A quick and easy evaporative crystallisation screen for drug candidate polymorphisms

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Introduction

During drug development initial identification of a new Active Pharmaceutical Ingredient (API) usually yields an amorphous form of the compound. However, compounds which are crystalline in nature often adopt a number of crystalline forms or polymorphs. The different physical characteristics of these polymorphs can impact on the manufacturing process as well as the efficacy of the drug and initial screening undertaken on an amorphous form may be misleading, as crystallisation may change properties such as dissolution rate and biological activity. Polymorph screening is therefore an important stage in the drug development process, the aim being to identify the different crystalline structures that a drug may adopt. Information gained is used to optimise the physical properties of the drug compound, to ensure efficacy, and provide formulation and manufacturing consistency.

In this study Medicinal Chemists from one of our pharmaceutical customers in Japan evaluated the Exalt Controlled Crystallisation system from Genevac as a method of polymorph screening in their drug discovery programme. To evaluate the process a widely available compound, Piroxicam, which is known form three different polymorphs [1], was chosen.

Figure 1. Exalt toolkit.

Method

Exalt is a method for evaporative crystallisation developed by Genevac which enables solutions of API in a wide range of solvents to be evaporated at the same time, and all at the same slow rate, producing crystals. Exalt uses a special holder for vials which allows a selection of baffles to be placed on top of each vial to slow the evaporation rate of volatile solvents (Figure 1). The size and number of baffles are selected to be most restrictive for the most volatile solvents, and least restrictive to the less volatile solvents. The holder is then placed in a Genevac HT series evaporator which cycles at atmosphere and at a slightly reduced pressure for the duration of the evaporation process.

Solutions of Piroxicam were prepared in a range of solvents (see table 1) to yield a solution of 2mg/ml. 3ml of each solution was placed into a vial and capped with a tower, containing baffles, as recommended by Genevac [2] . The lower volatility of six solvents meant that no tower was required. In addition, to ensure complete evaporation by the end of the run, three of these solvents also required a reduction in initial volume (concentration of these solutions was corrected to yield 6mg per vial). The complete holders were then placed in to a Genevac HT-4X evaporator, running the Exalt programme, for 72 hours.

Results

Table 1 lists the 20 solvents screened, the tower configuration, and physical appearance after 72hr evaporation.

No.	Solvent	Volume	Tower	Physical Appearance after 72hr
1	Dichloromethane	3ml	#21	Powder
2	Tertiary Butyl Methyl Ether	3ml	#17	Solid
3	Acetone	3ml	#16	Crystal
4	Methyl Acetate	3ml	#16	Solid
5	Chloroform	3ml	#14	Solid
6	Tetrahydrofuran	3ml	#12	Solution (1ml)
7	Hexane	3ml	#15	Solution (0.5ml)
8	Methanol	3ml	#10	Needle crystal
9	Cyclo Hexane	3ml	#9	Powder
10	Ethyl Acetate	3ml	#10	Solution (0.5ml) and needle crystal
11	Methyl Ethyl Ketone	3ml	#10	Solution (0.5ml)
12	Acetonitrile	3ml	#4	Solution (0.5ml)
13	1,2-Dimethoxy Ethane	3ml	#1	Solution (0.5ml)
14	Ethanol	3ml	None	Solid
15	Isopropyl Acetate	3ml	None	Candy
16	Heptane	3ml	None	Powder
17	Isopropyl Alcohol	1ml	None	Powder
18	Toluene	2ml	None	Solution (0.5ml)
20	1,4-Dioxane	1ml	None	Solid
21	Benzene	1ml	None	Cubic crystal

Table 1. Solvents screened, Exalt configuration, and physical appearance of product after 72hrs evaporation.

Seven vials had not fully evaporated and required further evaporation in the HT4X . Subsequently 19 out of the 20 solutions yielded a crystalline solid suitable for X-Ray Diffraction (XRD) analysis. Analysis of XRD results (Figure 2) indicates that the Exalt screening method was able to identify polymorphs of types I, II and III (Table 2). For three solvents results were inconclusive and whilst these were thought be solvates further investigation would be required to confirm this.

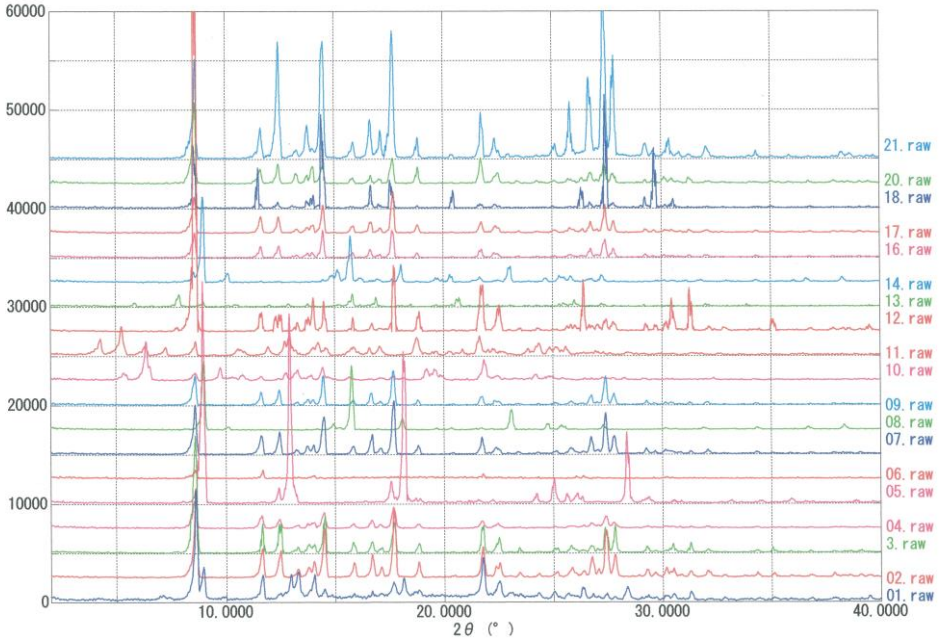

Figure 2. XRD results of crystals formed using Exalt controlled crystallisation

No.	Solvent	Crystal Polymorph Form
1	Dichloromethane	I & II
2	Tertiary Butyl Methyl Ether	I
3	Acetone	I
4	Methyl Acetate	I
5	Chloroform	III
6	Tetrahydrofuran	I
7	Hexane	I
8	Methanol	I
9	Cyclo Hexane	I
10	Ethyl Acetate	Not identified
11	Methyl Ethyl Ketone	Not identified
12	Acetonitrile	I
13	1,2-Dimethoxy Ethane	Not identified
14	Ethanol	II
16	Heptane	I
17	Isopropyl Alcohol	I
18	Toluene	I
20	1,4-Dioxane	I
21	Benzene	I

Table 2. Crystalline Polymorphisms as identified by XRD analysis.

Conclusion

Exalt Controlled Crystallisation allows the quick and easy screening of an API with minimal compound (6mg per vial). Piroxicam was screened using 20 solvents, identifying three polymorphisms, utilising just 150mg of compound. In addition the method is non-destructive meaning, where crystals are not formed, the compound may be re-dissolved for further use.

New compounds purified by reverse phase chromatography and dried by centrifugal evaporation are usually amorphous. Crystallisation changes properties such dissolution rate and biologically active so ideally polymorphisms should be identified before the compound is taken forward. In reality initial screening is often performed on amorphous compound. Erroneous results derived from amorphous compound may mean a candidate API is needlessly rejected, or is further developed only for the crystalline form to be found unsuitable at a later stage. Opportunity to obtain crystals easily at an early stage of drug discovery, with minimal compound, provides the ability to identify polymorphisms, work up potential hits more efficiently, and provide seed crystals / solvent data for scale up.

References

1.Vrecer F, Vrbinc M, Moden A (2003). Characterization of Piroxicam Crystal Modifications. International Journal of Pharmaceutics, Vol.256(1-2), 3–15.

2.Darrington R (2014). New technology for pharma small-molecule crystallisation. Lab Product News, http://www.labcanada.com/features/pharmaceutical-small-molecule-crystallization/.

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