High-throughput 96-well cocrystal screening workflow for active pharmaceutical ingredients

Increasingly, poorly soluble drug candidates are entering development, which has made identifying and selecting stable solid forms of an active pharmaceutical ingredient (API) with enhanced in vivo absorption more difficult. While pharmaceutical salts are the most common choice for solid forms, cocrystals are also known to improve "druggability" characteristics such as solubility, dissolution, bioavailability and hydration stability. In addition, cocrystals have advantages in terms of their structure diversity and the potential for intellectual property protection.

Since there are a large number of potential coformers for a given API, manual screening and selection of cocrystals is labor intensive, time consuming and requires large amounts of materials. In addition, it is known that pure cocrystal formation is favored when the API and coformers have similar saturation levels in the screening solvent. Therefore, a systematic and expansive exploration of a variety of solvents (e.g., aqueous, organic and aqueous/organic mixtures) increases the chance of forming pure cocrystal during the screen. Combining many solvents and coformers can result in an experimental design that would be overwhelming to perform manually. A high-throughput research (HTR) approach, coupled with rational experimental design, offers significant advantages and opens up new opportunities for exploring this experimental space.

HTR has been widely applied to solubility, crystallization, salt and polymorph screening, but high-throughput cocrystal screening has been used on a limited basis due to limitations of cocrystallization techniques at a miniaturized scale. Scientists at Amgen utilized Unchained Labs' freeslate system configured for small molecule preformulation and 96-well crystallization assembly with universal substrate to devise the cocrystallization HTR workflow shown in Figure 1. Following cocrystal formation and analysis by x-ray powder diffraction (XRPD), the solubility and solid/solution stability of the cocrystals were measured. The HTR workflow employed solvent mediated sonic blending to screen for cocrystal candidates with optimal properties. This application note describes the development and validation of a 96-well high-throughput cocrystallization workflow using solvent mediated sonic blending by H. Tan and coworkers.

Use of two model cocrystals to develop a cocrystallization workflow employing solvent mediated sonic blending

Since the crystal structure hydration stability at 98% relative humidity (RH) and solution stability are known for both 2:1 caffeine-oxalic acid (CO) and theophylline-oxalic acid (TO) cocrystals, they were used as models for the development of a sonic blending HTR cocrystallization workflow on a freeslate system configured for small molecule preformulation. To identify the optimal solvent volume to solid mass ratio, cocrystals of TO and CO were made using varying volumes (10, 20 and 40 µL) of six screening solvents: water, ethanol, 50:50 water:ethanol, 1,4-dioxane, 50:50 dioxane:water and ethyl acetate. To do this, 1 mg of the coformer (oxalic acid) was placed into each well of a 96-well crystallization assembly with glass universal substrate. After addition of oxalic acid, caffeine or theophylline was dispensed into each

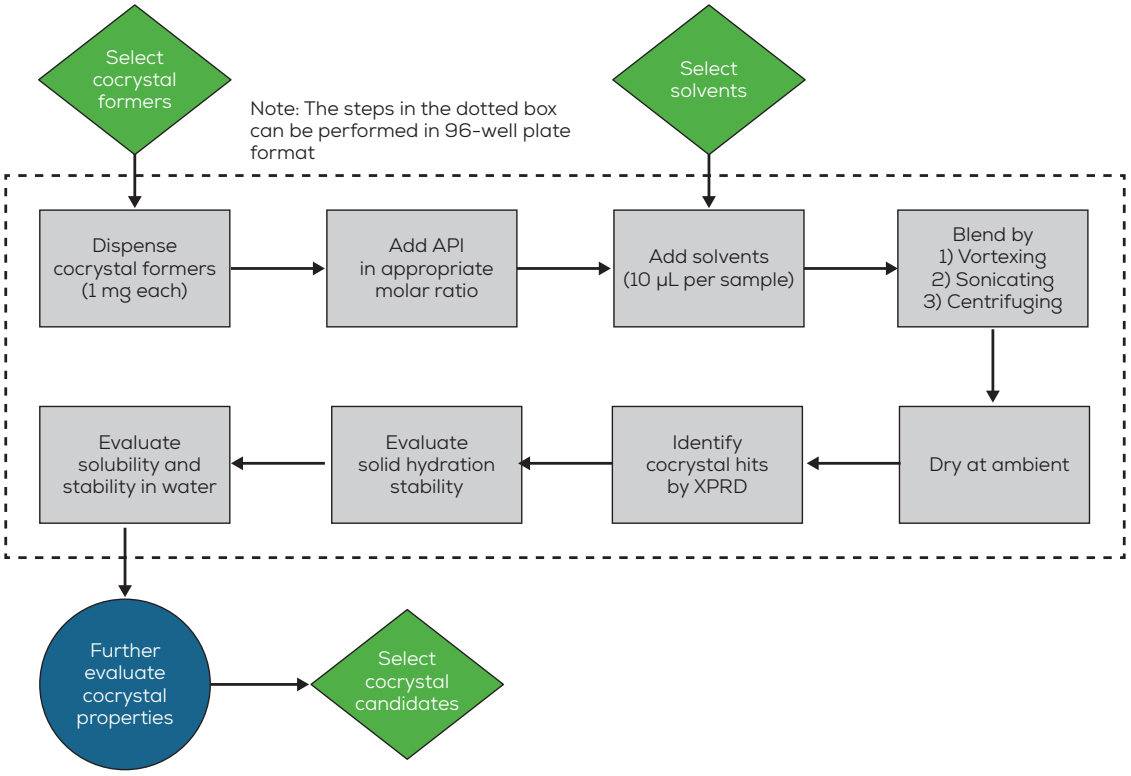

Figure 1: Diagram of 96-well high-throughput cocrystal screening workflow with solvent mediated sonic blending and on-plate solid/solution stability characterization[1].

well of the assembly using a targeted molar ratio of 2:1 oxalic acid:API. The assembly was sealed and 10, 20 or 40 µL of screening solvent was added to each well via a piercing needle. After the screening solvent was added, the assembly was agitated at 3,000 rpm for 30 minutes using the on-deck vortexer. The crystallization assembly was then removed from the freeslate and sonicated in a water bath (130 W, 40 kHz) for 60 minutes.

During sonication, the bath temperature was not allowed to exceed 40 °C. Post sonication, the assembly was centrifuged at 1,650 rpm for 10 minutes and left to stand overnight at room temperature. The next day, the crystallization assembly was opened and excess solvent in each well was removed by wick-drying with comb filter paper. The crystallization assembly was dismantled and the cocrystals were analyzed by XRPD directly on the universal substrate. Following XRPD analysis, the hydration stability of the cocrystals was measured by placing the glass universal substrate with cocrystals into a desiccator containing a saturated solution of potassium sulfate to create a 98% relative humidity environment. Cocrystals were incubated at 25 °C and analyzed at 4, 7 and 14 days by XRPD. Cocrystals that were stable for 14 days at 98% relative humidity were subjected to a 24 hour water slurry experiment to rank order their solubility and stability in water.

High-throughput solubility measurement of caffeine, theophylline and oxalic acid

To measure the solubility of caffeine, theophylline and oxalic acid in the various screening solvents, 50 mg of caffeine, theophylline or oxalic acid and 500 µL of the screening solvent were dispensed into each well of the crystallization assembly using the freeslate. The crystallization assembly was sealed, removed from the deck, sonicated for 60 minutes, equilibrated at room temperature until the next day and centrifuged. Next, 100 µL of supernatant from each well was transferred into tared glass vials in a separate 96-well plate. Vials were vacuum-dried and weighed with a resolution of 0.01 mg. The solubility of caffeine, theophylline and oxalic acid ranged from 3.9 mg/mL to over 100 mg/mL at room temperature. In addition to solubility, the Amgen scientists compared the solid forms from the solubility screen to the starting forms. After removal of the supernatant to measure solubility, the remaining solvent in each well of the crystallization assembly was aspirated and discarded. The solid was wick-dried with comb filter paper and analyzed by XRPD directly on the glass universal substrate. Table 1 shows the solubility results and remaining solid forms of caffeine, theophylline and oxalic acid found under the cocrystal screening conditions.

HTR cocrystallization results using solvent mediated sonic blending

The objective of this project was to develop an HTR cocrystallization workflow using solvent mediated sonic blending. Table 2 shows the results of the cocrystal screen at different

Solubility (mg/mL)/ residue form	Water	Ethanol	Water: Ethanol (50:50)	1,4-Dioxane	Dioxane: Water (50:50)	Ethyl Acetate
Caffeine	24.1/CM*	6.0/C	30.9/CM*	18.7/C	32.4/CM*	7.2/C
Theophylline	7.0/TM	3.9/T	18.0/TM	4.7/T	18.2/TM	4.6/T
Oxalic acid	93.9	>100	>100	>100	>100	>100

Table 1: Solubility and residual solid forms of caffeine, theophylline and oxalic acid in the screening solvents.[1] **C**: caffeine anhydrate, **CM***: caffeine monohydrate, gradually converted to caffeine anhydrate when exposed to ambient conditions, **T**: theophylline anhydrate, **TM**: theophylline monohydrate.

Reactant	Water	Ethanol	Water: Ethanol (50:50)	1,4-Dioxane	Dioxane: Water (50:50)	Ethyl Acetate
Caffeine (4.0 mg)	10	CO	CO	CO	CO	CO
	20	CO	CO	CO	CO	CO
	40	CO	CO	CO	CO	CO
Theophylline (4.3 mg)	10	TO+TM	TO	TO+TM	T+TO	TO
	20	TM	T	TM	TO	TO
	40	TM	T	TM	TO	TO

Table 2: Cocrystal screening results of caffeine and theophylline with 1 mg oxalic acid.[1] **CO**: caffeine-oxalic acid cocrystal, **T**: theophylline anhydrate, **TM**: theophylline monohydrate, **TO**: theophylline-oxalic acid cocrystal.

volumes of screening solvent. While it has been reported that caffeine anhydrate converts to a known monohydrate in the presence of water, CO cocrystals formed in quantitative yield with all the solvents and solvent volumes tested. However, for TO, depending on the solvent used and the saturation level of theophylline in the solvent, different cocrystals were formed.

While pure TO cocrystals formed under anhydrous conditions (e.g., dioxane and ethyl acetate), a mixture of TO cocrystals and TM were formed when the screening solvent contained water. This was consistent with previously reported results for CO and TO cocrystals under water slurry conditions. Based on the solubility data, both caffeine and theophylline were solid-saturated under the cocrystal screening conditions and oxalic acid was completely dissolved under all conditions except for 10 µL water. Therefore, 10 µL was selected as the solvent volume for the sonic blending workflow because that volume would maximize the saturation level of oxalic acid in the screening solvent and enhance the chance of forming pure cocrystals.

Reactant: AMG 517 Form A		Water	Ethanol	Water: Ethanol (50:50)	1,4-Dioxane	Dioxane: Water (50:50)	Ethyl Acetate
AMG 517 (Form A)	Sorbic Acid		●	●	●	●	●
	Citric Acid				■	■	
	Fumaric Acid		●	●	●	●	●
	Maleic Acid		●		●		●
	L-Malic Acid				●		●
	L-Tartaric Acid				■		
	Succinic Acid				●		●
	Glutaric Acid				●		●
	Adipic Acid		●	●	●	●	●
	Glycine						
	L-Aspartic Acid						
	L-Glutamic Acid						
	Benzoic Acid	●	●	●	●	●	●
	Benzamide			●	●	●	●
	Nicotinamide				■		

Table 3: Cocrystal screening results of AMG517 with 15 coformers.[1] AMG 517 or AMG 517 and conformer were blended with 10 µL of solvent. Formation of the cocrystal was detected by XRPD and hydration stability of the cocrystals formed was monitored by XRPD as the samples were subjected to 98% RH for two weeks. A blank cell indicates no cocrystal formed after blending. The symbol ● indicates formation of the cocrystal which was stable at 98% RH. The symbol ■ indicates formation of the cocrystal which was not stable at 98% RH.

Validating an integrated high-throughput cocrystal screening workflow

Amgen compound AMG517 was used to validate the HTR cocrystal workflow with solvent mediated sonic blending. Ten of the fifteen coformers selected were known to form cocrystals by either solvent-drop grinding or solution crystallization. Cocrystallization with the other five coformers had not been attempted before. The results using the same set of six solvents are shown in Table 3. For the 15 coformers screened, all 10 previously identified cocrystals were found and two new cocrystals were discovered. The two coformers that formed new cocrystals were nicotinamide and fumaric acid. Once XRPD analysis was completed, the cocrystals on the glass universal substrate were treated to 98% relative humidity to evaluate their hydration stability. Three cocrystals were unstable and were not analyzed further. The remaining cocrystals were stable for two weeks and were studied further for their solution stability and solubility.

Conclusion

Using the freeslate system, crystallization assembly with glass universal substrate and TO and CO as model cocrystals, Amgen scientists devised an HTR cocrystallization workflow based on solvent mediated sonic blending. For XRPD analysis, manual manipulation of delicate cocrystals was avoided by analyzing the crystals directly on the glass universal substrate of crystallization assembly. Since the cocrystals are not affected by this analysis, they can subsequently be subjected to additional experiments. In this case, the hydration stability of the cocrystals was evaluated by placing the glass universal substrate with cocrystals into a desiccator containing a saturated solution of potassium sulfate to create a 98% relative humidity environment. Cocrystals were analyzed after 4, 7 and 14 days by XRPD and those that demonstrated a hydration stability of greater than two weeks were studied further. Amgen scientists validated this workflow using a poorly soluble compound, AMG517 and 15 coformers. Ten previously known cocrystals were identified and two new cocrystals were discovered during the validation. Nine of the twelve cocrystals were stable for two weeks under 98% relative humidity and were studied further for their solution stability and solubility. By using an automated HTR approach, multiple APIs, coformers and solvents were studied under the same cocrystallization conditions simultaneously, which saved time and labor, minimized API usage and enabled the exploration of an expanded design space.

References

1 Reprinted from *International Journal of Pharmaceutics*, Vol 441, Issue 1-2, Van Luu, Janan Jona, Mary K. Stanton, Matthew L. Peterson, Henry G. Morrison, Karthik Nagapudi, Helming Tan, High-throughput 96-well solvent mediated sonic blending synthesis and on-plate solid/solution stability characterization of pharmaceutical cocrystals, p. 356–367, (2012) with permission from Elsevier

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