



High-throughput 96-well solvent mediated sonic blending synthesis and on-plate solid/solution stability characterization of pharmaceutical cocrystals

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ABSTRACT

A 96-well high-throughput cocrystal screening workflow has been developed consisting of solvent-mediated sonic blending synthesis and on-plate solid/solution stability characterization by XRPD. A strategy of cocrystallization screening in selected blend solvents including water mixtures is proposed to not only manipulate solubility of the cocrystal components but also differentiate physical stability of the cocrystal products. Caffeine–oxalic acid and theophylline–oxalic acid cocrystals were prepared and evaluated in relation to saturation levels of the cocrystal components and stability of the cocrystal products in anhydrous and hydrous solvents. AMG 517 was screened with a number of coformers, and solid/solution stability of the resulting cocrystals on the 96-well plate was investigated. A stability trend was observed and confirmed that cocrystals comprised of lower aqueous solubility coformers tended to be more stable in water. Furthermore, cocrystals which could be isolated under hydrous solvent blending condition exhibited superior physical stability to those which could only be obtained under anhydrous condition. This integrated HTS workflow provides an efficient route in an API-sparing approach to screen and identify cocrystal candidates with proper solubility and solid/solution stability properties.

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1. Introduction

Pharmaceutical cocrystals have been emerging as a new class of crystal forms in pharmaceutical development in the last decade (Shan and Zaworotko, 2008). With an increasing number of poorly soluble drug candidates entering into development, identification and selection of a stable solid form of active pharmaceutical ingredient (API) for enhancement of *in vivo* absorption has become more challenging in drug development (Kennedy, 1997). Formation of pharmaceutical salts represents the most common option in API form selection (Stahl and Wermuch, 2002). However, cocrystallization, through complexation of API with neutral molecules as opposed to ions, offers advantages over salt formation with regards to structural diversity and intellectual property, particularly when the options for forming pharmaceutically viable salts are limited (Stahly, 2007; Trask, 2007). Advancement in crystal engineering facilitates the design of cocrystallized molecules in the context of understanding and predicting hydrogen bonding interactions between an API and diversified coformers (Almarsson and Zaworotko, 2004; Vishweshwar et al., 2006), and pharmaceuti-

cal cocrystals have been reported to improve the physicochemical properties of API crystal forms such as solubility/dissolution (Childs et al., 2004; Blagden et al., 2007; Stanton and Bak, 2008; Schultheiss and Newman, 2009), bioavailability (McNamara et al., 2006; Bak et al., 2008; Stanton et al., 2010, 2011), and stability to hydration (Trask et al., 2005, 2006). Due to a large number of possible coformers for a given API, manual experimentation of a thorough cocrystal screen could be labor-intensive and time/material-consuming. An efficient high-throughput screening (HTS) approach, coupled with rational design by crystal engineering, can therefore be valuable in the process of identifying a viable cocrystal. HTS technologies have been extensively used in solubility, crystallization, salt and polymorph screens (Carlson et al., 2003; Morissette et al., 2004; Tan et al., 2005, 2008); however, high-throughput cocrystal screening has only been explored to a limited extent due to limitations of cocrystallization techniques at a miniaturized scale. The first example of cocrystals identified from a high-throughput screen was reported in 2003 (Remenar et al., 2003). Recent development in high-throughput cocrystal screening involved mechanochemical grinding in 48 sample vials (Bysouth et al., 2011), slurry and solvent drop sonication in 96-well plates (Kojima et al., 2010; Leung et al., 2012), which enabled a large number of cocrystallization experiments to be conducted simultaneously; nevertheless, successive cocrystal characterization often involves manual steps of

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sample preparation and thus becomes a limiting step in real life high-throughput screen.

Solvent crystallization (Zhang et al., 2007; Childs et al., 2008; Horst and Cains, 2008; Rager and Hilfiker, 2010) and solid state grinding (Pedireddi et al., 1996; Weyna et al., 2009) represent two most commonly used techniques for cocrystal synthesis at laboratory bench scales. In the past several years, solvent-drop or liquid-assisted grinding (LAG) evolved a preferable method in cocrystal screening because the solvent presented could considerably enhance the grinding efficiency or the cocrystallization kinetics (Shan et al., 2002; Trask and Jones, 2005; Jones et al., 2006). Furthermore, the solvent of choice could control the polymorphic nature of a cocrystal with an aim to obtain desired physicochemical properties (Trask et al., 2004). For example, inclusion of water in grinding was applicable to screening for hydrated forms of cocrystals with potentially improved hydration stability (Karki et al., 2007). Recently, new methods of slurry cocrystallization using ultrasound were introduced (Bucar and MacGillivray, 2007; Leung et al., 2012). Comparison of LAG and sonic slurry techniques indicates that formation of a pure cocrystal is related to the saturation levels of the reactants in the screening solvent but not to the processing method (Friscic et al., 2009). Consequently, selection of appropriate solvents with similar solubility or saturation levels of both cocrystal components is important to success of cocrystal screen.

Herein, we report the development of an integrated 96-well high-throughput cocrystal screening workflow employing a solvent-mediated sonic blending synthesis technique and on-plate X-ray powder diffraction (XRPD) characterization. Through parallel synthesis and analysis, stoichiometric ratios of APIs and cofomers were screened in selected solvents under sonic blending condition and formation of cocrystals was monitored by XRPD. In general, pharmaceutically accepted cofomers with lower molecular weights are more water soluble than the API, whereas the API tends to be more lipophilic and therefore more soluble in organic solvents. To increase the chance of cocrystal formation while decreasing the solubility differences between an API and different cofomers, multiple solvents including water, organic solvents and solvent–water mixtures were first time systematically evaluated in this screening workflow. Furthermore, we postulate that cocrystals which can be formed under anhydrous and/or hydrous solvent blending conditions may inherently have different hydration/solution stability properties. While a cocrystal is often intended to enhance the solubility of an API, sufficient cocrystal stability is required not only for solid form dosage but also for dissolution performance. A cocrystal with poor aqueous stability tends to dissociate quickly in water followed by precipitation of the API to render an undesirable dissolution profile. Hence we are interested in investigating solid/solution stability and solubility of the cocrystal hits through continuous screening and characterization in 96-well plate format to identify suitable cocrystal candidates for further characterization and development.

Crystal structures of 2:1 caffeine–oxalic acid (CO) and theophylline–oxalic acid (TO) cocrystals have been reported including the hydration stability at 98% relative humidity (RH) and the solution stability in water (Trask et al., 2005, 2006). As a part of screening workflow development, CO and TO cocrystals were used as model cocrystals to be made in six selected solvents with varying ratios of solvent volume to solid mass in one sonic blending experiment. The cocrystallization results were evaluated to derive an appropriate volume to solid ratio for this sonic blending technique. Utility of this integrated screening workflow was demonstrated for AMG 517, an Amgen compound which is a poorly soluble small molecule and whose cocrystal structures with a number of cofomers were previously reported with improved solubility and bioavailability (Stanton and Bak, 2008; Stanton et al., 2010,

2011). Approximately with 1 mg of conformer and equal molar amount of API in each well, AMG 517 was screened with 15 cofomers (five new and ten known to form the cocrystals) and six blend solvents on a 96-well plate in one run. The AMG 517 cocrystals formed on the plate were exposed to 98% relative humidity (RH) and solid-state stability was monitored by XRPD within a period of two weeks. Those cocrystals showing stability at 98% RH were subjected to a water slurry experiment on a 96-well plate for ranking solubility and stability in water. As a representative workflow diagram shown in Fig. 1, this 96-well solvent–water mediated sonic blending synthesis technique, integrated with on-plate XRPD analysis, was developed for screening and identifying cocrystal candidates with proper solubility and solid/solution stability profiles. Scale-up synthesis of the cocrystal hits and further characterization (i.e. single crystal structure, dissolution, bioavailability), which are typically performed on individual candidates for pharmaceutical development, were beyond the scope of this study.

2. Materials and methods

2.1. Materials

Anhydrate β form of Caffeine (Edwards et al., 1997), anhydrate theophylline (Ebisuzaki et al., 1997; Sun et al., 2002), and oxalic acid (anhydrate) were purchased from Sigma–Aldrich (St. Louis, MO) and used as supplied with a minimum chemical purity of 99%. AMG 517 (anhydrate Form A) was internally obtained (Amgen, Thousand Oaks, CA). Cofomers were commercially available and purchased with a minimum chemical purity of 99%. The XRPD patterns of the starting APIs and cofomers were collected prior to use in the screen.

2.2. High-throughput cocrystal screen in 96-well plate via solvent mediated sonic blending

A 96-well crystallization plate assembly (Freeslate, Sunnyvale, CA) was used, consisting of a flat glass piece in the bottom plate and 96-well inserts with O-ring seals in the top plate (Fig. 2). Following a screening design in 96-well format, approximately 1 mg of cofomer was individually weighed into each well of the assembled plate using a Core Module solid/liquid handler (Freeslate, Sunnyvale, CA) and the exact weight of the cofomer dispensed was recorded to the nearest 0.1 mg. API was then dispensed into the 96-well plate based on the targeted molar ratio of API to cofomer in each well (i.e. 2:1 for caffeine or theophylline to oxalic acid, 1:1 for AMG 517 to each cofomer). The crystallization plate was sealed with a 96-well cap mat and an appropriate volume (i.e. 10–40 μ L) of the screening solvent was dispensed into each well using a piercing needle. A typical sequence of 96-well plate sonic blending process was exemplified with the following operating parameters: (1) vortexing at 3000 rpm for 30 min using a plate vortexer, (2) sonicating in water bath of a ultrasonicator (130 W, 40 kHz) for 60 min during which the bath temperature did not exceed 40 °C, and (3) centrifuging at 1650 rpm for 10 min. After centrifuging, the sealed plate was allowed to stand at ambient conditions overnight. The cap mat was then removed and the wet solid packed at the bottom of the wells was wick-dried by comb paper. The plate was disassembled and 96 arrayed samples were obtained on a glass substrate (Fig. 2) for XRPD analysis.

2.3. High-throughput solubility measurement and residual solid form characterization for API and cofomers

Solubility was measured for APIs and cofomers in the solvents under screening using a high-throughput gravimetric weighing method. To a 96-well crystallization plate assembly, 50 mg of API

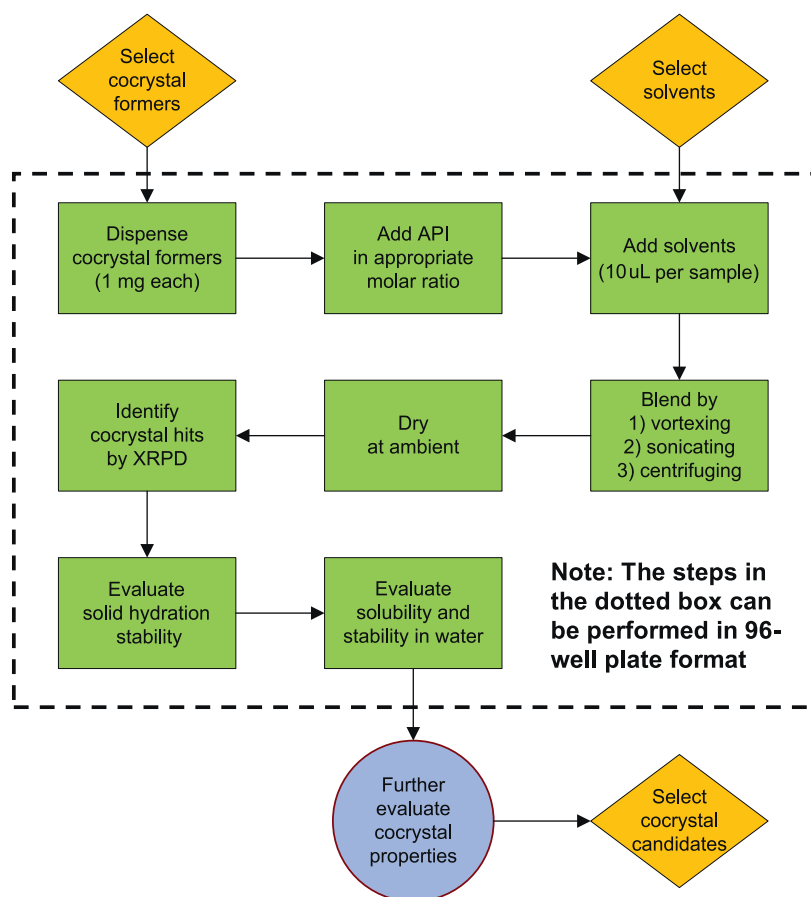


Fig. 1. Representative workflow diagram of 96-well high-throughput pharmaceutical cocrystal screening with solvent mediated sonic blending synthesis and on-plate solid/solution stability characterization.

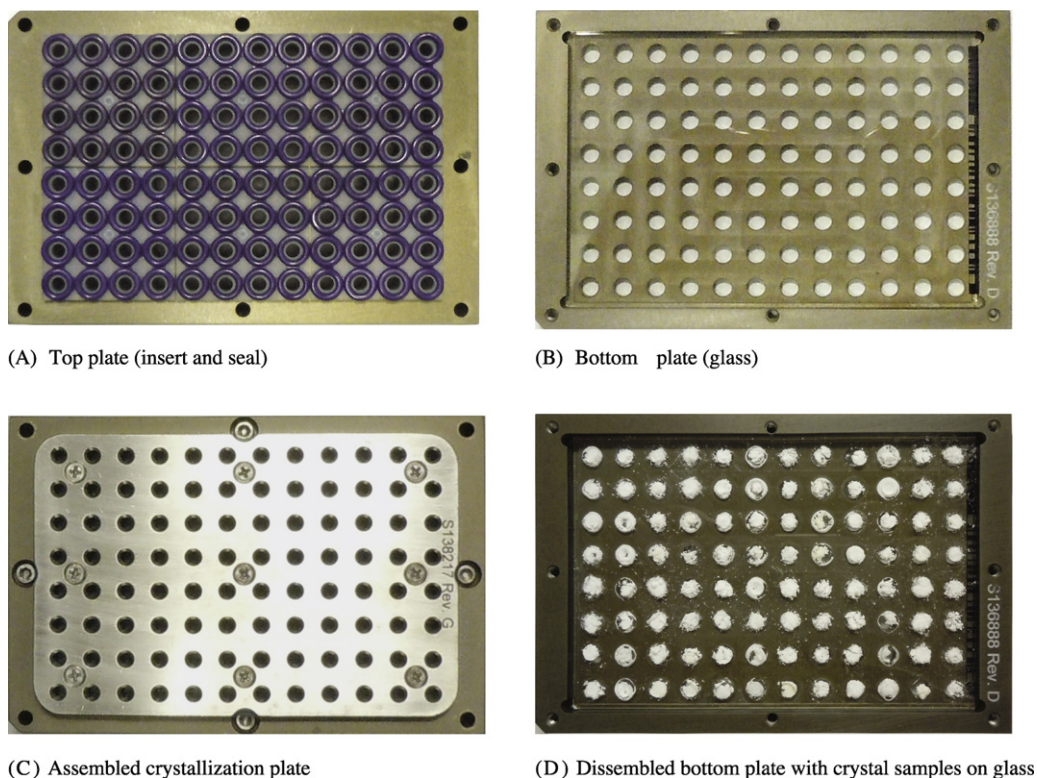


Fig. 2. A 96-well crystallization plate assembly (Freeslate, Sunnyvale, CA).

or coformers and 500 μL of the screening solvent were added to each well using a solid/liquid handler. The sealed plate was sonicated for 60 min followed by equilibration at ambient overnight. After centrifuging the plate, 100 μL of supernatant was taken from each well and transferred into the tare-weighted glass vials on a 96-well plate. The aliquots in the 96-well glass vial plate were vacuum-dried. The glass vials were then weighed to the nearest 0.01 mg and the weight difference from its tare weight was the solubility of API or coformer in 100 μL of solvent. The solubility range reported by this method was from 0.1 mg/mL to 100 mg/mL at ambient laboratory temperature.

In conjunction with solubility measurement, the crystal form of the residual solid was characterized by XRPD and compared to the API's starting solid form. After removal of the supernatant for solubility measurement, the remaining solvents in the 96-well crystallization plate assembly were aspirated and discarded. The residual solid was wick-dried by comb paper and recovered on a glass substrate for XRPD analysis after the plate was disassembled.

2.4. On-plate X-ray powder diffractometry (XRPD)

XRPD patterns were collected on a Bruker D8 Discover X-ray diffraction system fitted with a motorized xyz sample stage and a GADDS area detector. The 96-well screen samples on a flat glass plate were mapped and a sample area of 1 mm² was scanned in oscillating mode for 3 min from 5° to 48° 2 θ using Cu K α radiation (40 Kv, 40 mA) through a graphite monochromator and a collimator of 0.5 mm pinhole. The cocrystal hits were identified by comparing the powder patterns of the screen samples against the starting or known forms of API and coformers.

2.5. Cocrystal solid stability at 98% RH

A saturated solution of potassium sulfate in water was stored in a sealed desiccator to simulate an environmental condition of 98% RH at 25 °C. The 96-well screen samples on a flat glass plate were placed inside the desiccator. After storage of 4, 7, and 14 days, respectively, the plate was taken out of the desiccator, X-ray scan was performed, and solid-state stability of the cocrystal samples was monitored by XRPD. Once X-ray scan was finished at each time point, the plate samples were returned to the storage condition for continuing hydration stability study at 98% RH.

2.6. Cocrystal stability and solubility in water

The cocrystal hit showing hydration stability at 98% RH after 14 days was further investigated for its physical stability in water by a 24 h slurry experiment, and at the same time its solubility was determined and compared to that of the API. To a 96-well crystallization plate assembly, approximately 5 mg of the cocrystal sample (pooled if necessary) was added, followed by addition of 500 μL water. The slurry sample on the plate was shaken at 400 rpm for 24 h at ambient laboratory temperature prior to phase separation by centrifuging. The supernatant was withdrawn and filtered through a 0.2 μm , 4 mm PTFE Millipore Millex syringe filter. The first 300 μL filtered volume was discarded and the remaining volume was filtered into a conical bottom HPLC vial for solubility measurement by HPLC. The residual solid was wick-dried by comb paper and recovered on a glass substrate after the plate was disassembled. The physical stability of the cocrystal hit in water was then evaluated by XRPD by comparing its crystal form before and after slurry.

3. Results and discussion

3.1. High-throughput cocrystal screening workflow development with model cocrystals

CO and TO cocrystals were used as model cocrystals in this sonic blending HTS workflow development and their simulated powder patterns are showed in Figs. 3 and 4, respectively. With the intention to minimize the API material required in 96-well cocrystal screening while allowing accurate solid weighing by a robotic handler, the target weight of coformer (oxalic acid) was set to 1 mg and the stoichiometric amount of API, approximately ca. 4.0 mg of caffeine or ca. 4.3 mg of theophylline, was then added for cocrystallization in each well. Six solvents (water, ethanol, ethanol/water (50/50, v/v), 1,4-dioxane, dioxane/water (50/50, v/v), ethyl acetate), each in three different volumes (10, 20, 40 μL), were evaluated for formation of CO and TO cocrystals under sonic blending condition. The cocrystal screening results are showed in Table 1, while Table 2 list the solubility and remaining solid form of caffeine, theophylline and oxalic acid determined under the same screening condition.

Based on the solubility data, caffeine and theophylline were believed to remain solid-saturated under the defined cocrystal screening conditions whereas oxalic acid should be completely dissolved except in 10 μL of water. CO cocrystal was readily obtained in a quantitative yield by sonic blending with anhydrous or hydrous solvents at different saturation levels regardless of the fact that caffeine anhydrate can convert to a known monohydrate in the presence of water (Edwards et al., 1997). The powder pattern of CO cocrystal sample obtained by sonic blending with water, shown in Fig. 3, matched the simulated pattern of the known CO cocrystal. On the other hand, formation of TO cocrystal depended upon the used solvent and the saturation level of theophylline in solvent. A pure TO cocrystal formed via mediation of anhydrous solvents such as dioxane and ethyl acetate. However in the presence of water, a mixture of TO cocrystal and theophylline monohydrate, a known hydrate form of theophylline (Sun et al., 2002), was obtained as showed in Fig. 4. With decreased saturation of theophylline and increased volume of water presented, the cocrystal purity decreased in dioxane/water as a result of partial conversion to theophylline hydrate, whereas the conversion was complete in water or ethanol/water therefore no cocrystal formed. Although both CO and TO cocrystals were obtained under anhydrous conditions, the outcomes of cocrystal formation in the presence of water were quite different. In contrast to CO cocrystal, TO cocrystal was not easily formed when water was present. This observation was in agreement with the reported stability of CO and TO cocrystals under water slurry condition: the former was stable in water but the latter was not (Trask et al., 2005, 2006). It is interesting to note that formation of a cocrystal during screening in the presence of water could be indicative of its stability tendency against dissociation in water.

3.2. Solvent-mediated sonic blending conditions

By evaluating the screening outcomes of CO and TO cocrystals, the solvent volume used in this sonic blending workflow was chosen to be 10 μL with two rationales: (1) to increase the chance of cocrystal formation by increasing the saturation levels of reactants; and (2) to allow accurate dispensing of the solvent volume by a liquid handler. From HTS perspective, it is practical and beneficial to screen multiple solvents including water mixtures, aiming to manipulate solubility of cocrystal components and also to differentiate stability of cocrystal products under anhydrous vs. hydrous processing conditions. Given the complexity that an API may exist in multiple forms including hydrate, it is highly recommended to include a limited polymorph screen without the use of conformers

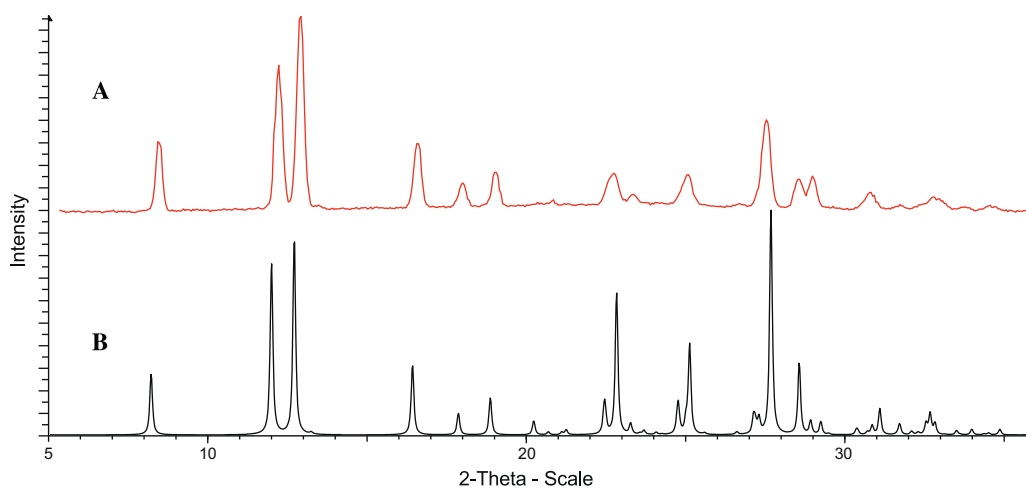


Fig. 3. Powder pattern (A) CO cocrystal screening sample by sonic blending with water; (B) simulated, CO cocrystal.

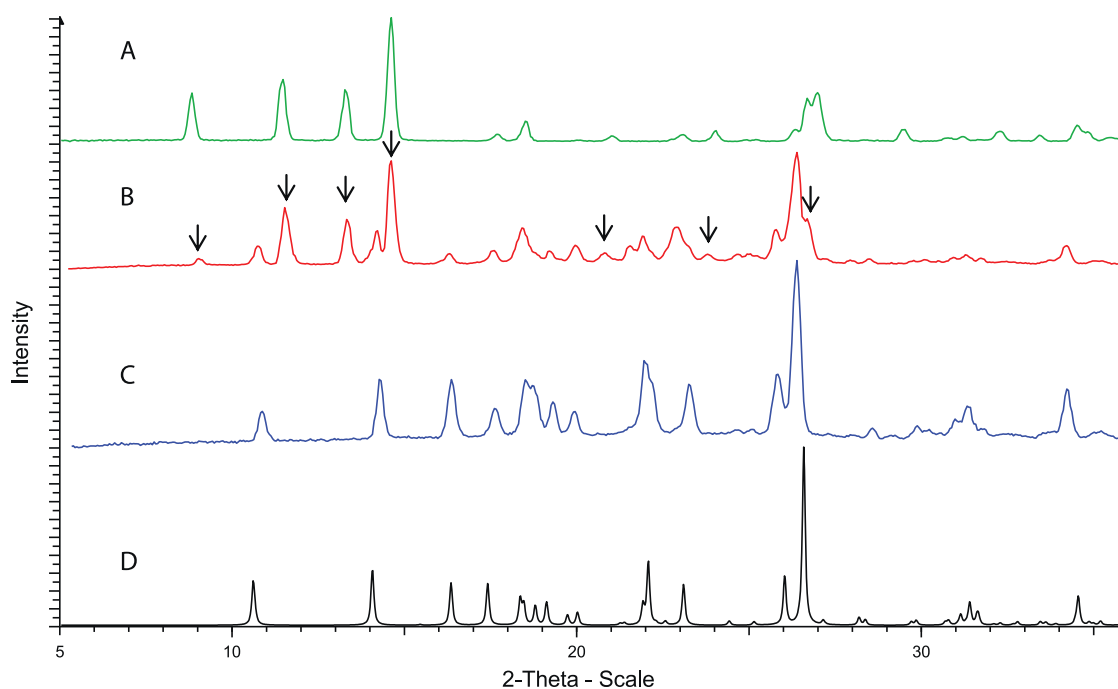


Fig. 4. Powder pattern (A) theophylline monohydrate; (B) TO cocrystal screening sample by sonic blending with water, showing a mixture of TO cocrystal and theophylline monohydrate whose peaks were indicated by the arrows; (C) TO cocrystal screening sample by sonic blending with dioxane; (D) simulated, TO cocrystal.

Table 1

Cocrystal screening results of caffeine and theophylline with oxalic acid.

Reactant	Solvent (μL)	Water	Ethanol	Ethanol/water (50/50)	1,4-Dioxane	Dioxane/water (50/50)	Ethyl acetate
Caffeine (4.0 mg)	10	CO	CO	CO	CO	CO	CO
	20	CO	CO	CO	CO	CO	CO
	40	CO	CO	CO	CO	CO	CO
Theophylline (4.3 mg)	10	TO + TM	TO	TO + TM	TO TOA	TO + TM	TO
	20	TM	T	TM	TO	TO + TM	TO
	40	TM	T	TM	TO	TO + TM	TO

Note: CO: caffeine oxalic acid cocrystal; TO: theophylline oxalic acid cocrystal; T: theophylline anhydrate; TM: theophylline monohydrate.

Table 2

Solubility and residual solid forms of caffeine, theophylline, and oxalic acid in selected solvents.

Solubility (mg/mL)/residue form	Water	Ethanol	Ethanol/water (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

Note: C: caffeine anhydrate; CM*: caffeine monohydrate, gradually converted to caffeine anhydrate when exposed to ambient; T: theophylline anhydrate; TM: theophylline monohydrate.

Table 3

Cocrystal screening results of AMG 517 with 15 cofomers.

Reactant	Water	Ethanol	Ethanol/water (50/50)	1,4-Dioxane	Dioxane/water (50/50)	Ethyl acetate
AMG 517	Form A	Form A	Form A	Form A	Form A	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				×		

AMG 517 or AMG 517 and cofomer were blended with 10 μ L 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.

during cocrystal screening on a 96-well plate in selected solvents under the same sonic blending condition.

3.3. High-throughput cocrystal screen of AMG 517 via solvent mediated sonic blending

AMG 517 was used as a model compound in validation of this high-throughput cocrystal screen employing solvent mediated sonic blending technique. Fifteen cofomers were chosen as shown in Table 3 in which ten of them have been reported to form cocrystals with AMG 517 by means of solvent-drop grinding or solution crystallization (Stanton and Bak, 2008; Stanton et al., 2010, 2011). Five cofomers which were not attempted previously in manual screen were also included and they were nicotinamide, fumaric acid, glycine, L-aspartic and L-glutamic acid. The screening results of AMG 517 with 15 cofomers in 6 solvents on a 96-well plate under sonic blending condition were summarized in Table 3. Specifically, 1 mg of the cofomer and equal molar amount of AMG 517 to cofomer was blended in 10 μ L of the solvent in each well. Solubility of AMG 517 and cofomers in the screening solvents were determined as shown in Table 4. Under the screening condition, AMG 517 maintained saturation and its starting solid form (Form A), whereas a cofomer was saturated unless its solubility was greater than 100 mg/mL in that solvent. Presumably the cocrystal stoichiometry is unknown, it is appropriate to conduct a screen with equal molar ratio of cofomer to API. Under the circumstance that a 2:1 cocrystal of API to di-acid cofomer does form, excess of the cofomer can be distinguished by the XRPD pattern of the screen sample.

Out of 15 cofomers screened with AMG 517 under sonic blending condition, all ten known cocrystals previously reported (Stanton and Bak, 2008; Stanton et al., 2010, 2011) were successfully obtained, and two new cocrystals with nicotinamide and fumaric acid were found in this screen. Glycine, L-aspartic and L-glutamic acid were tried but no cocrystal formation with AMG 517 was observed. Benzoic acid gave the highest hit rate for cocrystal formation in all six solvents including water followed by adipic, fumaric and sorbic acid in five of six solvents except water. It seemed that AMG 517 benzoic acid cocrystal was easily obtained regardless of solubility of both components being less than 0.1 mg/mL in water or greater than 100 mg/mL in dioxane. However, the solvent of choice deemed to affect the outcome of cocrystal formation, and individual cocrystals could be preferably obtained in the presence of one solvent over the other. In this screen, all 12 AMG 517 cocrystals formed in dioxane, followed by

ethyl acetate (10 crystals), ethanol (5 cocrystals), ethanol/water or dioxane/water (5 cocrystals), and water (1 cocrystal). Given the unpredictability of solvent effect on cocrystal formation, it is prudent to screen multiple solvents in order to diversify solvent properties and to increase the chance of cocrystal formation. Based on the AMG 517 cocrystal screening results, the reproducibility of the hit samples was greater than 90% under the same solvent condition from the same plate run or between different plate runs. Not only being more efficient, this 96-well solvent mediated sonic blending technique was also validated through formation of the AMG 517 cocrystals which were prepared manually via conventional solvent-drop grinding or solution crystallization methods.

3.4. Stability of AMG 517 cocrystals at 98% RH

After 12 different cocrystal hits were generated on a plate, they were subjected to 98% RH condition and their hydration stability was monitored by XRPD. After four days at 98% RH, AMG 517 cocrystals of nicotinamide, citric and tartaric acid were found to be unstable and dissociated to physical mixtures of AMG 517 (Form A) and the corresponding cofomer. It is noticed that these hydration instable cocrystals was only made under anhydrous solvent blending condition. The instability at high RH eliminated these three cocrystals as candidates for further characterization or development. The remaining nine cocrystals were stable at 98% RH for 2 weeks. These were cocrystals with L-malic, glutaric, succinic, maleic, sorbic, adipic, fumaric, benzoic acid, and benzamide. To rank-order the solubility and solution stability of these cocrystal hits, a 24 h slurry experiment in water was performed. We think RH stability is one of the most critical pharmaceutical properties of a cocrystal API. Unlike reversible moisture sorption/desorption often observed for a free form API, RH instability usually leads to irreversible dissociation of a cocrystal API. From shelf-life perspective a cocrystal may not be a viable development candidate if it is not stable at high RH. On the other hand, if a cocrystal is stable at high RH and exhibits a balance profile of solution stability and solubility, it may warrant further investigation to achieve an ultimate goal of pharmacokinetics/bioavailability enhancement.

3.5. Solution stability and solubility trends of AMG 517 cocrystals

As shown in Table 5, AMG 517 anhydrate Form A converted to a monohydrate (a known form) after slurry in water for 24 h. Solubility of AMG 517 was nearly unchanged (0.01–0.03 μ g/mL) in

Table 4

Solubility of AMG 517 and coformers in selected solvents.

Solubility (mg/mL)	Water	Ethanol	Ethanol/water (50/50)	1,4-Dioxane	Dioxane/water (50/50)	Ethyl acetate
AMG 517 (Form A)	<0.1	7.5	0.3	>100	1.1	30.3
Sorbic acid	1.9	>100	23.2	>100	47.9	40.9
Citric acid	>100	>100	>100	>100	>100	17.4
Fumaric acid	5.5	35.2	21.6	36.7	52.7	6.3
Maleic acid	>100	>100	>100	>100	>100	42.1
L-Malic acid	>100	>100	>100	>100	>100	66.8
L-Tartaric acid	>100	>100	>100	>100	>100	13.0
Succinic acid	68.7	73.0	>100	74.5	>100	6.4
Glutaric acid	>100	>100	>100	>100	>100	87.3
Adipic acid	22.2	86.4	94.0	>100	>100	11.0
Glycine	>100	1.8	19.3	0.8	12.4	1.0
L-Aspartic acid	3.4	1.6	0.3	0.8	1.0	1.1
L-Glutamic acid	9.0	1.2	1.5	1.0	1.3	0.7
Benzoic acid	<0.1	>100	92.9	>100	98.9	>100
Benzamide	17.7	>100	>100	>100	>100	41.1
Nicotinamide	98.8	88.7	>100	40.5	>100	11.0

Table 5

Solubility and physical stability of AMG 517 cocrystals after slurry in water for 24 h.

Pre-slurry form	Solubility ($\mu\text{g/mL}$)	Post-slurry form
AMG 517 Form A	0.02	AMG 517 monohydrate
L-Malic acid cocrystal	0.25	AMG 517 monohydrate
Glutaric acid cocrystal	0.08	Glutaric acid cocrystal + AMG 517 monohydrate
Benzamide cocrystal	0.03	Benzamide cocrystal + AMG 517 monohydrate
Succinic acid cocrystal	0.02	Succinic acid cocrystal + AMG 517 monohydrate
Maleic acid cocrystal	0.02	Maleic acid cocrystal + AMG 517 monohydrate
Sorbic acid cocrystal	0.02	Sorbic acid cocrystal + AMG 517 monohydrate
Adipic acid cocrystal	0.02	Adipic acid cocrystal
Benzoic acid cocrystal	0.02	Benzoic acid cocrystal
Fumaric acid cocrystal	<0.01	Fumaric acid cocrystal

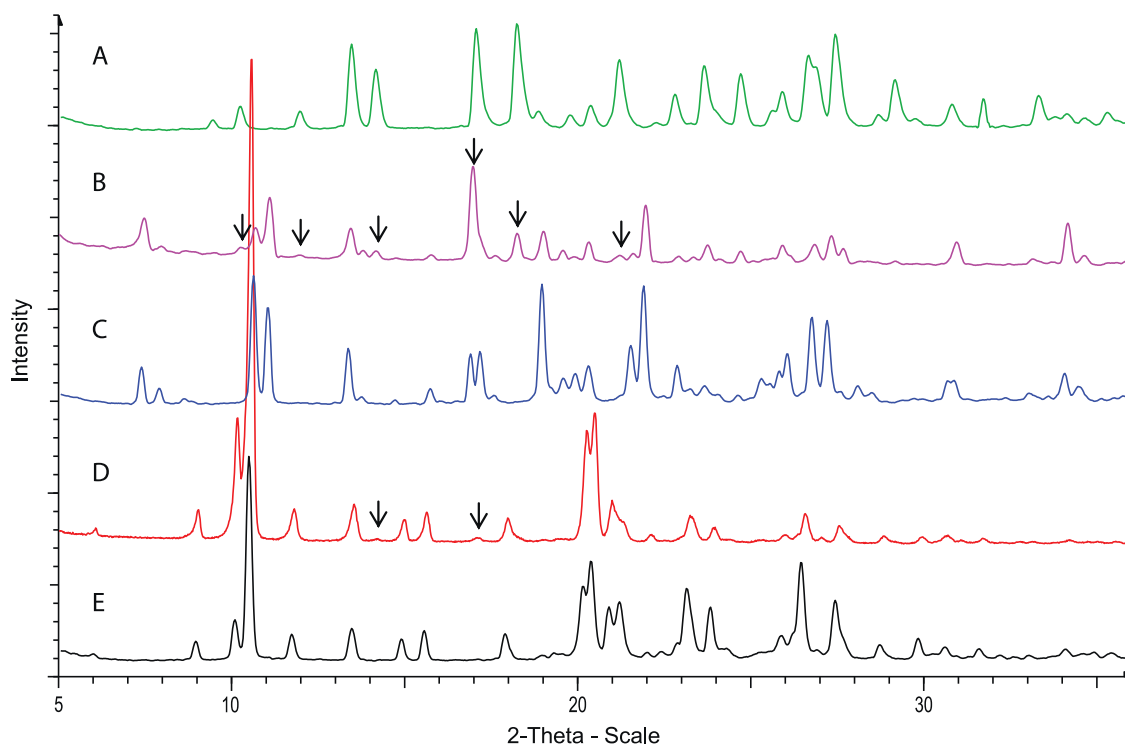


Fig. 5. Powder pattern (A) AMG 517 monohydrate; (B) post-slurry sample of AMG 517 glutaric acid cocrystal, showing a mixture of cocrystal and AMG 517 monohydrate whose peaks were indicated by the arrows; (C) AMG 517 glutaric acid cocrystal; (D) post-slurry sample of AMG 517 sorbic acid cocrystal, showing a mixture of cocrystal and AMG 517 monohydrate whose peaks were indicated by the arrows; (E) AMG 517 sorbic acid cocrystal.

the cocrystal samples as compared to AMG 517 itself (0.02 µg/mL), except that L-malic and glutaric acid cocrystals exhibited 12-fold and 4-fold of solubility increase, respectively. XRPD analysis of pre- and post-slurry samples indicated that adipic, benzoic and fumaric acid cocrystals were physically stable in water for 24 h, while L-malic acid cocrystal completely dissociated to AMG 517 monohydrate. Mixtures of cocrystal and AMG 517 monohydrate were detected in the post-slurry samples of other five cocrystals whose stability was approximately ranked in the order of glutaric < benzamide < succinic < maleic < sorbic acid, based on qualitative assessment of AMG 517 monohydrate content in the mixture by XRPD. Powder patterns of pre- and post-slurry samples, represented by glutaric and sorbic acid cocrystals, are shown in Fig. 5. It appeared that less stable cocrystals were more soluble in water as noted for L-malic and glutaric acid cocrystals, and this solubility enhancement was likely due to the counterion effect of the cofomer in solution. Furthermore, the cocrystals comprised of lower water solubility cofomers (fumaric, benzoic, sorbic, adipic acid, solubility < 25 mg/mL) showed better physical stability than those of higher solubility cofomers (L-malic, glutaric, maleic, succinic acid, solubility > 65 mg/mL). This observed cocrystal stability trend was consistent with the solubility trend of the cofomers and further complemented the limited stability data of AMG 517 cocrystals in FaSIF (Schultheiss and Newman, 2009). Benzamide as a cofomer was an exception whose solubility was relatively low but its cocrystal was not very stable. This is probably because hydrogen bonding is weaker in amide than in carboxylic acid. Most interestingly, it was found through screening that AMG 517 cocrystals which were made under both hydrous and anhydrous conditions (fumaric, benzoic, adipic, and sorbic acid) exhibited superior stability in water to those generated only under anhydrous condition (maleic, succinic, glutaric, L-malic acid). The differentiation power in stability of the resulting cocrystals by screening in the absence or presence of water could be useful in identifying and selecting cocrystal candidates with proper stability and solubility properties for further testing (i.e. dissolution, bioavailability).

4. Conclusions

We have developed an integrated 96-well high-throughput cocrystal screening workflow employing a solvent-mediated sonic blending synthesis technique and on-plate XRPD characterization. Taking advantage of expanded design space in 96-well array format, multiple APIs and cofomers in user-selected blend solvents can be screened under the same cocrystallization condition while minimizing API material and saving time and labor. A diversified set of solvents, including water mixtures in particular, were selected not only to level out the solubility difference between API and different cofomers but also to differentiate the hydration/solution stability of the resulting cocrystals. The well-known CO and TO cocrystals were used as model compounds in this screening workflow development, and their cocrystallization outcomes were evaluated and an appropriate volume of solvent for blending was derived with regards to saturation levels of the cocrystal components and physical stability of the cocrystal products under solvent-mediated blending conditions.

To demonstrate the utility and the validity of this HTS workflow, AMG 517 was screened with a number of cofomers in selected blend solvents. In addition to known AMG 517 cocrystals previously obtained, new cocrystals were identified from this screen. In an integrated screen by subsequent exposure of 96-well plate samples to 98% RH and slurry in water, the solid/solution stability and the solubility of the resulting cocrystal samples were investigated by XRPD and HPLC. A stability trend was consistently observed for those stable cocrystals comprised of low solubility cofomers.

The screening results also revealed that the cocrystals formed under hydrous solvent blending condition could possess superior hydration/solution stability to those formed only under anhydrous condition. These findings implicate cocrystallization screening at the presence of water as a means for identifying stable cocrystal candidates that are preferable for further development. Overall this API-sparing HTS workflow, capable of parallel synthesis and subsequent characterization in 96-well plate format, can be an efficient tool in the integrated process of cocrystal design, screen and selection.

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