An automated, high-throughput approach to generating cocrystals with solvent-drop grinding (SDG)

Introduction

Increasing demand for drug candidates has expanded the range of exploration beyond modifications of ionizable active pharmaceutical ingredients (APIs). As a result, there is a growing need for new approaches to polymorphism studies, since screens that target pharmaceutical salts are limited to this category of materials. Cocrystalization techniques are an emerging alternative approach for weakly ionizable or non-ionizable drug candidates. Cocrystalization is a particularly attractive approach because virtually all APIs are potentially capable of cocrystal formation. More importantly, cocrystals are known to improve the "druggability" characteristics of an API, such as solubility, bioavailability and stability.[1]

There are many methods for forming cocrystals, but solvent-drop grinding (SDG) is the preferred approach due to a higher probability of generating cocrystals.[1] To increase the chances of forming and selecting optimal cocrystals, a systematic and expansive screening of variables including different coformers, solvents and solvent combinations is required. Currently, most SDG methods are performed manually and involve a number of tedious and time-consuming steps such as weighing, mortar and pestle grinding and manual transfer for x-ray diffraction (XRD) analysis. While some attempts have been made to automate the method using mechanical milling with steel balls, the compound loading and analytics are still manual. The time and effort required for these manual methods make it difficult – if not impossible – to cover a meaningful fraction of the experimental space. An automated SDG method would eliminate the labor-intensive and time-consuming steps and, when combined with a rational high-throughput approach, would enable exploration of a much broader experimental space. In this application note, we describe the development of an automated high-throughput method for cocrystal screening with Unchained Labs' freeslate system for small molecule preformulation.

Experimental conditions

The goal of the study was to create an automated process to replicate the manual method of cocrystal formation by SDG of glutaric acid/1,2-di(pyridin-4yl) ethane with MeOH (Figure 1) as grinding solvent.

Manual method

Solvent-drop grinding involves adding both coformers and a solvent to a mortar and manually grinding with a pestle. Due to time constraints resulting from the manual mortar and pestle grinding method, no experimental variables other than the number of grinding cycles were explored.

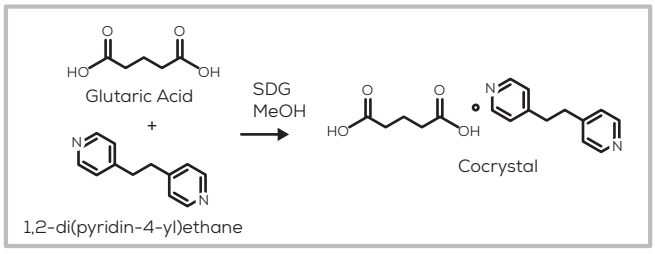


Figure 1: Cocrystal formation by SDG of glutaric acid/1,2-di(pyridin-4yl) ethane with MeOH as grinding solvent.

Row	MeOH µL	Total material loading		# Steel balls	Steel ball size
A	10	10 mg	20 mg	2	Small (2.4 mm)
B	20	10 mg	20 mg		
C	10	10 mg	20 mg	3	
D	20	10 mg	20 mg		
E	10	10 mg	20 mg	1	Medium (3.9 mm)
F	20	10 mg	20 mg		
G	10	10 mg	20 mg	2	
H	20	10 mg	20 mg		

Table 1: Screening conditions for development of an automated SDG method. Typically screens would be performed in an 96-well plate format to evaluate 96 conditions in parallel, but this test case only evaluated 16 conditions in a 96-well plate.

Automated method

The first step in developing an automated method was to evaluate which combination of variables would yield full conversion to the desired cocrystal. The experimental design is shown in Table 1. Typically 96 conditions could be screened at once, but for this test case only 16 conditions were screened in a 96-well plate.

Variables and experimental conditions

- Material loading: [1:1 molar ratio of glutaric acid: 1,2-di(pyridin-4-yl)ethane]
  - 10 mg total: 4.2 mg of glutaric acid and 5.8 mg of 1,2-di(pyridin-4-yl)ethane
  - 20 mg total: 8.4 mg of glutaric acid and 11.6 mg of 1,2-di(pyridin-4-yl)ethane
- Size and number of steel balls for grinding:
  - Small size (2.4 mm): 2 vs. 3
  - Medium size (3.9 mm): 1 vs. 2
- MeOH (grinding solvent) amount: 10 µL vs. 20 µL
- Grinding condition: Vortex with steel balls at 900 rpm for one hour at room temperature

Experiment design

The first step in the automated workflow is to setup the experiment in Library Studio®, which is part of Unchained Labs' Lab Execution and Analysis (LEA) software suite (Figure 2).

The experiment designs are stored in the LEA database and then used by Automation Studio™ to control the system.

Equipment

The SDG method was automated using a freeslate system (Figures 3–4), which includes all the capabilities required for this process, including liquid and solid dispensing, vortexing, weighing and crystallization using a proprietary crystallizer assembly (Figure 4).

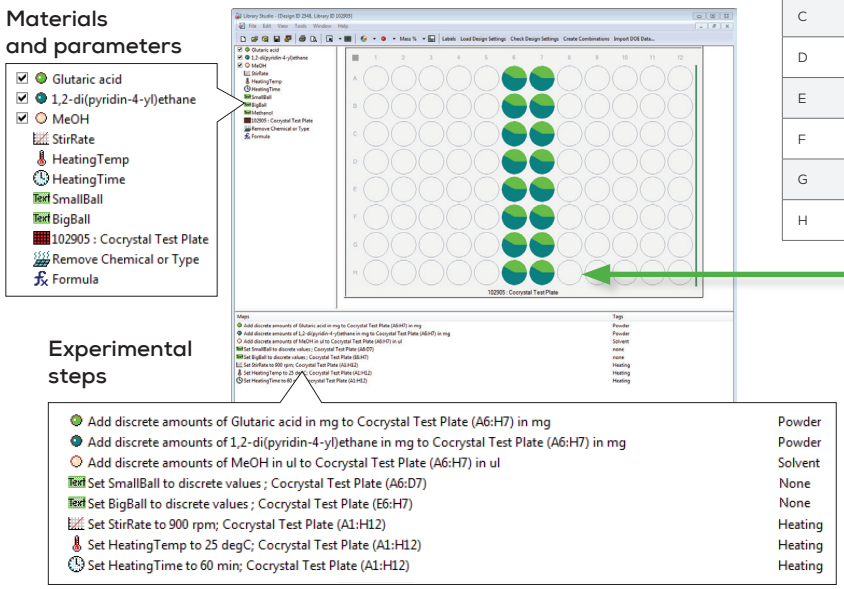
Workflow

An overview of the automated workflow is shown in Figure 5.

1 **Solid dispense**
The 96-well crystallizer assembly is automatically moved to the on-deck balance, and the requested amount of each coformer (glutaric acid and 1,2-di(pyridin-4-yl)ethane) is dispensed (Figure 6).

2 **Addition of steel balls**
Steel balls were added manually to the crystallizer assembly.

3 **Liquid dispense**
The plate is sealed and the freeslate adds MeOH as the grinding solvent to the wells containing the coformers and steel balls (Figure 7).



Row	MeOH µL	Total material loading		# Steel balls	Steel ball size
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E	10	10 mg	20 mg	1	Medium (3.9 mm)
F	20	10 mg	20 mg		
G	10	10 mg	20 mg	2	
H	20	10 mg	20 mg		

Figure 2: The experimental design is entered into Library Studio. The specified recipe includes both chemical (names and amounts) and processing (vortexing) steps in the order they will be executed.

Figure 3: The freeslate system is a fully configurable, automated system for preparing, processing and testing chemically complex samples and mixtures. The freeslate system can be used to automate SDG cocrystal generation using the system's liquid and solid dispensing, vortexing and weighing capabilities.

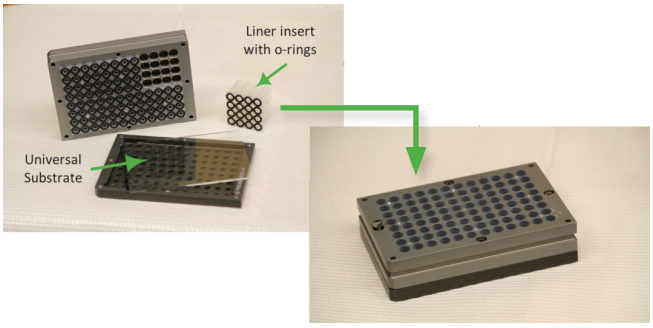


Figure 4: The crystallizer assembly is composed of a 96-well plate, a glass or quartz plate (universal substrate™) and disposable liner inserts. The steel balls and the compounds will be deposited in the liner insert. O-rings at the bottom of the insert will ensure that the material in each well remains isolated. The cocrystals will be formed on the universal substrate, which can be removed for analytical testing without further manipulation of the cocrystals.

Figure 5: Automated method workflow. Steps 1, 3 and 4 are automated by the freeslate for small molecule preformulation.

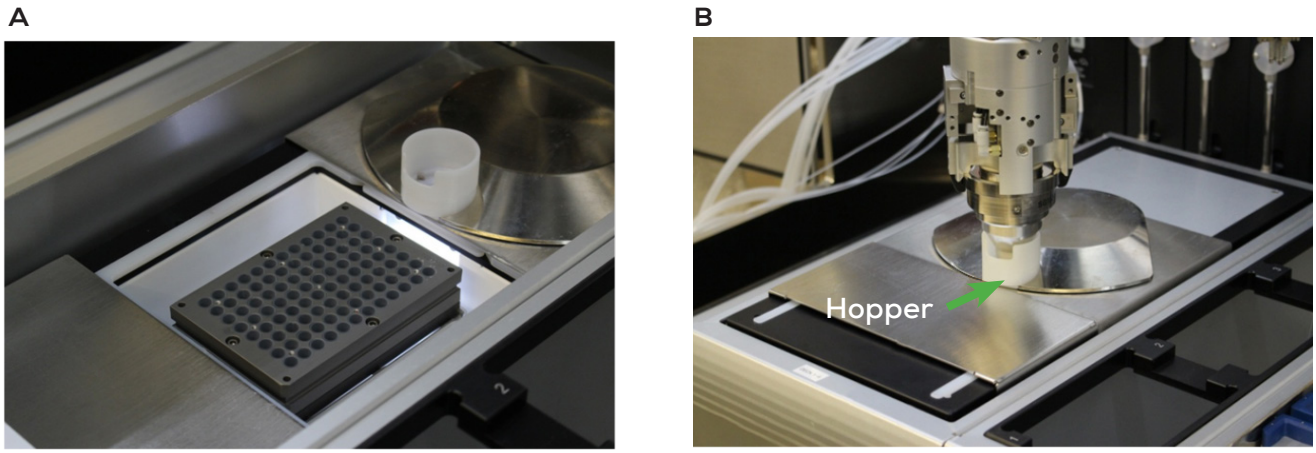


Figure 6: (**A**) The deck of the freeslate with the crystallizer assembly in the on-deck balance. (**B**) The solid dispensing arm with hopper containing powder to be dispensed.

4 **Grinding**
The freeslate vortexes the crystallizer assembly at 900 rpm for one hour at room temperature.

5 **Preparation for analytics**
The steel balls are manually removed using a 96-pin magnetic retriever and the crystallizer assembly is opened as shown in Figure 8.

6 **XRD analysis**
After the experiment is complete, the universal substrate containing the cocrystals is ready for analysis by the XRD. The first step is to run the mapping protocol. This protocol maps the location of the cocrystals in each well and saves this information in the LEA database. Once the plate is mapped, the XRD protocol is run. The XRD will scan each well in the specified location and store the results in the LEA database.

Results

Manual method

The XRD results from the manual method are shown in Figure 9. As the desired cocrystal forms, the peaks related to the individual coformers gradually disappear over four grinding cycles. After four grinding cycles, the individual coformer peaks disappear, indicating complete formation of the cocrystal.

Automated method

LEA provides complete data management by collecting and consolidating data from a wide variety of sources into a single Library ID. For this automated SDG method, the Library Studio design, experimental data from the freeslate and analytics from the Bruker XRD are linked together, allowing easy data viewing and the ability to track back to

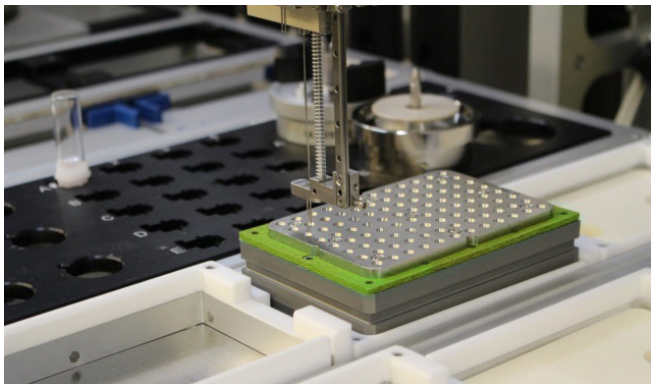


Figure 7: The freeslate automatically dispenses the required amount of solvent to the plate.

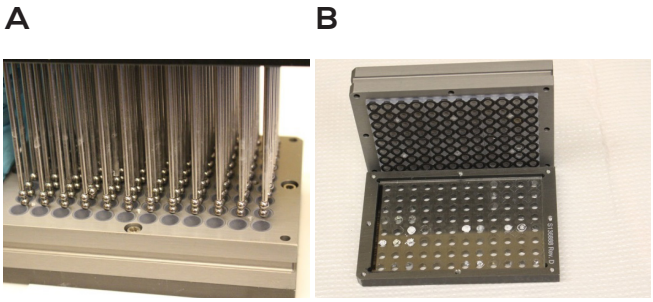


Figure 8: (**A**) Steel ball removal. (**B**) Opening the crystallizer assembly.

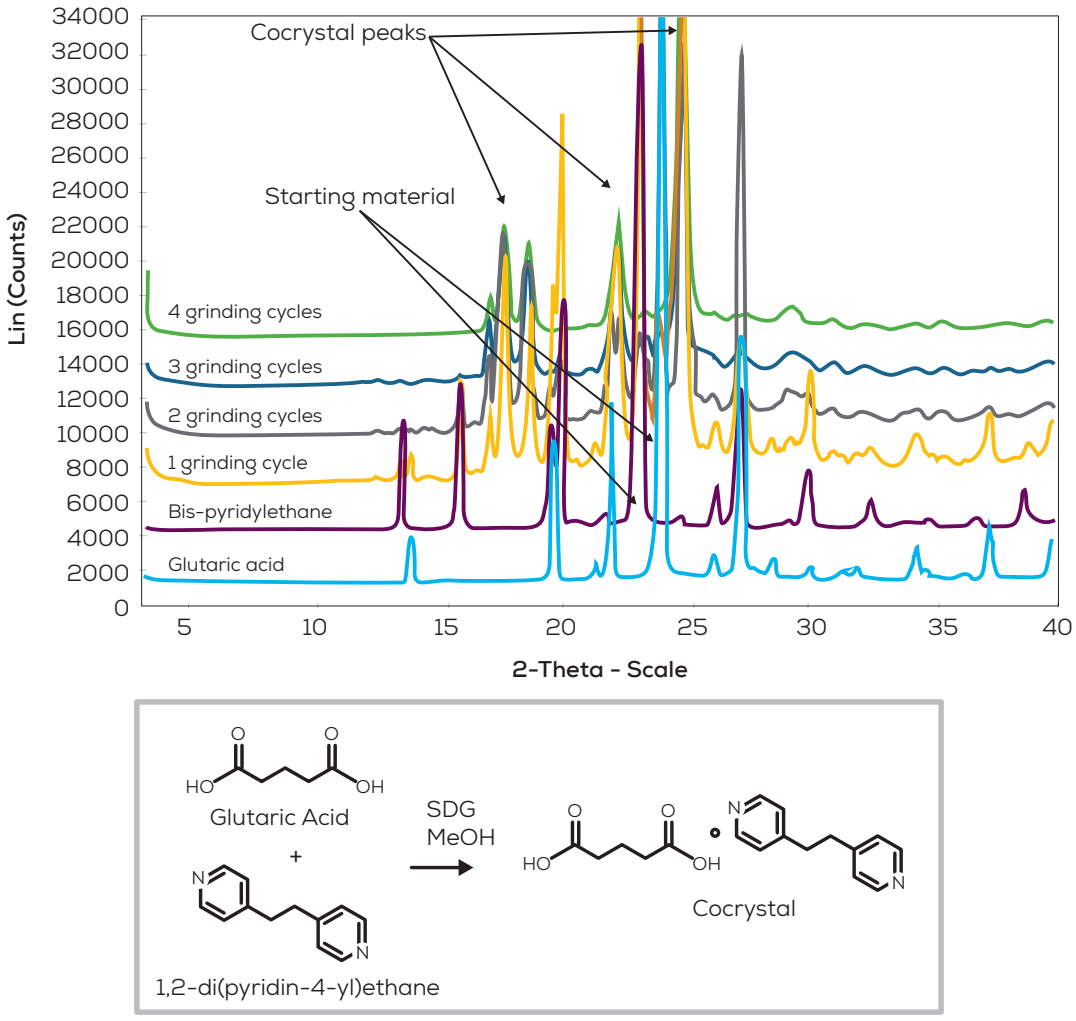


Figure 9: XRD data from the manual SDG method demonstrates that after four grinding cycles, cocrystal formation was complete.

the initial experimental design and conditions, as shown in Figure 10. Data analysis is performed with Spectra Studio™, which allows easy overlay and comparison of the XRD data.

Using this approach, the data was analyzed to determine the best conditions for creating co-crystals using the SDG method. The effects of material loading and volume of grinding solvents are compared in Figure 11. In all cases, the original coformer peaks are less intense, indicating cocrystal formation. The lower intensity of the original coformer peaks indicates more complete cocrystal formation. Comparing the four conditions shows that less total material loading (10 mg) with more grinding solvent (20 µL) provides more complete cocrystal formation.

Comparing the data from the four conditions that used 20 µL MeOH and a total material loading of 10 mg, we can evaluate the effect of the number and size of the steel balls on cocrystal formation. The overlay of these four traces with the original coformers is shown in Figure 12. In this case, trace H6 has a more complete cocrystal formation and the least amount of individual starting material peaks, indicating that more steel balls and larger size steel balls lead to more effective cocrystal formation.

Comparison of the optimized conditions (10 mg total loading, 20 µL of MeOH, with two medium steel balls) with the original materials indicates excellent conversion to the desired cocrystal as shown in Figure 13.

Conclusion

Based on the results of the test cases shown here, SDG cocrystal screening can be automated using Unchained Labs' freeslate system configured for small molecule preformulation. This automated method clearly identified the impact of different variables on cocrystal formation as well as the best condition for cocrystal conversion. After analyzing the XRD data, the following conclusions were clear:

- More steel balls and larger size steel balls resulted in more complete cocrystal formation
- The best cocrystal conversion was seen with 20 µL of MeOH, 10 mg total loading, and two medium size steel balls (Figure 12, condition H6)
- Combined with the other conditions, vortexing at 900 rpm for one hour provides sufficient grinding to generate cocrystals

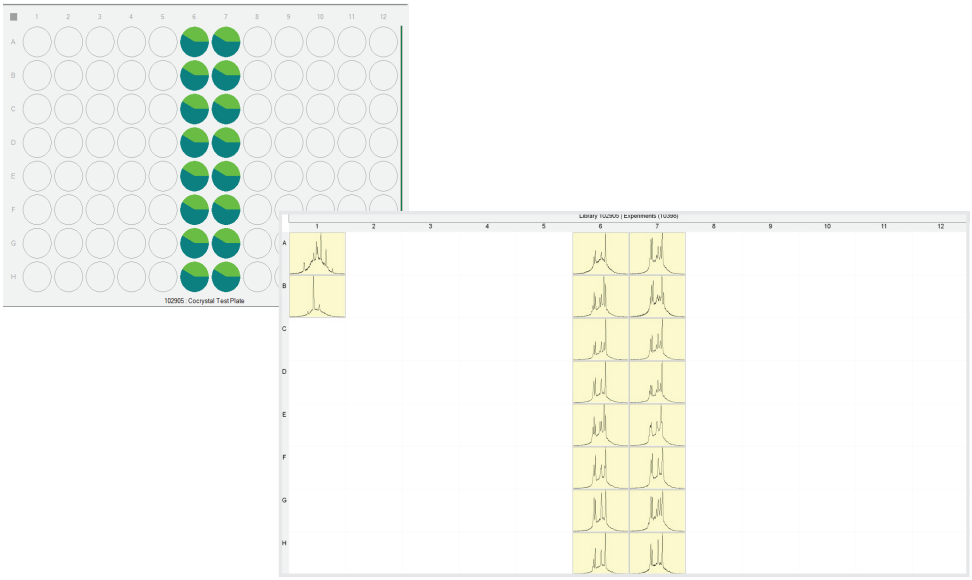
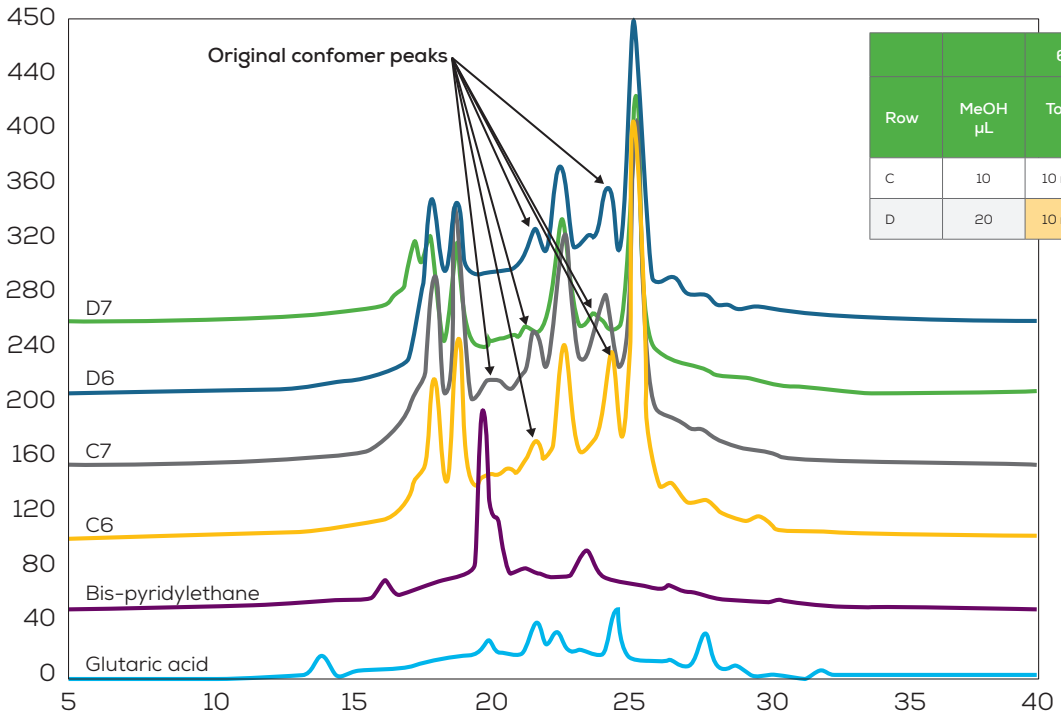
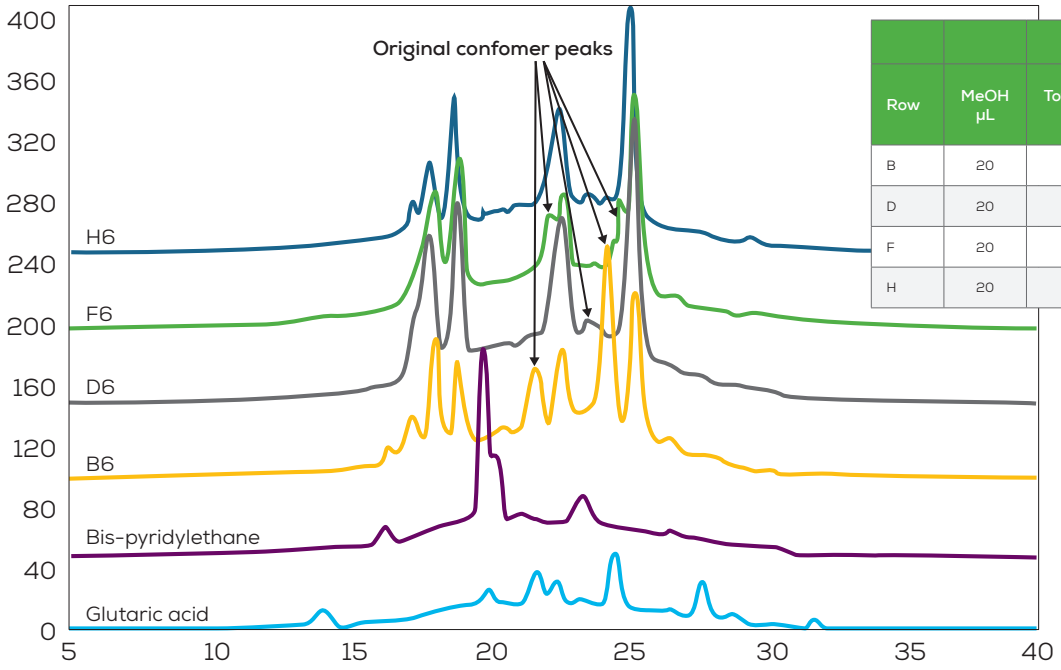


Figure 10: LEA software suite links initial experimental design to results, allowing for easy data viewing and sample tracking.



		6	7		
Row	MeOH µL	Total material loading		# Steel balls	Steel ball size
C	10	10 mg	20 mg	3	Small (2.4 mm)
D	20	10 mg	20 mg		

Figure 11: Comparison of total material loading and volume of grinding solvent. The original coformer peaks are less intense indicating cocrystal formation. Of the four wells compared, the conditions in well D6 (10 mg of material and 20 µl of grinding solvent) provides the most complete cocrystal formation.



		6		
Row	MeOH µL	Total material loading	# Steel balls	Steel ball size
B	20	10 mg	2	Small (2.4 mm)
D	20	10 mg	3	
F	20	10 mg	1	Medium (3.9 mm)
H	20	10 mg	2	

Figure 12: Evaluation of the effect of the number and size of steel balls on cocrystal formation with 20 µl of grinding solvent and 10 mg of material. H6 has the least amount of starting material indicating more complete cocrystal formation demonstrating that more and larger size steel balls lead to more effective cocrystal formation.

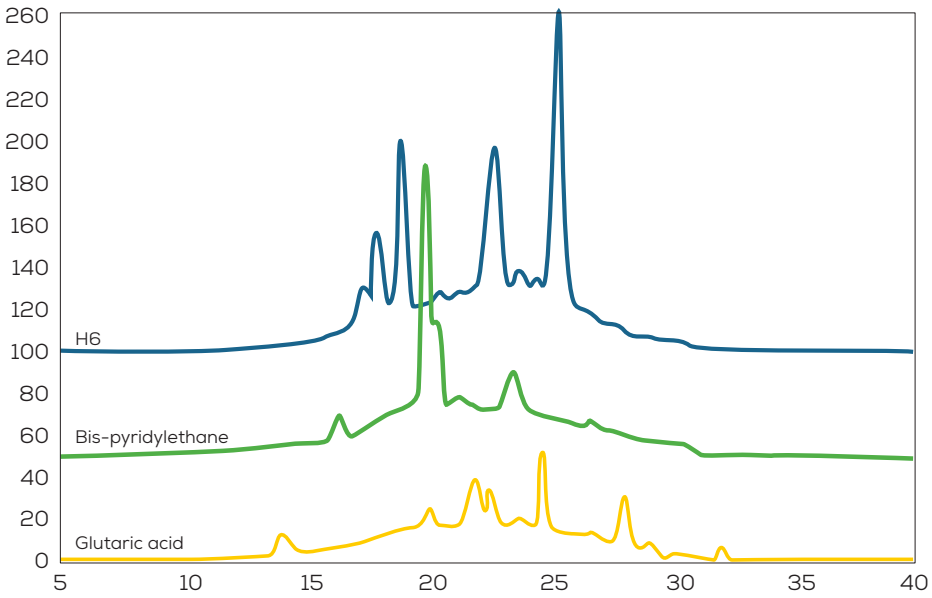


Figure 13: Comparison of optimized method conditions with starting materials. The optimized condition (10 mg total loading, 20 µL of MeOH, with two medium steel balls) has little to no peaks from the original starting materials indicating excellent conversion to the desired cocrystal.

This automated method can now be used for full plate screening involving novel APIs and a broad spectrum of coformers and grinding solvents. By automating SDG, it is possible to eliminate the labor intensive steps, reduce material and solvent usage and quickly implement a truly high-throughput approach to the engineering of pharmaceutical cocrystals.

Using a freeslate system, a scientist can accelerate polymorph screens, finding suitable crystalline forms faster while reducing the risk of late stage failure from emergence of new metastable forms. With automated heating, cooling and mixing, SDG, slurry, precipitation, cooling and evaporation experiments can be completed on a single platform. Data from birefringence, X-ray powder diffraction and Raman spectroscopy can be easily linked to reaction conditions and solvent systems resulting in rapid understanding that can inform sound scientific decision making.

References

1 High-throughput 96-well solvent mediated sonic blending synthesis and on-plate solid/solution stability characterization of pharmaceutical cocrystals, Luu et al., *International Journal of Pharmaceutics*, **441**, (2013), 356–364.

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