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Process Research and Development of a MTP Inhibitor: Another Case of Disappearing Polymorphs upon Scale-up

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Abstract:

LAB687 is an inhibitor of microsomal triglyceride transfer protein (MTP) designed to lower triglycerides and LDL cholesterol. The discovery of its polymorphic forms closely intertwines with the synthesis development of the molecule. At the early development stage, LAB687 was known to crystallize in two modifications, Forms A and B. Knowledge of the molecule's polymorphic nature prompted extensive polymorphic screening using drug substance produced by the earlier synthesis routes. These studies revealed the existence of a third polymorph, Form C. Subsequently, Form C was selected for further development based on data from the additional formulation and polymorphic studies. Surprisingly, a new modification, Form D, appeared when the crystallization process known to routinely produce Form C was scaled up in the pilot plant. Once Form D was introduced to the laboratory, Forms A and C could no longer be made. We hypothesize that a change in drug substance impurity profile due to the changes in synthesis, led to the emergence of the most stable Form D.

Introduction

Polymorphism, the ability of a solid material to exist in more than one crystal structure, was first discovered in minerals by German chemist Martin Heinrich Klaproth in 1798. Since then, this phenomenon has been encountered in many areas of drug development. It is the variation in the properties of organic compounds, such as the melting point, solid-state chemical reactivity, and bioavailability that makes polymorphism such a potentially important issue for the pharmaceutical industry. Numerous drug substances, which are mostly small organic molecules with molecular weights below 600, have been discovered to exhibit polymorphism. McCrone even suggested that the number of forms known for a given compound is proportional to the time and money spent in research on the compound.[1] Its occurrence introduces complications during manufacturing and adds yet another challenge to the complexity of drug development. Often process chemists find themselves not only facing the complexity of achieving chemical purity, but also the challenges of understanding and controlling the crystal polymorph through crystallization.[2−4]

As the search for new therapies intensifies, drug candidates are becoming more conformationally flexible with greater number of functional groups that may form a number of hydrogen bonds are predisposed to polymorphism.[3] When a compound exhibits polymorphism, cases of difficulties in obtaining crystals of a particular known form or irreproducibility of the experimentation abound.[4−7] A celebrated tale of the serendipitous nature of polymorphs is the protease inhibitor ritonavir (Abbott Laboratories) where a given polymorphic form could not be produced even though it had previously been obtained routinely over long time periods, resulting in drug product shortage.[4] It is believed that once a particular polymorph has been obtained, it is always possible to obtain it again given the right conditions.[5,6] Numerous factors, including the solvent, solution concentration, degree of supersaturation, heating and cooling rates, seeding and mixing, may affect the crystallization process and result in the production of different polymorphs.[8−14] Quite often our ability to manipulate the kinetic processes of nucleation and growth in polymorphic systems is poor, and the consequence of this is that the level of process control is limited.[15]

To further explain why a process that has remained stable and under control for years can suddenly go out of control, it has been argued that the presence or absence of impurities or byproduct could direct the polymorphic outcome of the crystallization process. In the case of a polymorphic drug, sulfathiazole, the polymorphic purity of the crystallized product could be affected by the final hydrolysis byproduct, ethamidosulfathiazole, at concentrations as low as 1 mol %.[16] Changes in the supplier of an intermediate could cause a change in the impurity profile, thus allowing another polymorph to appear.[4] In the context of process development and route selection, these findings have profound implications. The continual process improvement could give rise to increasingly selective chemistry,

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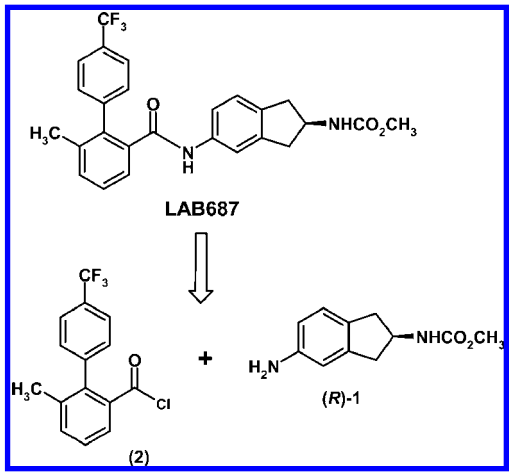

Figure 1. **Molecular structures of LAB687 and its key intermediates.**

whereby the decreasing level of an important reaction byproduct could affect the polymorphic purity of the product. The byproduct that directs the crystallization of a specific polymorph during pilot scale or early manufacturing trials could be eliminated at the full scale, again allowing a different polymorph to appear.[17] It is therefore imperative that development chemists are vigilant to these issues during the development of a synthetic route.[2]

In this paper, we present a case study on the polymorphic behavior of LAB687 where the discovery of its polymorphic forms closely intertwines with the synthesis development of the drug molecule.

Process Development of LAB687

LAB687 is an inhibitor of microsomal triglyceride transfer protein (MTP) designed to decrease the production of gut-derived chylomicrometers and hepatic very low density lipoproteins (VLDL); thus, to lower triglycerides and LDL cholesterol.[18] LAB687 was synthesized by the coupling of the amine **(R)-1** and the biaryl acid chloride **2** (Figure 1). Significant improvements were attained in the synthesis of the key enantiomerically pure intermediate **(R)-1** at various development stages to develop an economical route. The first synthetic route used for producing gram scale of LAB687 is referred to as the early route (Figure 2). Major modifications have led to an efficient and practical synthesis of this enantiomerically pure compound **(R)-1** for the phase I synthesis (Figure 3). This new route consequently introduced a new dimeric urea impurity **6** to the drug substance, which was not present in the batches produced by previous routes. The compound **6** originated from byproduct **5** (Figure 4,[19]). that was formed in the conversion of compounds **3** to **4** via Schiff base **5-P** (Figure 3).

Polymorphism of LAB687

The first anhydrous crystalline LAB687 was isolated by a research chemist. It became apparent that the compound exhibited polymorphism when the early route produced a new crystalline form, which was named Form A. The research batch was named Form B. To search for all potential polymorphs of LAB687, a polymorphic screening study was performed using Form A drug substance, (purity 98.9%). A series of approaches (Table 1), namely slurry equilibration, evaporative crystallization, cooling crystallization and antisolvent crystallization, produced a new polymorph (Form C) and a toluene solvate. Seeded crystallization processes were successfully developed to routinely produce Forms A or C at larger scale (100 g).

Table 1. **Summary of polymorph screen study**

method	solvent	temperature [°C]	results
slurry	heptane	25	Form A
	hexane	25	Form A
	methyl *tert*-butyl ether	25	Form A
	isopropyl ether	25	Form A
	water	25	Form A
	ethanol/water (1:1)	25	Form A
	heptane	50	Form A
	hexane	50	Form A
	methyl *tert*-butyl ether	50	Form A
	toluene	50	Form A
	water	50	Form A
	ethanol/water (1:1)	50	Forms A and C
evaporation	acetone	25	amorphous
	acetonitrile	25	amorphous
	ethanol abs.	25	amorphous
	ethanol 95%	25	amorphous
	ethyl ether	25	Form A
	ethyl acetate	25	amorphous
	methanol	25	amorphous
	methylene chloride	25	amorphous
	2-propanol	25	amorphous
	tetrahydrofuran	25	amorphous
	toluene	25	Form A
crash cool	ethanol/water (1:1)	60−4	Form B
	toluene	60−4	Form S_A
	methyl *tert*-butyl ether	60−4	Form A
antisolvent	acetone/water	20	oil
	tetrahydrofuran/water	20	oil
	ethanol/water	20	Form A
	ethyl acetate/heptane	20	Form A
	2-propanol/water	20	Form A
	tetrahydrofuran/heptane	20	Form A
	tetrahydrofuran/heptane	50	Form A
	ethanol/water	50	Form A

Table 2. **Thermal properties of LAB687 polymorphs**

analysis	Form A	Form C	Form D
melting point (onset) [°C]	130.6	120.5	157.4
heat of fusion [mJ/mg]	57.5	54.3	71.6

Table 3. **Solubility of LAB687 polymorphs in common solvents at 25°C**

solvent	Form C	Form A	Form D
water	0.0012	0.0011	0.0009
heptane	0.64	0.59	0.41
ethanol/water (1:1)	1.93	1.8	1.4
methanol/water (2:1)	8.9	7.8	6.8
ethanol/water (2:1)	22.6	21.3	11.2
methyl *tert*-butyl ether	32.4	17.8	15.6
toluene	33.5	26.4	15.4

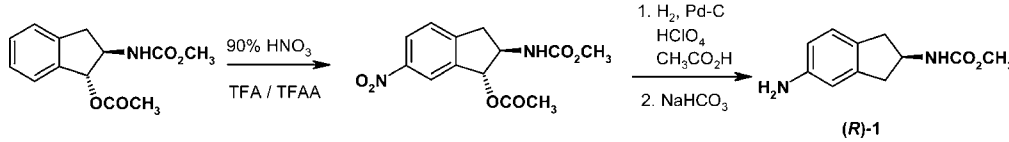

Figure 2. **Early synthesis route for key intermediate (R)-1.**

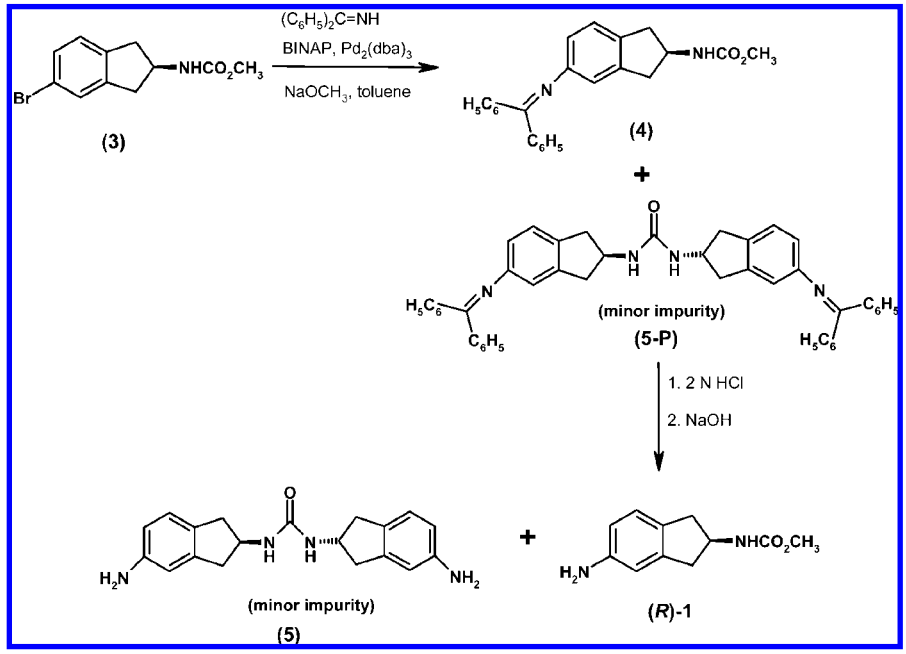

Figure 3. **Phase I synthesis route for key intermediate (R)-1.**

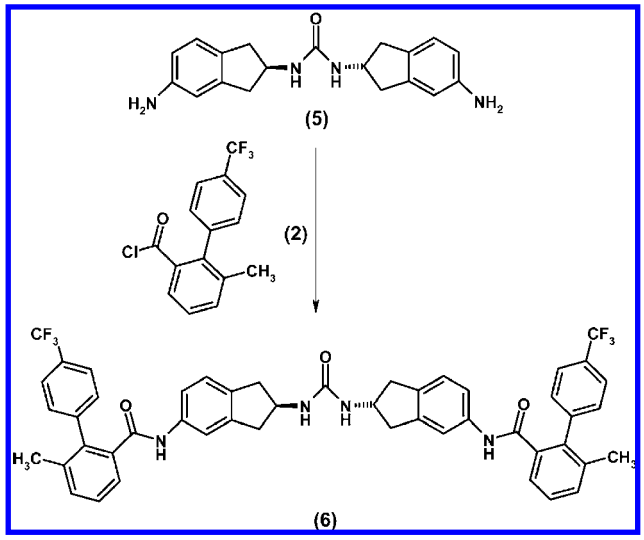

Figure 4. **Molecular structure of the new dimeric impurity (6) and its origin.**

During these development activities, two more solvates (heptane and methylcyclohexane) were also identified. Interestingly, after the point of the discovery of Forms A and C, Form B could no longer be produced.

Table 4. **Results of dynamic vapor absorption studies of LAB687 polymorphs**

RH%	Form A	Form C	Form D
45	0.04	0.49	0.03
65	0.06	0.68	0.08
90	0.16	0.88	0.17

Additional polymorphic studies were carried out to investigate the thermodynamic relationship between Forms A and C and to facilitate the form selection. Though the chemical and physical stabilities and the intrinsic solubility of Forms A and C are very similar, the trigonal Form C with superior filterability and flowability was selected for further development. However, when the Form C crystallization process was scaled up to multikilogram scale in the pilot plant, a new polymorph, Form D, was produced. Since the emergence of Form D, those seeded crystallization processes that consistently produced Forms A and C started to produce predominately Form D in the laboratory. Shortly after the pilot plant campaign during which one multikilogram batch of Form D was produced, LAB687 was terminated due to toxicity concerns.

Clearly, the conclusions from the polymorphic investigations that were performed early in the laboratory did not predict the existence of the most stable Form D. We hypothesize that the

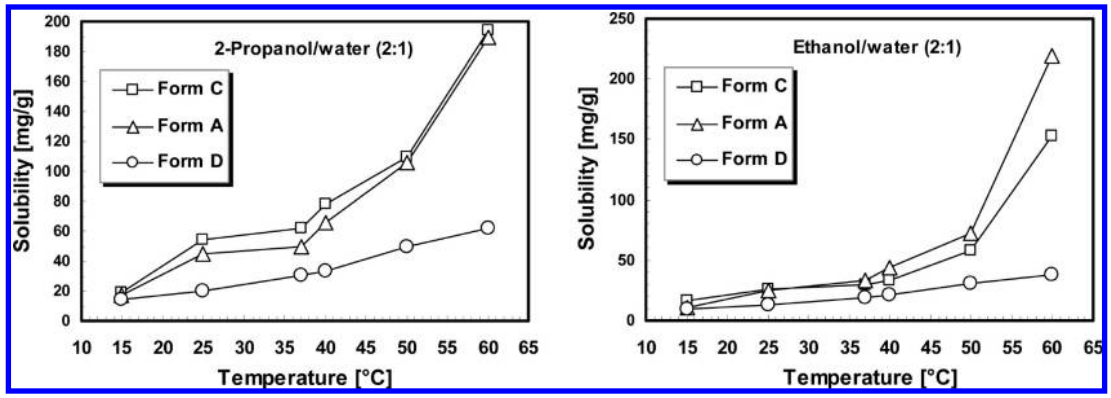

Figure 5. **Solubility of Forms A, C, and D in 2-propanol/water (2:1) and ethanol/water (2:1).**

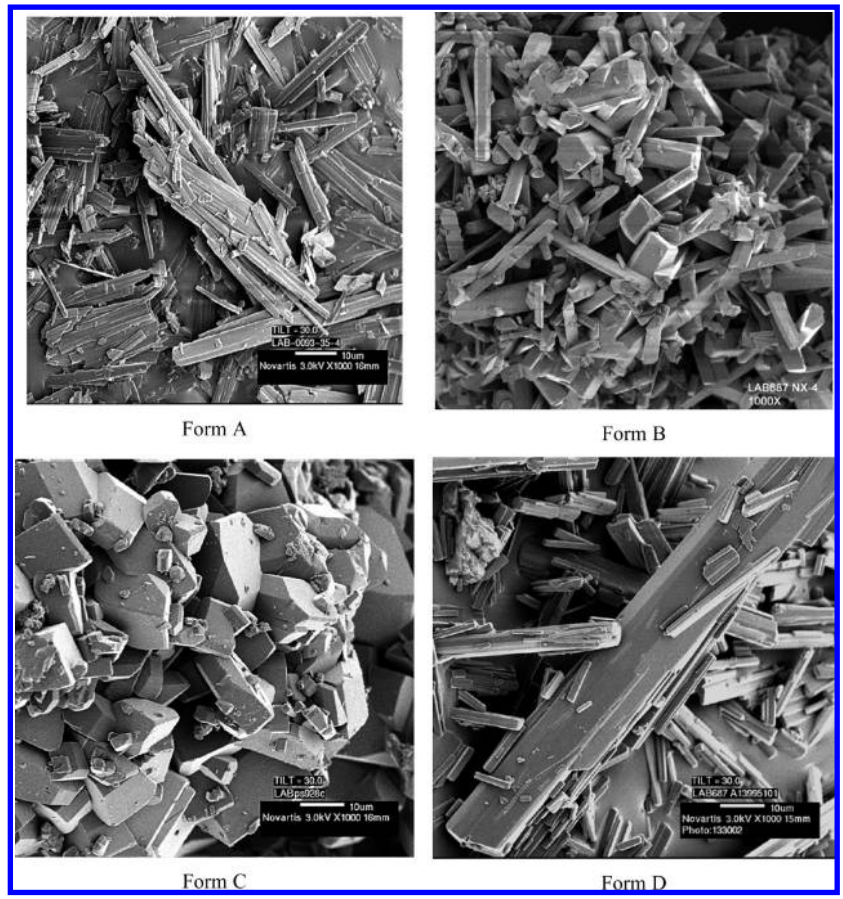

Figure 6. **Scanning electron microscopic images of LAB687 polymorphs.**

appearance of Form D could be attributed to the lack of certain impurities due to the changes in the synthesis route, or presence of the new dimeric urea impurity (**6**) unique to the phase I route.

Characterization of LAB687 Polymorphs. LAB687 polymorphs were characterized using several established techniques. Data on Form B are limited due to insufficient material.

Thermal Properties. Thermal properties of the three forms were determined by differential scanning calorimetry (DSC). The results are listed in Table 2. Form D exhibits the highest melting point (157.4 °C), and heat of fusion (71.6 mJ/mg). The melting point and heat of fusion of Form A are significantly greater than those of Form C. According to heat of fusion rule,[20] these thermal data suggest that Form D is the thermodynamically most stable form and is monotropic with respect to Forms A and C.

Solubility of Forms A, C, and D. A comparison of the equilibrium solubility of Forms A, C, and D in common organic solvents at room temperature (Table 3) shows that Form D is the least soluble polymorph. The solubility profiles of these polymorphs in alcohol/water (Figure 5) also indicate that Form D is significantly less soluble than Forms A and C over a wide range of temperatures. The solubility of Forms A and C on the other hand are relatively similar. In ethanol/water system, the solubility curve of Form A seems to intercept with that of Form C suggesting an enantiotropic relationship. However, the trend was not observed in the 2-propanol/water system. Given the small difference in their solubilities, the relationship between Forms A and C is not conclusive.

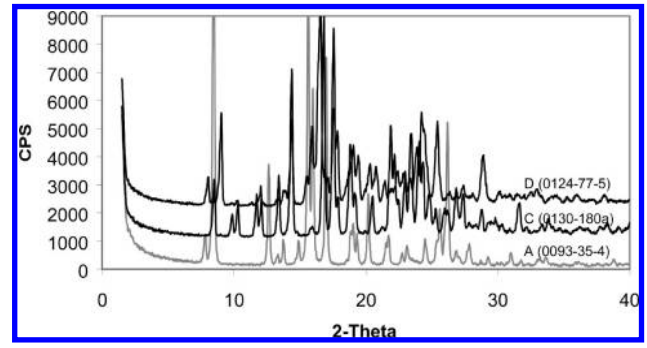

Figure 7. **Powder X-ray diffraction patterns of the LAB687 polymorphic forms.**

Hygroscopicity. Dynamic vapor absorption studies show that the LAB687 polymorphs are not hygroscopic. At room temperature, Forms A and D pick up less than 0.2% of water at 90% RH, while Form C is able to uptake 0.88% of water (Table 4.).

Crystal Morphology. As shown in Figure 6, the crystal morphology of Form A is blade-like, Form B is acicular, Form C is trigonal, and Form D is columnar. As shown in Figure 7, the polymorphic forms have distinctively different powder X-ray diffraction patterns.

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Table 5. **Phase equilibration of mixtures of Forms D/A and Forms D/C**

	D/A mixture			D/C mixture		
solvent	5 °C	25 °C	50 °C	5 °C	25 °C	50 °C
heptane	D/A	D/A	–	C/D	C/D	–
methyl *tert*-butyl ether	D/A	D	–	D	D	–
toluene	D	D	D	D	D	D
water	D/A	D/A	D	C/D	C/D	C/D
ethanol/water (1:1)	D	D	D	D	D	D

Thermodynamic Relationships. Solubility data and thermal properties of these polymorphs indicate that Form D is the thermodynamically most stable form and is monotropic to Forms A and C. This finding is further supported by a series of equilibration experiments (Table 5) during which the mixtures of Forms D/C and D/A were slurried in common solvents at various temperatures for at least 24 h. Enrichment of Form D was seen in all experiments. Previous equilibration experiments of mixtures of Forms A and C indicate that they are equally stable; however, when these experiments were repeated after the discovery of Form D, the mixtures of Forms A and C converted to Form D instead.

Experimental Section

The typical processes to generate Forms A and C are described here. However, these processes are of historical significance only. Once Form D was obtained, they no longer produced the intended polymorph.

Form A Process. Charge 4 g of LAB687 to a crystallizer, then add 25 mL of 2-propanol. Heat the mixture to 50 °C to yield a clear solution. Filter the solution and charge it to a clean crystallizer. Cool the solution to 10 °C rapidly and add 10 mg of Form A seeds. Stir for 2 h at 10 °C and cool to 3 °C over ∼15 min. Add 25 mL of distilled water over ∼30 min. Stir for 2 h at 3 °C. Isolate the solid by filtration and wash the cake twice with 10 mL of 2-propanol/water (1:1 v/v). Dry the cake in an oven at ∼75 °C, 35 mmHg for at least 16 h to obtain 3.4 g of Form A.

Form C Process. Charge 30.0 g of LAB687 to a crystallizer, then add 217.5 g of methanol and stir at room temperature until a clear solution is obtained. Filter the solution and charge it to a clean crystallizer. Heat the solution to ∼50 °C. Add 100 g of distilled water over ∼30 min with vigorous agitation while maintaining the temperature at 45 °C. Cool the solution to 40 °C and add 30.0 mg of Form C. Stir the mixture for 15 min. Cool the mixture to ∼33 °C over ∼1 h and stir at 33 °C for 1 h. Add 100 g of distilled water over ∼40 min. Stir for 2 h at 30 °C. Isolate the solid by filtration and wash the cake twice with 60 mL of methanol/water (3:2 v/v). Dry the cake in an oven at ∼75 °C, 35 mmHg for at least 16 h to obtain 28 g of Form C.

Prior to the discovery of Form D, the above processes were developed to generate Forms A and C, respectively. However, once Form D was introduced to the laboratory, these processes no longer produced Forms A or C, but primarily Form D.

Summary

The development of LAB687 demonstrates that dealing with polymorphs is an unpredictable process. Although we have carried out extensive research early in the development to screen potential polymorphs, Mother Nature surprised us upon scale-up. In reflection, once the final route of synthesis has been chosen, additional polymorph screening studies may be carried out to seek the potential new polymorphs due to the changes in the impurity profile of the drug substance. Nevertheless, considering the precarious nature of the drug development, process chemists should always be prepared for surprises.

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