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Application of the Quality by Design Principles for the Development of the Crystallization Process for a Piperazinyl-Quinoline and Definition of the Control Strategy for Form 1

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Supporting Information

ABSTRACT: The studies carried out to develop a robust crystallization method for the substituted piperazinyl-quinoline (**1**) a compound potentially active in the treatment of depression, are described in this contribution. These studies include the control of a solvate that could have potentially formed in the crystallization process. The principles of quality by design (QbD) were applied to generate the process understanding and to define the control strategy for the control of the formation of the solvate during the crystallization. The application of process analytical technology (PAT) tools was key in achieving the desired process control.

1. INTRODUCTION

Depression is a chronic, recurring, and potentially life-threatening illness that affects many people worldwide (up to 20% of the population).[1]

Selective serotonin reuptake inhibitors (SSRIs) have a widespread utility in the treatment of depression and other mental illnesses, but their therapeutic use showed latency in the onset of clinically meaningful effects. It was identified that approaches based on inhibition of serotonin reuptake in conjunction with antagonism of 5-HT_1 (5-hydroxytryptamine) autoreceptors offer advantages over the current antidepressants in terms of a faster onset of therapeutic effect and improved efficacy.[2] 1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone, **1**, is a presynaptic inhibitor 5-HT_1 receptor antagonist selected for the cure of depression and anxiety that reached early phase II trials.[3]

The studies carried out to develop a robust crystallization method for the drug substance **1** are described in this contribution. These studies include the control of a solvate with *n*-propanol (*n*-propanolate) that could have potentially formed in the crystallization process. The quality by design (QbD) principles were applied to generate the process understanding and to define the control strategy for the control of the formation of the *n*-propanolate during the crystallization.[4] The application of process analytical technology (PAT) tools was the key to achieve the desired process control and to assess the amount of *n*-propanolate in the drug substance **1** during the crystallization, enabling a flexible control strategy for the control of the *n*-propanolate.

Regarding the QbD principles, they are described in a number of regulatory guidelines (ICHQ8,[5a] ICHQ9,[5b] and ICHQ10[5c]). These guidelines are focused on different aspects of QbD. For example, ICH Q8[5a] describes an enhanced approach by the use of process understanding. ICH Q9[5b] describes the risk management tools that can be used to successfully manage the risk, and ICH Q10[5c] introduces the concept of a control strategy, defined as a set of controls derived from current product and process understanding, that assures process performance and obtain drug substance that meets the critical quality attributes (the measurable properties that are critical to ensuring patient safety and efficacy).

The development of a robust control strategy supported by process understanding and by the use of the appropriate risk assessment tools is therefore key to ensuring that the quality of the drug substance or drug product is appropriate and consistent.

Regulatory agencies[6] fully support this approach and encourage its adoption during the development of drug substance and drug product manufacturing processes. More details on this approach have been recently reported in a previous contribution,[7] where a detailed description of the elements of control (the attributes of the input materials, the process parameters, and the procedure) is also given.[8]

2. SYNTHETIC PROCESS TO THE DRUG SUBSTANCE 1

The synthetic process to the drug substance **1** was introduced in a previous contribution.[9] In Scheme 1 the final synthetic process is reported, Stage 3 shows the crystallization process. The activities to optimise Stage 3 are summarized in this contribution. The drug substance **1** is obtained from the intermediate grade **1** (**IG-1**), which is obtained after a nucleophilic substitution starting from the compounds **2** and **3**. Compound **3** comes from the alcohol **4** after a mesylation step (Step 1). The form in development for the final active pharmaceutical ingredient (API) is defined as anhydrous **Form 1**.

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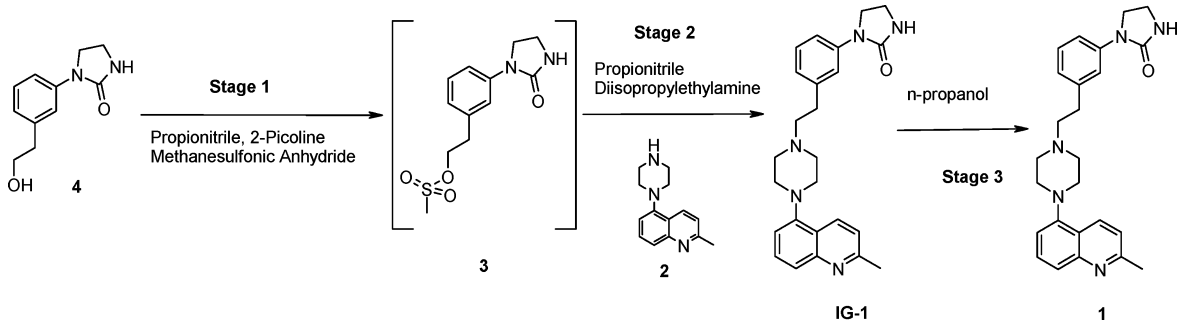
Scheme 1



3. STAGE 3: SOLVENT SCREENING

Solvent selection is a key step in the design of a crystallization process to get the drug substance characterized by suitable recovery, purity, crystal form, and appropriate physical properties.

The first step of a good solvent selection is the drug substance solubility investigation in different solvents aiming to identify, by first intent, a solvent to be employed for a seeded cooling crystallization. The definition of a single-solvent, seeded, cooling crystallization process is a highly desirable outcome to give a process which is easy to control and scale up. Most of the investigated systems were selected considering class 3 solvents[10] in order to minimize the risks for human health associated with potential residual solvents in the final drug substance.

The drug substance **1** solubility was measured in the solvents reported in Table 1, different temperature points were evaluated

Table 1. Drug substance 1 solubility

	solubility (mg/mL) at different temperatures				
solvent	20 °C	50 °C	60 °C	70 °C	80 °C
acetone	4	9			
MEK	5		10		
MIBK	2		6		
anisole	8		29		
cumene	0				3
n-propanol	8		42		
2-propanol	3			14	
isoamyl alcohol	6		30		
n-butanol	7		37		
DMSO	21		62		
i-PrOAc	1		2		
THF	17		40		
MTBE	1	1			

in order to identify the solubility dependence with temperature. The solubility data were generated through concentration measurement by HPLC[11] in the different solvents saturated with the API at different temperatures.

From the screening carried out it was clear that the drug substance **1** was characterized by low solubility in most solvents. *n*-Propanol and *n*-butanol[12] seemed to be the most promising solvents for developing a robust crystallization step considering the low solubility at 20 °C and the good dependence of solubility with temperature. Anisole could be considered another interesting option but it was discarded after some crystallization trials: a small amount of anisole (about 1% w/w) was present in the dried solids and it was not possible to remove it even applying extensive washing with a different solvent after filtration and before drying. The residual solvent and the risk of its bad smell in the final API were not considered appropriate and the attention was focused on *n*-propanol and *n*-butanol. *n*-Propanol was finally preferred over *n*-butanol due to its lower viscosity (that could have impacted the filtration step) and lower boiling point (that could have impacted the drying step).

4. DEFINITION OF THE CRYSTALLIZATION METHOD

Development activities started with the definition of a complete solubility curve and metastable zone width data (MSZW) in *n*-propanol. The turbidity method was used for the definition of the MSZW; the turbidity signal was acquired while heating and cooling very slowly HPLC vials containing a known amount of API and *n*-propanol. The change in the turbidity signal during the heating and cooling ramps could be associated with the dissolution and the precipitation of the active ingredient (see Figure 1).

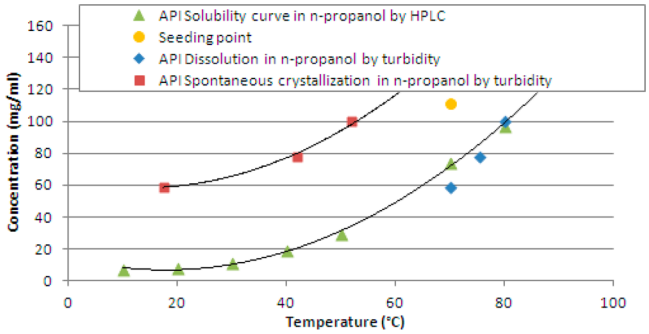


Figure 1. Solubility curve and metastable zone width for drug substance **1** in *n*-propanol.

Considering the obtained data, a crystallization process was designed as follows:

- The IG material was suspended in 15 volumes of *n*-propanol and heated up to 85 °C. The solution was clarified and distilled to 9 volumes. This step was introduced in the process in order to easily clarify the solution at 85 °C without risk of precipitation and to minimize the possible amount of water in the solution taking advantage of the distillation step. This was a key point in order to minimize the risk of formation of the monohydrate. It was already known from previous experiments that the precipitation of the monohydrate did not occur when the water present in the solution was limited to 0.5% w/w. The distillation step was easily able to control the water content to this level.[9]
- A seeding point of 70 °C was selected, and the risk of self-nucleation was assessed by checking the stability of the

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supersaturated solution on different scale. Self-nucleation depends on several factors such as reactor geometry, mixing properties, and the presence of foreign particles; therefore, it is difficult to mimic. However, self-nucleation was never observed on a small scale and during the scale-up work. During the scale-up runs and the pilot-plant campaigns the crystallization step was always FBRM monitored and self-nucleation before seeding was never highlighted, therefore the risk of precipitation induced by self-nucleation was considered very low.

- An amount of 0.025 wt of seed was added at seeding temperature (70 °C), the effect of the seed amount was not submitted to further studies in this project phase.
- According to the procedure, after the crystallization, the obtained solid was isolated at 20 °C and washed according to the procedure reported in the Experimental Procedures.
  - First, wash with 2 volumes of *n*-propanol in order to remove effectively the impurities present in the mother liquor.
  - Second, wash with 2 volumes of a *n*-propanol/isooctane 1/1 mixture.
  - Third and last, wash with 2 volumes of isooctane.

Isooctane was selected as the solvent for final washing to displace *n*-propanol from the cake before drying, giving the low API solubility.

- The solid obtained was finally dried under vacuum (20−50 mm/hg) at 50−55 °C for 10−15 h.

The described crystallization method (Figure 2) was successfully employed to prepare the batches needed for development,

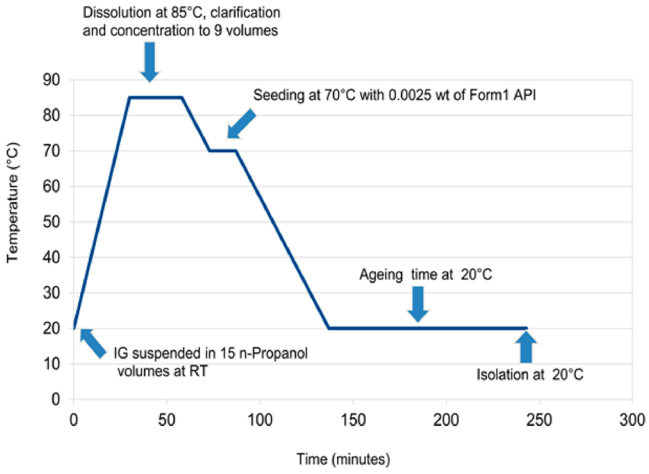

Figure 2. Final crystallization process.

and it was successfully scaled up from laboratory to pilot plant. The selected anhydrous **Form 1** was always obtained, and the process was able to deliver a yield higher than 85% w/w and very good purity of the final drug substance.

5. IDENTIFICATION AND PREPARATION OF THE NEW SOLVATE

In order to get more data on the identified crystallization system, an extensive polymorph investigation taking into account *n*-propanol was carried out[13] and an *n*-propanol solvate was identified. The solvate was obtained by temperature cycling experiments of drug substance slurries in *n*-propanol (temperature range 0−40 °C, slurring time 48−60 h) as well as by rapid cooling of a solution prepared at room temperature.[13]

The pure *n*-propanolate was then obtained from a slurry of drug substance **1** in *n*-propanol stored for more than one year at 0 °C. It was clear that in view of the next pilot plant campaigns, the risk associated with *n*-propanolate formation needed a deeper understanding as the solvate could potentially contaminate the drug substance, therefore the *n*-propanolate content in the drug substance **1** was defined as a drug substance critical quality attribute (drug substance-CQA). This decision was taken considering that the drug substance would have been formulated as a tablet and after having carried out a risk assessment exercise where the failure mode and effect analysis (FMEA)[5b] tool was used. The risk that the presence of the *n*-propanolate in the drug substance **1** could impact the drug product performance and ultimately the patient safety (no information on the effect of the clinical performance of the drug product in the presence of the *n*-propanolate were available) was considered too high, therefore the amount of the *n*-propanolate in the drug substance had to be controlled.

Some attempts to prepare pure *n*-propanolate in larger quantities to support more experimental studies were carried out, the aim of which was to start some studies to understand the temperature range where the solvate was more stable with respect to the anhydrous **Form 1** in the crystallization environment.

The first attempt was to apply the current crystallization procedure using pure *n*-propanolate as a seed (3% w/w loading), but the obtained drug substance was pure **Form 1**.

This experiment was important because it showed that the risk of formation of *n*-propanolate in the current crystallization was quite low. This aspect was also confirmed by the batches of drug substance prepared for preclinical and clinical uses where only **Form 1** had always been seen. Moreover, to further reduce this low risk of *n*-propanolate formation, **Form 1** was selected to be used as a seed, to ensure the control of this process step.

Even if the risk of formation of the *n*-propanolate was considered to be low, it was decided to improve the understanding of the crystallization by using an *online* (PAT) technique that could monitor the process in real time. Real-time data could help to explain if a phase transformation was occurring during the process. At first Raman was considered as a possible PAT technique. The solid phases were checked, and some differences in the spectra that could be used to discriminate **Form 1** from the *n*-propanolate were highlighted. The online monitoring technique was applied, without any quantification, to follow the crystallization trials carried out to produce the *n*-propanol solvate.

Considering the available data, the *n*-propanolate had only been obtained at low temperatures, and we supposed that in these conditions it could be more stable. On this basis, a new experiment was set up, lowering the seeding temperature down to 40 °C and enhancing the solvent volumes from 8 to 20 in order to avoid self-nucleation before the seeding temperature. The process was followed by Raman monitoring. As can be observed in Figure 3 and Figure 4, it was clear that the *n*-propanolate was obtained during the crystallization process; the spectra recorded after seed addition showed the formation of *n*-propanolate highlighted by the shoulder growing at 1355 cm^{-1}. The solid was filtered and dried under vacuum at room temperature; XRPD analysis showed it was pure *n*-propanolate.

The *n*-propanolate crystallization method was repeated several times on a 4-g scale, always leading to 100% *n*-propanolate pure form (by XRPD).

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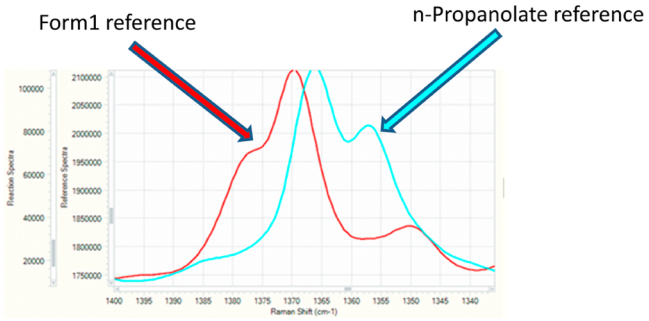

Figure 3. Formation of the *n*-propanolate.

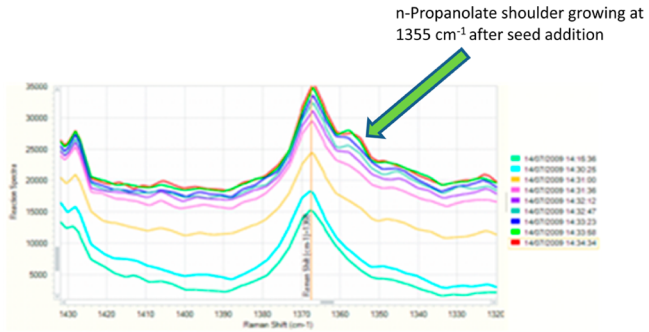

Figure 4. Formation of the *n*-propanolate.

6. CHARACTERISATION OF THE NEW SOLVATE

The *n*-propanolate solid-state form was characterised with the help of different techniques such as XRPD, differential scanning calorimetry, and thermogravimetric analysis.

The solid appeared to be highly crystalline by XRPD (Figure 5) with a desolvation onset temperature at around 70 °C as shown in

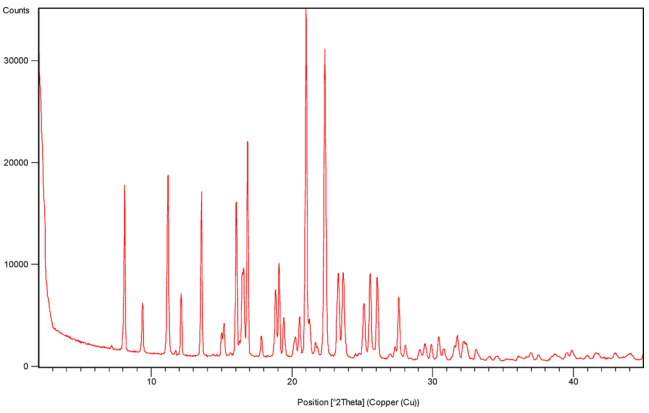

Figure 5. XRPD trace of the *n*-propanol solvate form.

the DSC/TGA traces (Figure 6). Once desolvated the material recrystallises into a mixture of unsolvated phases of which one is **Form 1**.

From TGA weight loss calculation it appears that the *n*-propanolate is not a stoichiometric solvate.

A quantification method to eventually define the amount of *n*-propanolate form present in a bulk material of **Form 1** was required.

Therefore, a reliable quantification procedure for the mixture of these two solid forms was developed using X-ray powder diffraction.

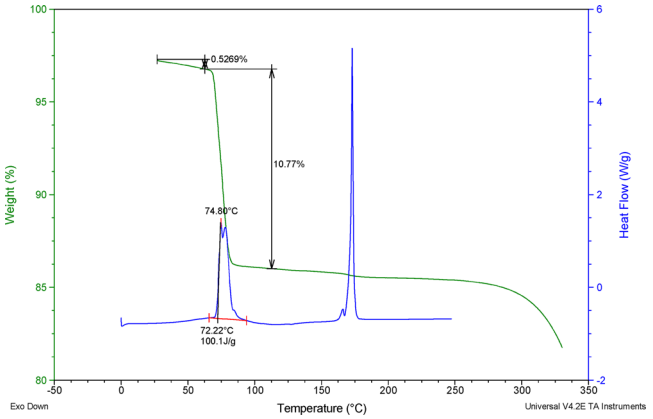

Figure 6. DSC (blue line) and TGA (green line) traces of a batch of *n*-propanolate form.

Quantitative analysis of pharmaceutical solids by XRPD has been well described in the literature by employing uni- or bivariate approaches based on either the heights or areas of a single or several characteristic peaks.[14−16]

Pure batches of **Form 1** and of *n*-propanolate were used as input materials. To prepare solid mixtures with uniform particle size, the raw materials of **Form 1** and *n*-propanolate were lightly ground in a mortar. However, after the grinding process, a partial amorphisation of the *n*-propanolate was found by XRPD (see Figure 7).

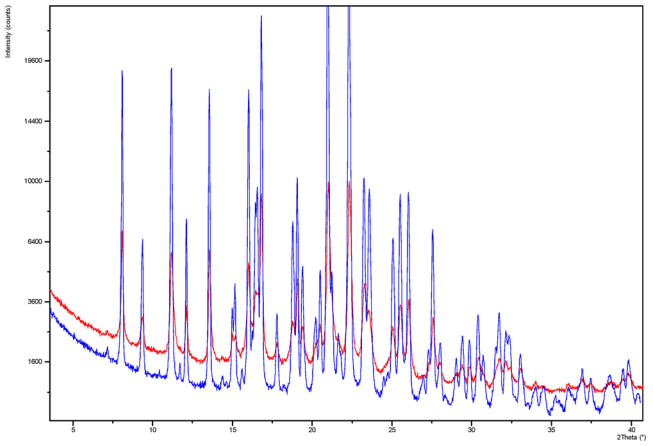

Figure 7. XRPD traces of *n*-propanolate before (blue trace) and after (red trace) the grinding process with mortar and pestle.

For this reason binary mixtures of **Form 1** and *n*-propanolate at appropriate ratios were prepared using gentle manual mixing with a spatula.

The peak at 8.10° 2θ is specific peak for the *n*-propanolate form, while the peak at 14.5° 2θ is of **Form 1** (see Figures 8 and 9).

The nominal % w/w concentration of *n*-propanolate prepared in binary mixtures with **Form 1** were the following: 2.5, 5, 10, 25, 50, 70, 100% w/w. The calibration samples were analysed in duplicates by XRPD in transmission mode to avoid sample orientation. The *n*-propanolate solid was quantified by calculating both the height and the area of the characteristic peak of the *n*-propanolate at 8.10° 2θ. The integration of the peaks was carried out using the X'pert HighScore software. Table 2 reports the calculated data for each of the binary mixtures analysed.

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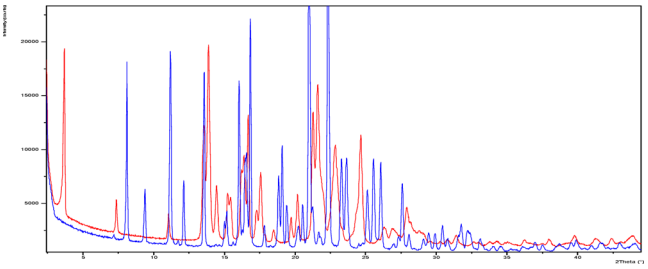

Figure 8. XRPD overlays of *n*-propanolate (blue trace) and **Form 1** (red trace). Highlighted are the distinctive peaks of the *n*-propanolate (8.10° 2θ) and **Form 1** (14.46° 2θ).

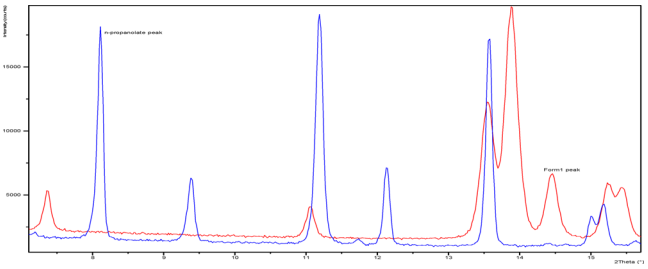

Figure 9. XRPD overlays of *n*-propanolate (blue trace) and **Form 1** (red trace). Highlighted are the distinctive peaks of the *n*-propanolate (8.10° 2θ) and of **Form 1** (14.46° 2θ).

Table 2. Calculated height and area of *n*-propanolate peak at 8.10° 2θ in the prepared *n*-propanolate/Form 1 mixture

nominal w/w % *n*-propanolate in **Form 1**	actual w/w % *n*-propanolate in **Form 1**	*n*-propanolate peak height counts (8.10° 2θ)	*n*-propanolate peak area (8.10° 2θ)
2.5	2.4	188.28	23.30
2.5	2.4	207.08	30.15
5	5.5	421.51	52.18
5	5.5	380.46	45.38
10	9.9	888.82	92.23
10	9.9	830.28	98.11
25	24.8	1867.08	213.66
25	24.8	1967.09	222.25
50	50.3	3769.47	373.21
50	50.3	3693.00	429.00
66	66.7	4930.12	567.42
66	66.7	5004.06	541.88
100	100.0	7013.50	774.35
100	100.0	6999.09	780.86

Figures 10 and 11 show the linearity plots calculated using the *n*-propanolate distinctive peak height and the peak area, respectively.

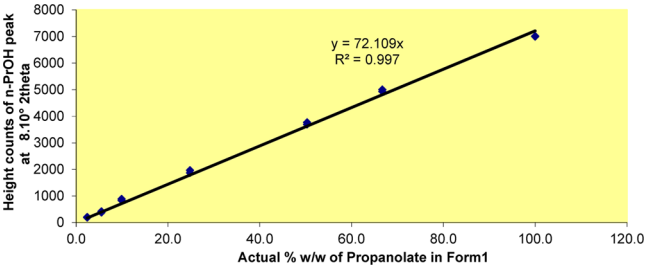

Figure 10. Linearity plot calculated using *n*-propanolate 8.10° 2θ peak height.

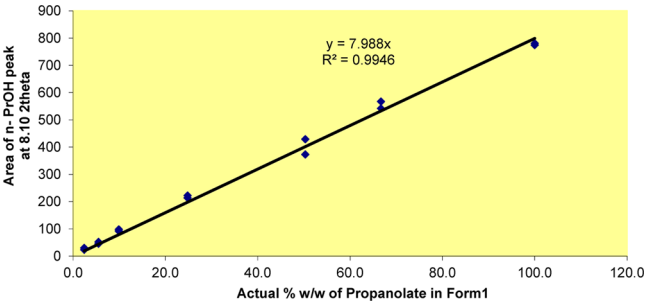

Figure 11. Linearity plot calculated using *n*-propanolate 8.10° 2θ peak area.

The linearity curve achieved with both the height and the area of the characteristic peak of the *n*-propanolate form was good enough for the purposes of quantifying the low amount of *n*-propanolate in the **Form 1** bulk sample. The limit of detection by XRPD of the *n*-propanol solvate in binary mixtures with **Form 1** was not determined.

7. STUDIES FOR THE DEFINITION OF THE CONTROL STRATEGY FOR THE CONTROL OF THE *N*-PROPANOLATE

In this section, the studies for the definition of the control strategy for the control of the *n*-propanolate in the drug substance **1** are described. These studies allowed the definition of the different elements of control (categorized by procedures, parameters, and attributes).[8] Pure *n*-propanolate and pure **Form 1** were employed to carry out slurry ripening experiments in *n*-propanol at different temperatures in order to understand the temperature range where the solvate was stable and to have a better understanding of the risks associated with the current process.

The competitive slurry experiments were carried out starting from pure **Form 1** and pure *n*-propanolate in 50/50 wt/wt mixture. The two components were suspended in dry toluene presaturated with **Form 1**. Toluene was selected as a solvent in order to avoid water contamination and consequently to minimize the risk of hydrate formation. The objective of the study was to highlight the thermodynamic stability of the propanolate; no kinetic impacting factors (i.e., particle size, mixing rate) were taken into account.

The results showed that, from 20 °C (isolation temperature) to 40 °C, **Form 1** converted to *n*-propanolate, whereas from 40 °C to a higher temperature, the *n*-propanolate converted to **Form 1**. At 40 °C 35 days were needed to obtain a complete conversion from the solvate to **Form 1**. The samples analyzed after 1, 3, 7, 15, and 22 days were *n*-propanolate and **Form 1** mixtures where the solvate was decreasing and **Form 1** was becoming the principal component. At 50 °C in just 24 h the solvate had completely converted to the nonsolvate form. As a result of the experiments carried out, it was clear that the highest risk for the process to give the *n*-propanolate was from 40 °C to the isolation temperature (see Figure 12). On the basis of these observations, a risk assessment was carried out, and the seeding temperature (70 °C), the cooling time (from 70 to 20 °C), the ageing time, and the filtration temperature (20 °C) were defined as critical process parameters. Due to the initial phase of the project, the studies for the selection of the PARs were postponed to a later phase.

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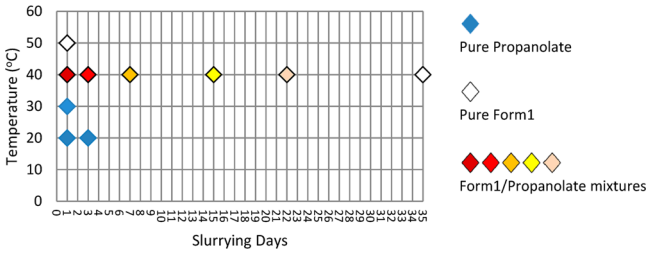

Figure 12. Slurry of **Form 1** and *n*-propanolate at different temperatures.

Some studies were also carried out to understand the amount of material that would have precipitated in the range 40−20 °C; this information would have allowed a better assessment of the risk of having *n*-propanolate in the final drug substance.

As can be observed in Figure 13, about 90% w/w of the drug substance is crystallized from the seeding point to 40 °C

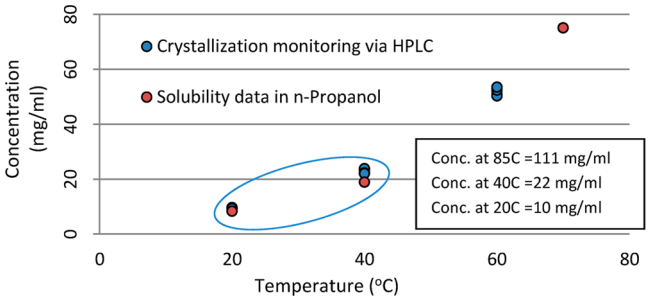

Figure 13. Amount of solid precipitated in the range 40−20 °C.

as **Form 1**, and the amount of drug substance **1** crystallized in the temperature range 40−20 °C is slightly higher than 10% w/w. Therefore, considering that in this range the *n*-propanolate was more stable than **Form 1**, the risk of slurry-to-slurry interconversion could not be eliminated. However, the data on the batches available, where only **Form 1** was present, confirmed that this risk was low.

By considering all the data collected on the *n*-propanolate and **Form 1**, it was possible to assess that the risk of contamination of the drug substance with the *n*-propanolate was low, and this was based on the following:

- *n*-Propanolate had never been identified either in the samples produced on a lab scale (10−15 batches) or in the pilot-plant batches.
- Even if the experiment was on a small scale, the kinetics of interconversion between the *n*-propanolate and **Form 1** was very slow. It required 35 days to have complete conversion at 40 °C (see Figure 12).
- The temperature range where the solvate is stable was associated with the precipitation of approximately 10% w/w of drug substance
- Once filtered, the drug substance was washed, and according to the procedure, *n*-propanol was displaced by the isooctane, lowering the risk of solvate formation during deliquoring and drying.

Among the approaches proposed by the Q9,[5b] the failure mode effect analysis (FMEA) tool was used to help in the assessment.

8. DEVELOPMENT OF AN NIR METHOD IN SUPPORT OF THE CONTROL STRATEGY

Even if the formation of the *n*-propanolate was considered a low risk in the optimized crystallization process, the detection of *n*-propanolate formation before isolation would be a key point for the definition of the control strategy to control the amount of *n*-propanolate in the drug substance. Therefore, the development of a method to quantify the *n*-propanolate in **Form 1** during the crystallization step was necessary. After some screening, the technique chosen to build a quantification model was NIR using a diffuse reflectance probe. NIR was chosen basically for two reasons:

- This is a common technique in a plant and for this reason is relatively easy to install in a reactor.
- This is a "safe" technique that does not require a particular risk assessment to install a probe in a reactor; whereas Raman, being a more energetic technique, might have introduced a higher safety risk.[17]

A preliminary test was performed on the solid state with the NIR equipment,[18] to understand the possibility to discriminate the *n*-propanolate from **Form 1**. Overlapping the spectra of *n*-propanolate form and **Form 1**, it is possible to see some differences in the profiles of the two compounds at 20 °C (see Figure 14). By processing the spectra by first derivative

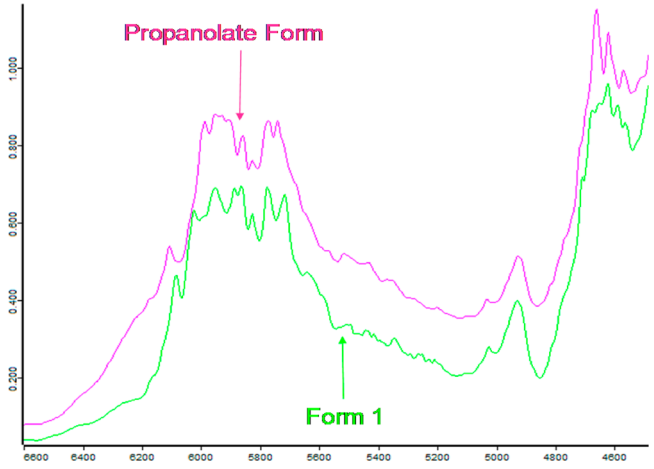

Figure 14. NIR spectra of Form 1 and the *n*-propanolate.

(smoothing points 9) and using the function of multiplicative scatter correction (MSC), these differences are more appreciable (see Figure 15).

This preliminary test was useful to understand that NIR, with a diffuse reflectance probe, was able to discriminate the *n*-propanolate and **Form 1** in the solid state. The same result was then confirmed when the two forms were in suspension (to mimic the real crystallization conditions). The spectral region from 4759.4 to 5420.3 cm^{-1} was then selected, and the spectra were processed by first derivative + MSC (multiplicative scatter correction) with a smoothing point of 9 (mean centering function active). This procedure allowed the definition of the method that was then applied to a series of suspensions containing the *n*-propanolate up to 15% w/w. This allowed setting the LOQ (limit of quantitation) for this method at 2% that was considered acceptable for this process development phase. The details related to the development of this model are available in the SI (see also the Conclusion).

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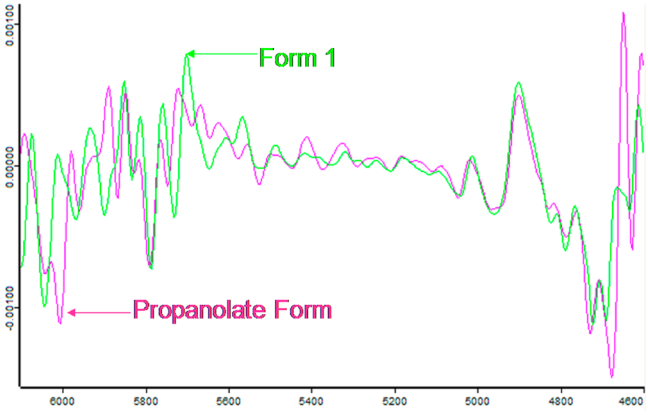

Figure 15. Image of the processed spectra reported in Figure 12.

9. CONTROL STRATEGY FOR THE CONTROL OF THE *N*-PROPANOLATE

The studies carried out in sections 7 and 8 gave all the information to build a robust control strategy. In particular, the NIR method developed would allow to control the process for the formation of **Form 1** in the crystallization of the drug substance **1**; if the presence of *n*-propanolate is detected at 20 °C before the filtration, the crystallization could be repeated by heating the slurry to 85 °C, dissolve the slurry, and start again with the seeded precipitation. Once the suspension is at 20 °C, a new check could be made. The iteration could be repeated until **Form 1** only is formed. Alternatively, it is recommended to operate the filtration step at 40 °C, a temperature where **Form 1** is more stable than the *n*-propanolate and only 10% of yield will be lost.

In summary, the studies carried out have allowed the definition of the different elements of control in any process step:

- at seeding, attribute (**Form 1** seed) and parametric (seed amount and seeding temperature) controls,
- during cooling from 70 to 20 °C, parametric (cooling time) control,
- after cooling to 20 °C, parametric (ageing time) and attribute control (NIR in process control),
- during filtration, by procedure (washing sequence) and parametric (filtration temperature).

Figure 16 summarizes the control strategy for the *n*-propanolate in the drug substance **1** crystallization process.

10. CONCLUSION

The studies carried out to develop a robust crystallization method for the drug substance **1** are described in this contribution. During the process development studies a solvate of *n*-propanol was identified and characterized. As a result, some studies were carried out to assess the risk of formation of the solvate in the crystallization. Process control was significantly improved when a PAT method (based on NIR) to control the amount of solvate in the drug substance during the crystallization was developed; this allowed a flexible control strategy and a mitigation plan in case of *n*-propanol solvate formation.

Nevertheless, a PAT method, based on NIR, for the control of the formation of the *n*-propanolate in solution was developed and validated; it is recommended that the control of the

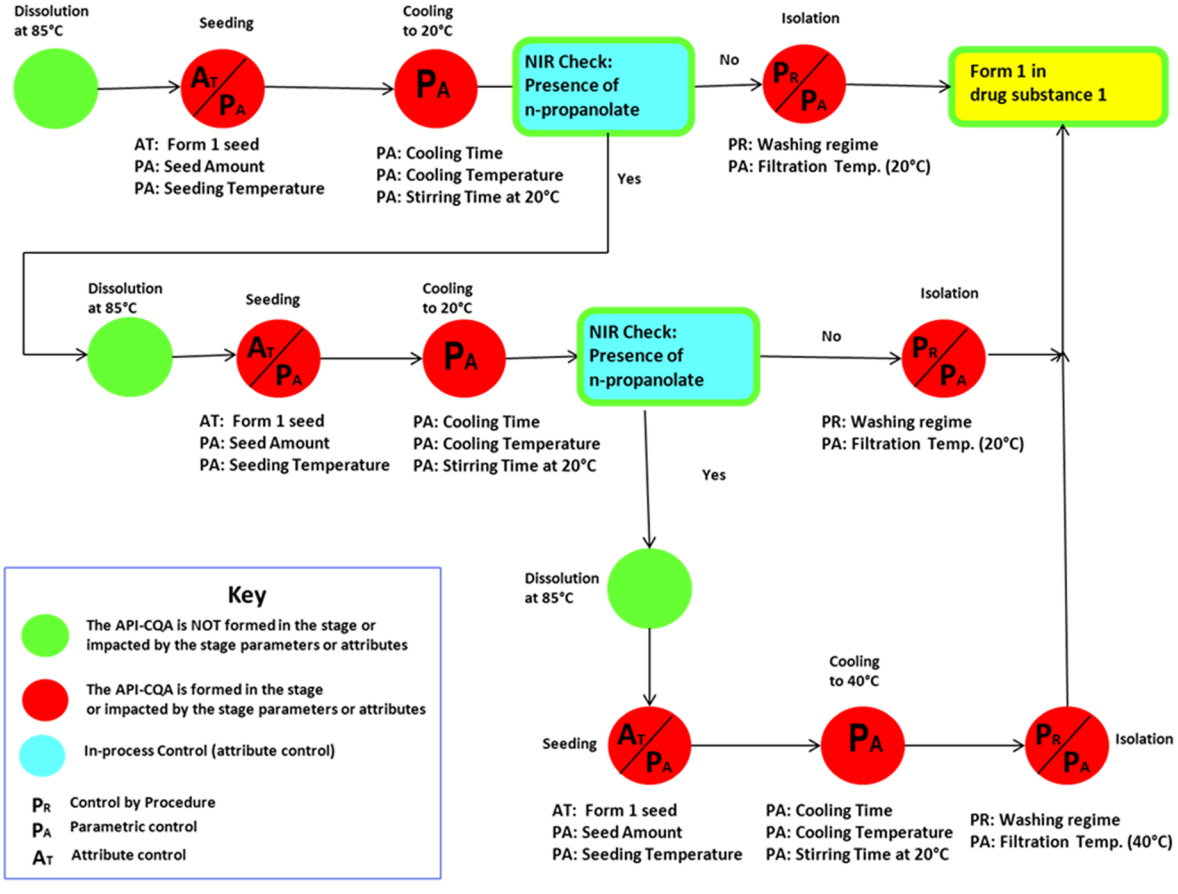

Figure 16. Control strategy for the *n*-propanolate solvate in the drug substance **1**.

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n-propanolate level in the drug substance is carried out as a routine test for the drug substance release. The discontinuation of this test is recommended only when a suitable set of data based on the development batches is available and when more biopharmaceutical and clinical data are available on the effect of the *n*-propanolate in the drug product performance.

12. EXPERIMENTAL PROCEDURES

Drug Substance 1 (Form 1) Preparation. **IG-1** (1 wt) is dissolved in 1-propanol (15 vol) by heating to 85 °C; then it is passed through a clarification line-filter (5 μm). The line-filter is washed with hot 1-propanol (2 vol). The collected filtrates are concentrated to 9 vol by distillation at atmospheric pressure. The resulting solution is then cooled to 70 °C (internal temperature) and seeded with **1** (0.0025 wt). The suspension is cooled down to 20 °C (±2 °C) (internal temperature) over approximately 1 h (1 °C/min ramp). The slurry is stirred for 2 h at 20 °C (±2 °C) (internal temperature), and then the solid is collected by filtration. The cake is washed successively with 1-propanol (2 vol), 1-propanol/isooctane 1:1 (2 vol), and isooctane (2 vol). All the washes are carried out at 20 °C (±2 °C). The damp cake is dried in a vacuum oven at 50−55 °C overnight (yield about 85% w/w).

^{1}H NMR (600 MHz, DMSO-d_6) δ 2.64 (m, 2 H), 2.63 (s, 3 H), 2.74 (br, s, 4 H), 2.78 (m, 2 H), 3.04 (br s, 4 H), 3.39 (m, 2 H), 3.84 (dd, J = 8.94, 7.01 Hz, 2 H), 6.89 (d, J = 7.70 Hz, 1 H), 6.91 (m, 1 H), 7.11 (dd, J = 6.60, 1.92 Hz, 1 H), 7.21 (t, J = 7.97 Hz, 1 H), 7.39 (m, 2 H), 7.46 (t, J = 1.65 Hz, 1 H), 7.59 (m, 2 H), 8.34 (d, J = 8.80 Hz, 1 H). *m/z;* 416

Drug Substance 1 (*n*-Propanolate) Preparation. *Procedure 1.* A sealed and saturated solution of drug substance **1** in *n*-propanol is aged at −20 °C for about 10 days. The solution of drug substance **1** crystallizes as *n*-propanol solvate.

Procedure 2. A microfluidized solution of drug substance **1** (**Form 1**) is left ageing at 4 °C for about 1 year. After the ageing period the drug substance **1** is isolated as an *n*-propanol solvate.

Procedure 3. Drug substance **1** (**Form 1**, 1 wt) is dissolved in *n*-propanol (20 vol) at 70 °C. The resulting solution is then cooled to 40 °C (internal temperature) and seeded with drug substance **1** (*n*-propanolate, 0.03 wt). The suspension is cooled down to an internal temperature of 10 °C (±2 °C) over approximately 20 min. The slurry is stirred for 3 h at 10 °C (±2 °C), and then the solid is collected by filtration. The cake is washed twice with 2 vol of *n*-propanol at room temperature. The wet solid is dried in a vacuum oven at room temperature.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Stability data mentioned in section 8, Development of an NIR Method in Support of the Control Strategy. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(4) It is worth noting that this work is related to a process developed to support the initial toxicology and clinical requirements, and therefore, it might not be seen as ready for technical transfer or regulatory submission. What is stressed here is the help that the application of the QbD principles has given to achieve process understanding and control and to define a robust control strategy for the solvate.

(5) (a) *ICH Q8 Pharmaceutical Development*, (R2); U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, Aug 2009. (b) *ICH Q9 Quality Risk Management*; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, June 2006. (c) *ICH Q10 Pharmaceutical Quality System*; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, April 2009.

(6) See for example U.S. Food and Drug Administration *Pharmaceutical cGMPs for the 21st Century − A Risk Based Approach* (initiative launched in 2002); U.S. Food and Drug Administration, Silver Spring, MD, 2003; http://www.fda.gov/drugs/developmentapprovalprocess/manufacturing/questionsandanswersoncurrentgoodmanufacturingpracticescgmpfordrugs/ucm071836 (accessed Sept 18, 2012).

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(8) Each of the elements of control can be categorised into three control *modes*, as follows: (i) attribute controls, which include in-process controls (IPCs), and specifications for starting materials, intermediates, solvents, and drug substance (ii) parametric controls, which involve operation within proven acceptable ranges (PARs) for critical process parameters (CPPs) which are linked to CQAs, it is worth noting that, in this case the PARs were not defined due to the early phase of the project (iii) procedural controls, which describe operations linked to CQAs such as facilities setup, equipment configuration, order of addition, reagent, and solvent choice, sequence of events, etc.

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(11) The HPLC method used in the solubility screening is a generic HPLC gradient method with Phenomenex Luna C18 column. Mobile phases: A1 = TFA 0.05% vol/vol in water, B1 = TFA 0.05% vol/vol in acetonitrile. Gradient from 0% B1 to 95% B1 in 8 min. UV detection at 220 nm.

(12) Ethanol was initially considered, but it was decided not to test it as its water affinity (water activity) was considered an additional risk with respect to the formation of a monohydrate form; this aspect is discussed in the paper cited in reference 9.

(13) A polymorph study was carried out, after the selection of *n*-propanol as a crystallization solvent, on 48 solvents and different experiment types; the result of this study highlighted the possible existence of the *n*-propanolate, but it was decided to continue with the use of this solvent for the initial phases of development.

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(17) RAMAN technique uses a very energetic laser that could be generating a significant heating in the solution and this might have process safety implications
(18) To develop this quantification model a NIR Bruker Matric-F with a Diffuse Reflectance probe made from Solvias was used. The peculiarity of this probe was the fiber bundle used to make it; in this probe Solvias has used a bundle with seven optical fibers inside rather than one, and this increases, the sensitivity of the instrument a lot. The software used to control the instrument and to develop the model was *OPUS build 5, 5, 60 (20050210)*, version 5.5; Bruker Optics, Inc.