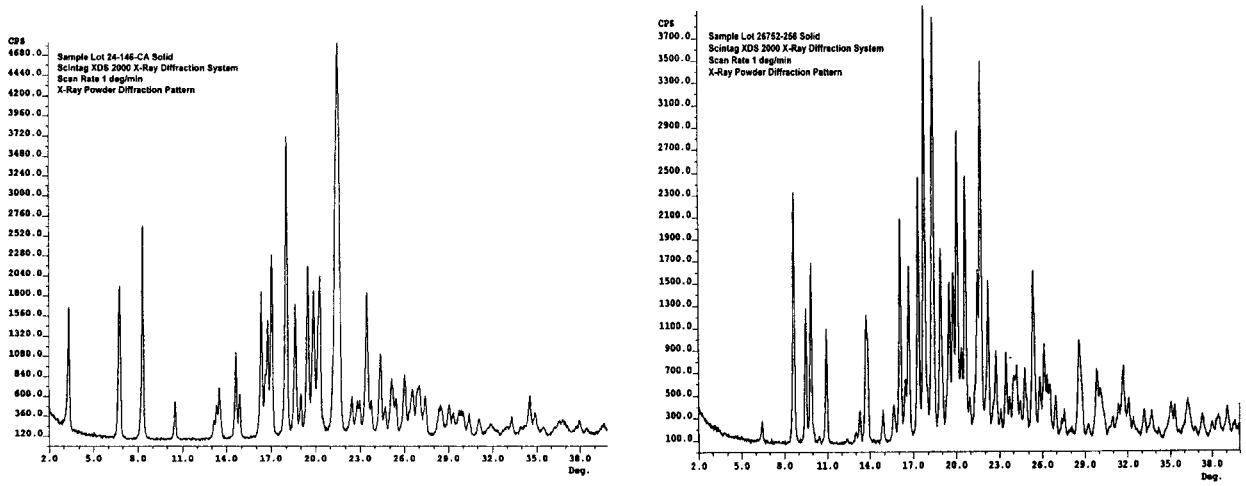

Figure 2. **X-ray powder diffraction of Form I (left) and Form II (right).**

and different crystallization techniques. Results from these studies indicated that ritonavir (Form I or Form II) recrystallized predominantly as Form I or amorphous material. These findings are consistent with the Ostwaldt rule which states that the less stable form precipitates first and crystallizes more easily and preferably.

Solid-state characterization by means of ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy as well as NIR (near infrared spectroscopy) and mid-IR confirmed that ritonavir can exist in two polymorphic forms. Similar spectroscopic studies using both NMR and IR on solutions of both Forms I and II ritonavir confirm that once in solution the ritonavir is identical regardless of the original polymorph dissolved. Solutions prepared using Form I and solutions prepared using Form II gave identical NMR and IR spectra.

Therefore, Form I or Form II ritonavir drug substance are both considered suitable for use in production of Norvir soft gelatin capsules as long as the manufacturing conditions ensure complete dissolution of the drug.

Form I and Form II Characterization and Analytical Information

The Form II crystals were analyzed by using several established techniques for characterizing polymorphic forms. The results of the comparison of Forms I and II are detailed below.

Optical Microscopy. As shown in Figure 1 the crystal habits of Forms I and II differ when examined using a polarized light microscope. Form I is usually observed as lath crystals or rods, while form II is almost always fine needles.

As shown in Figure 2, the two polymorphic forms have very different and distinctive patterns. Form I has a characteristic combination of peaks at 3.32 and 6.75 2θ, while Form II is missing these peaks and has characteristic peaks at 9.51, 9.88, and 22.2 2θ.

Solid-State Near Infrared Spectroscopy. The primary peaks for Forms I and II differ by approximately 19 wavenumbers: 6085.6 wavenumbers (1.653 μm) for Form I and 6066.7 wavenumbers (1.648 μm) for Form II.

Solid-State ^{13}C Nuclear Magnetic Resonance (NMR). The solid state NMR spectra for the two forms differ significantly in the ranges of 150−180 ppm and 190−215 ppm.

Melting Points. There is an approximately 2−3 °C higher melting point for Form II (approximately 125 °C) versus Form I (approximately 122 °C). The heat of fusion for Form II (87.8 J/g) is greater than for Form I (78.2 J/g).

Forms I and II Solubilities

A comparison of the solubility profiles of the two forms in a series of ethanol:water solvent mixtures at 5 °C is

Table 1. **Solubility profile in various hydroalcoholic solvent systems at 5 °C**

ethanol/ water	100/1 (mg/mL)	95/5	90/10	85/15	80/20	75/25
Form I	90	188	234	294	236	170
Form II	19	41	60	61	45	30

Table 2. **Ritonavir polymorph I and II solubility**

solvent	ratio	mg/mL (Form I)	mg/mL (Form II)
Temperature = 70 °C			
ethyl acetate	NA	1250	825
ethyl acetate:heptanes	2:1	266	125
ethyl acetate:heptanes	1:1	62.5	31
ethyl acetate:heptanes	1:2	11	6
Temperature = 50 °C			
ethyl acetate	NA	ND[a]	26.85
ethyl acetate:heptanes	2:1	9.26	6.67
ethyl acetate:heptanes	1:1	ND	2.38
ethyl acetate:heptanes	1:2	ND	0.51
Temperature = 25 °C			
ethyl acetate	NA	14.87	5.45
ethyl acetate:heptanes	2:1	4.43	1.85
ethyl acetate:heptanes	1:1	ND	0.66
ethyl acetate:heptanes	1:2	0.33	0.21

[a] ND = Not Determined.

presented in Table 1. As shown by these data the solubility profiles parallel each other, with Form II having significantly lower solubility throughout the series.

The solubilities of ritonavir polymorphs I and II in the bulk drug manufacturing process crystallization system are shown in Table 2. The solubility of polymorph I is significantly higher than that of polymorph II.

Evaluation of Bulk Drug Manufacturing for a Correlation to Form II Found in the Semisolid Formulation

After Form II was found in the semisolid formulation, an investigation was done to correlate manufacturing changes and the presence of Form II. The only significant process change implemented in manufacturing was during the final washing as described below.

Normal wash sequence was the following: aqueous potassium carbonate, aqueous citric acid, aqueous potassium carbonate, aqueous citric acid, and water followed by crystallization.

Modified wash sequence was the following: aqueous potassium carbonate, aqueous citric acid, aqueous potassium carbonate, aqueous citric acid, dilute aqueous potassium carbonate, and water followed by crystallization.

The modified wash sequence was implemented to minimize early eluting impurities in the bulk drug. After the modification, at least 12 lots were used in the formulation without any failure for dissolution; therefore, this modification could not be directly responsible for the generation of Form II. Graphs were plotted to find the correlation of Form II appearance with bulk drug potency, total impurities, individual impurities, largest unknown impurities, pH of the bulk drug, and amorphous content. All of the studies indicated no correlation between these factors and the presence of Form II.

Impact of Form II on the Manufacturing of Bulk Drug. Manufacturing lots that generated Form II during final crystallization had a 50% failure rate. Failures were mainly for three reasons: solvent front impurities, residual solvents, and ethyl carbamate impurity. Extended drying time was needed to remove the residual solvents. It was found that early eluting impurities could be removed by an additional potassium carbonate wash prior to final crystallization. The carbamate impurity was linked to the source of the starting material (5-wing HCl containing the precursor impurity ethyl-*p*-nitrophenyl carbonate). This impurity was always formed whenever the source contained the precursor impurity. If the final product crystallized out as Form I, the carbamate impurity was washed away with the mother liquors; on the other hand, it cocrystallized with Form II. Thus, this impurity could be eliminated either by controlling the impurity profile of the starting material (5-wing HCl) or by controlling the final crystallization process to give Form I. In conclusion, even though a new formulation was developed to accommodate Form II solubility and no specification on the crystalline form of the bulk drug was required, it was still desirable from the manufacturing point of view to target Form I as the final crystal form. This also reduced processing time during formulation since Form I dissolves much faster than Form II.

Back to the lab. First, we decided to address memory retention of Form II in solution form. We found that, although Form II was much less soluble than Form I, it could be sonicated to form a highly supersaturated solution with respect to Form II and this solution could be maintained under a closed system to prevent any external contamination of Form II. This solution was then seeded with Form I to cause crystallization. The powder X-ray results showed only Form I as the product. This clearly suggested that the crystal form memory was not retained in the solution (Note: it is known that in the case of ritonavir if there is any contamination of Form II, the product is always Form II even if it is seeded with Form I).

Encouraged by this observation we moved on to pursue the process to selectively generate Form I. Super seeding is a common approach used to achieve the formation of the less thermodynamically stable, yet desired, polymorph. As high as 50% seeding was considered for this purpose. By adding such a high amount of seed the throughput was reduced by 50% which was a huge drawback. We developed a very interesting idea to simulate super seeding without actually using large amounts of seeds, by using a reverse addition technique. The reverse addition technique was as follows: a small amount of seeds was stirred in the required amount of antisolvent. To this, the solution of product, in a crystallizing solvent, was slowly added. Since a very small amount of solution was added to the small amount of seeds originally present, this created the same effect as superseeding, and as the addition progressed, the product that

Table 3

ethyl acetate	heptanes	results
2	1	>90% polymorph II
1	1	50−50 polymorph I and II (solvent volume was 1/3 higher than normal)
1	2	mostly polymorph I
0	1	mostly polymorph I

crystallized in turn acted as seeds, giving the effect of an extreme case of super-seeding.

We put this idea into practice and developed a very reliable crystallization process on a laboratory scale to generate desired meta-stable polymorph I, using less than 5% seeds (as little as 0.5% was also demonstrated in the lab). This process not only assured generation of Form I (even in the area contaminated with Form II) during recrystallization, but it was also used to generate Form I, starting with 100% Form II. We also discovered that by choosing an appropriate ratio of solvent to antisolvent the equilibrium leading to conversion of Form I to Form II could also be controlled with time.

The typical process is described as follows:

Charge 1 kg of ritonavir to reactor A. Then charge 4 L of ethyl acetate to the reactor and reflux until all the solids dissolve. Charge 0.005 kg of seed crystals (of Form I) to reactor B. Charge 4 L of heptanes to reactor B and agitate at ambient temperature.

Slowly filter, using 0.2 μm filter cartridge, the hot solution (ritonavir in ethyl acetate) from reactor A to reactor B (containing seed crystals of Form I as a slurry in heptanes) over NLT 2 h. (It is not critical to maintain any particular temperature).

Note: An initial slower addition will increase the chance of success. Cool the slurry in reactor B to an ambient temperature, agitate for NLT 3 h, filter, wash with heptanes, and dry. Following this process we were consistently successful in obtaining Form I.

Solvent Ratio Effects on Rates of Equilibration. To reduce the propagation of polymorph I to polymorph II, an equilibration study was carried out with different solvent ratios (ethyl acetate:heptanes) at room temperature.

The results are as follows (Table 3):

Isolated polymorph I was contaminated with 1% polymorph II and stirred at room temperature for 21 h.

Conclusion: Equilibration from polymorph I to polymorph II is reduced as the percentage of heptanes increases.

Manufacturing Process to Control Form I. From lab studies it was clear that Form I could be generated during crystallization as long as the solution and the reactor were free of any Form II contamination. Form I, being the kinetic form, will always crystallize out first, and seeding with Form I crystals free of Form II could further control this. The following process was successfully developed and implemented in the manufacturing of the bulk drug to consistently obtain the crystalline product with less than 3% Form II contamination:

A solution of ritonavir in ethyl acetate was refluxed for at least 1 h. To this heptanes were charged at the rate to control reflux. After the addition of heptanes was over, reflux was continued for at least 2 h. The solution was cooled to 45 °C and seeded with crystalline ritonavir (Form I) and then stirred for not less than 3 h at that temperature. This was then cooled to 22 °C at the rate of not more than 8 °C/h. The solids were filtered at 22 °C.

Manufacturing Process to Control Form II. We also have designed a manufacturing process to produce exclusively the thermodynamically stable Form II, which is described below.

A solution of ritonavir in ethyl acetate was heated to 70 °C. It was filtered and cooled to 52 °C (rate 2−10 °C/h) and seeded with Form II crystals of ritonavir and agitated for not less than 1 h at 52 °C. It was then cooled to 40 °C (rate 10 °C/h) and heptanes was charged and the mixture cooled to 25 °C and stirred for 12 h and filtered. It was dried at 55 °C for 18 h to give exclusively Form II.

Summary

Although the polymorphism phenomenon is not new to the pharmaceutical and chemical field, Mother Nature continues to surprise the scientific community. One cannot be too careful in dealing with crystalline pharmaceutical bulk drug substances.[6] It is highly advisable to put enough resources to carry on exhaustive research to identify the most stable and all possible polymorphs. Moral of the story is: Dealing with **P**olymorphs is **P**otentially **P**recarious **P**ractice and the **P**roper way to **P**lay this game is with **P**atience and **P**erseverance.

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