High-throughput polymorph screening of active pharmaceutical ingredients

Introduction

Many active pharmaceutical ingredients (API) exhibit polymorphism. Since each polymorph can exhibit different properties, such as solubility and bioavailability, it is important to systematically explore conditions to discover as many potential new forms as possible, as early as possible. To identify potential new forms, the API is typically isolated using a wide range of solvents, processing conditions and crystallization methods. In early development, limited compound supply and time constraints can discourage a comprehensive exploration of conditions. Later in development, polymorph screening can be used to identify whether an API's polymorphic behavior has changed, ensure broad intellectual property coverage and confirm that the most stable form was found. The freeslate system configured for preformulation is an automated platform that can be used from early to late development to perform high-throughput polymorph screening. Using the freeslate system, process development scientists can explore up to 384 different crystallization conditions at a time, using approximately one gram of API to discover potential new crystalline forms during development in as little as three days.

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Powerful software to design, execute and analyze data from polymorph screens

The goal of a high-throughput polymorph screen is to explore as many diverse experimental conditions as possible to discover potential new crystalline forms of the API. Therefore, designing a library that covers a broad range of crystallization methods (e.g., slurry, cooling, evaporation and precipitation), conditions (e.g., time, temperature) and solvents is essential. Figure 1 shows an overview of the polymorph screening workflow that was used for this study.

Prior to starting the polymorph screen, a solvent library consisting of an 8 x 12 array of solvent mixtures was designed using Library Studio®, which is part of Unchained Labs' Lab Execution and Analysis (LEA) software suite. The library consisted of four main solvents (cyclohexane, THF, water and 1,1,2-trichloroethene), which were combined in varying ratios with 16 co-solvents to cover a broad range of crystallization conditions. By using a built-in combination generator and a recipe oriented approach, Library Studio simplifies the design of complex polymorph screens and provides the user with a flexible method to create solvent libraries. Designs are stored in the LEA database and can be recalled by Automation Studio™, the LEA component that controls the instruments that execute the polymorph screen. During the screen, process and analytical data are stored in the LEA database for easy searching and reporting by two additional programs in the software suite, PolyView® and Spectra Studio™.

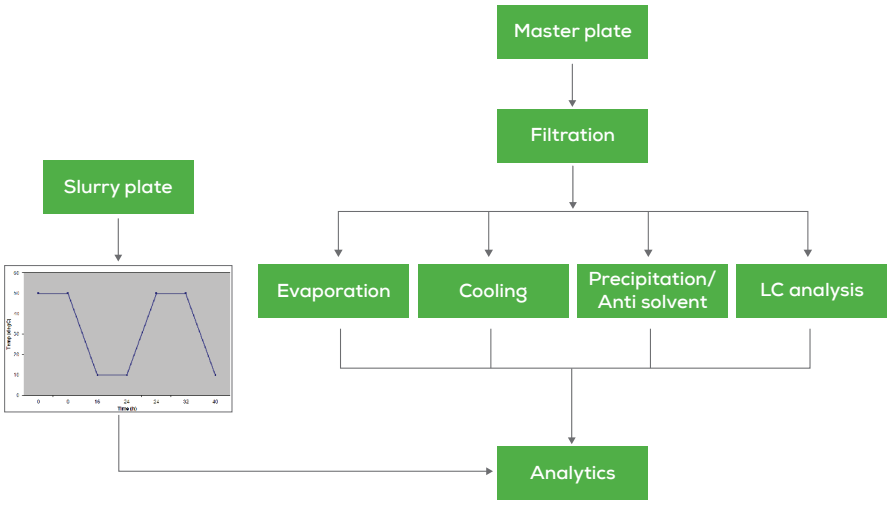


Figure 1: Overview of the polymorph screening workflow using the freeslate system for preformulation.

Screen a broad range of crystallization conditions with the freeslate system configured for preformulation

The freeslate system uses Unchained Labs' proprietary crystallization assembly with universal substrate. The universal substrate enables direct analysis of deposited crystals using techniques such as birefringence imaging, powder x-ray diffraction (PXRD), Raman spectroscopy and visual inspection without disturbing or manually manipulating individual solid samples. For this polymorph screen, four crystallization assemblies, one for each crystallization condition (i.e., evaporation, cooling, precipitation and slurry), were used with the freeslate system.

Slurry experiment

For the slurry experiment, 3 mg of API was added to each of the 96-wells of the crystallization assembly using Powdernium™ solid dispensing technology. For dispensing solvents and viscous liquids, the freeslate system has a single-tip liquid dispenser and a positive displacement tip (PDT) pipette. Using the single-tip liquid dispenser for this experiment, solvents were added to each well of the crystallization assembly as per the Library Studio design (Figure 2). The total volume in each well was 150 µL. Once the solvents were added, plates were heated to 50 °C and stirred for eight hours followed by cooling to 25 °C , at 8 hours were cooled down to 10 °C, maintained at 10 °C from 16 hours to 24 hours, reheated at 24 hours back to 50 °C, maintained at 50 °C from hours 24 to 32 hours and finally, cooled back down to 10 °C on the system's heating/cooling/stirring station (Figure 1). Supernatant was removed from each well by wicking with filter paper and the solids were air dried. The universal substrate was removed from the crystallization assembly for analysis by birefringence imaging, PXRD and Raman spectroscopy.

Master plate

To create the Master Plate for the cooling, evaporation, precipitation and LC Analysis experiments, 8 mg of API was added to each well of a 96-well microplate using Powdernium solid dispensing technology. Using the freeslate system's single-tip liquid dispenser and PDT pipette, solvents were added to each well of the microplate to create binary mixtures as per the Library Studio design (Figure 3). The total volume in each well was 800 µL. Once solvents were added, the microplate was heated to 50 °C on the system's heating/cooling/stirring station. After two hours, the supersaturated solutions containing API were transferred and filtered at 50 °C using the heated 4-tip liquid dispenser and heated filter block. Filtrate was collected in 1 mL glass vials. Filtrate was transferred at 50 °C to three crystallization assemblies for the evaporation, cooling and precipitation experiments and to a microplate for LC analysis.

Main solvents: Water | THF | Cyclohexane | Trichloroethene

Main solvent volume (µL)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	24	48	72	96	120	130	104	78	52	26	0
B	0	24	48	72	96	120	130	104	78	52	26	0
C	0	24	48	72	96	120	130	104	78	52	26	0
D	0	24	48	72	96	120	150	120	90	60	30	0
E	0	30	60	90	120	150	150	120	90	60	30	0
F	0	30	60	90	120	150	130	104	78	52	26	0
G	0	26	52	78	104	130	130	104	78	52	26	0
H	0	26	52	78	104	130	130	104	78	52	26	0

Co-solvent volume (µL)

		1	2	3	4	5	6	7	8	9	10	11	12	
DMF	A	150	126	102	78	54	30	20	46	72	98	124	150	H_2O
Ethanol	B	150	126	102	78	54	30	20	46	72	98	124	150	Toluene
2-Propanol	C	150	126	102	78	54	30	20	46	72	98	124	150	1-Butanol
Acetonitrile	D	150	126	102	78	54	30	0	30	60	90	120	150	n-Butylacetate
2-Butanone	E	150	120	90	60	30	0	0	30	60	90	120	150	Trifluoroethanol
Cyclopentyl methyl ether	F	150	120	90	60	30	0	20	46	72	98	124	150	Ethyl acetate
1, 2-Dimethoxyethane	G	150	124	98	72	46	20	20	46	72	98	124	150	1, 4-Dioxane
1, 2-Dichloroethane	H	150	124	98	72	46	20	20	46	72	98	124	150	Nitromethane

Figure 2: Design of the slurry experiment solvent library.

Main solvents: Water | THF | Cyclohexane | Trichloroethene

Main solvent volume (µL)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	128	256	384	512	640	760	608	456	304	152	0
B	0	128	256	384	512	640	760	608	456	304	152	0
C	0	128	256	384	512	640	800	640	480	320	160	0
D	0	128	256	384	512	640	800	640	480	320	160	0
E	0	160	60	90	120	150	800	640	480	320	160	0
F	0	160	60	90	120	150	800	640	480	320	160	0
G	0	152	52	78	104	130	760	608	456	304	152	0
H	0	152	52	78	104	130	760	608	456	304	152	0

Co-solvent volume (µL)

		1	2	3	4	5	6	7	8	9	10	11	12	
DMF	A	800	672	544	416	288	160	40	192	344	496	648	800	H_2O
Ethanol	B	800	672	544	416	288	160	40	192	344	496	648	800	Toluene
2-Propanol	C	800	672	544	416	288	160	0	160	320	480	640	800	1-Butanol
Acetonitrile	D	800	672	544	416	288	160	0	160	320	480	640	800	n-Butylacetate
2-Butanone	E	800	640	480	320	160	0	0	160	320	480	640	800	Trifluoroethanol
Cyclopentyl methyl ether	F	800	640	480	320	160	0	0	160	320	480	640	800	Ethyl acetate
1, 2-Dimethoxyethane	G	800	648	496	344	192	40	40	192	344	496	648	800	1, 4-Dioxane
1, 2-Dichloroethane	H	800	648	496	344	192	40	40	192	344	496	648	800	Nitromethane

Figure 3: Design of evaporation, precipitation and cooling solvent library.

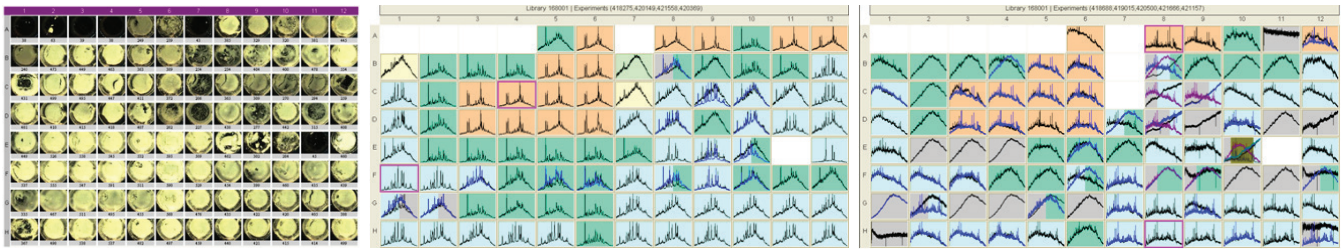
	1	2	3	4	5	6	7	8	9	10	11	12
A	10.13	10.60	10.23	Invalid	10.44	10.54	11.13	Invalid	Invalid	10.69	10.70	3.43
B	2.25	10.53	11.42	11.28	11.16	9.06	4.33	2.31	1.15	3.26	0.14	0.02
C	1.23	10.15	9.76	9.92	10.65	9.98	5.08	5.96	4.82	4.03	2.49	2.26
D	6.83	10.46	10.74	9.88	9.66	10.14	4.73	3.21	1.67	1.20	0.74	2.27
E	2.95	1.45	0.62	0.20	0.01	0.00	0.02	6.12	10.74	10.53	10.59	9.27
F	0.09	0.03	0.02	0.00	0.00	0.01	0.00	0.06	0.77	0.84	1.27	1.10
G	9.58	6.17	2.56	0.79	0.23	0.00	0.20	0.49	0.82	2.97	5.78	10.57
H	0.85	0.41	0.15	0.06	0.00	ND	0.04	0.96	3.00	6.02	8.21	9.37

Table 1: Solubility of the API (mg/mL) measured by HPLC.

The conditions for the evaporation, precipitation, cooling and LC analysis were the following:

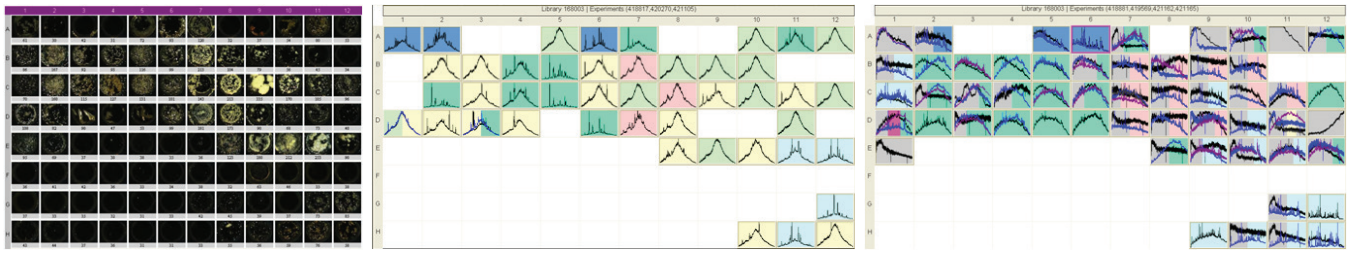
- **Precipitation experiment:** To prepare the precipitation experiment, 300 µL of water was dispensed to wells A1–12, B1–6, C1–6 and D1–6 and 300 µL of heptane was dispensed to wells B7–12, C7–12, D7–12 and E1–H12 of a crystallization assembly to act as anti-solvents. Using the heated 4-tip liquid dispenser, 100 µL of filtrate from each heated filter block vial was transferred at 50 °C to the precipitation crystallization assembly. The resulting plate was allowed to cool at room temperature overnight. Supernatant was removed from each well by wicking with filter paper and the crystals were air dried.
- **Evaporation experiment:** For the evaporation experiment, 200 µL of filtrate from each heated filter block vial was transferred at 50 °C using the heated 4-tip liquid dispenser to the evaporation crystallization assembly. Solvent was allowed to evaporate under ambient conditions.
- **Cooling experiment:** For the cooling experiment, 200 µL of filtrate was transferred from each heated filter block vial at 50 °C using the heated 4-tip liquid dispenser to a crystallization assembly, that was pre-heated to 50 °C. After adding the filtrate, the crystallization assembly was slowly cooled to 5 °C over an eight hour period and then equilibrated at 5 °C for an additional two hours. A constant level of supersaturation was achieved by using a non-linear cooling profile throughout the experiment. Solvent was removed by wicking and the crystals were air dried.
- **LC analysis:** To gauge API's stability under each condition, the remaining filtrates are allowed to dry under ambient conditions, followed by redissolution using 30%THF/70%MeCN and LC analysis. LC traces are used to identify any potential degradation (Table 1).

At the end of each crystallization experiment, the universal substrates were removed from each crystallization assembly for analysis by birefringence imaging, PXRD and Raman spectroscopy. Birefringence images were taken of each well of the universal substrates from the slurry, evaporation, cooling and precipitation experiments using a Zeiss AxioVert 200M microscope. All wells were imaged at ~80X magnification to capture the full diameter of each well. Wells with dark images indicate the absence of crystalline material, and these wells were not analyzed further. Wells with crystalline material were further analyzed using a Horiba Raman spectrometer and a Bruker D8 Discover X-Ray Spectrometer with GADDS. Figures 4–7 show the birefringence images, PXRD and Raman spectra from the analysis of the slurry, evaporation, cooling and precipitation experiments.



Birefringence PXRD Raman spectroscopy

Figure 4: Birefringence, PXRD and Raman Spectroscopy data from slurry crystallization conditions.

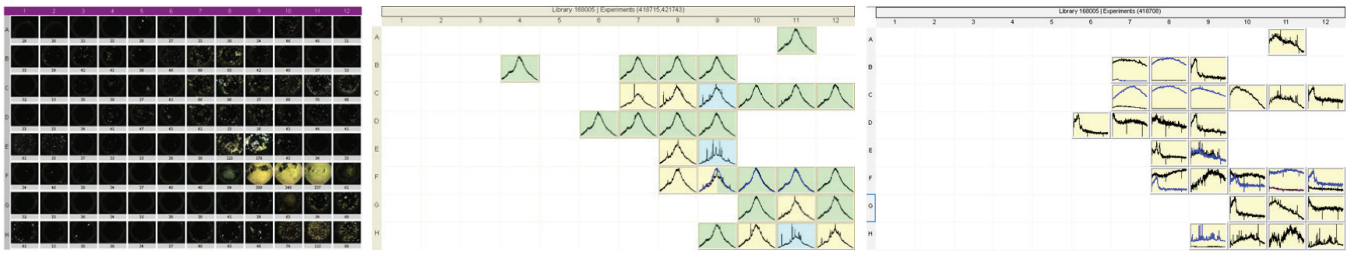


Birefringence PXRD Raman spectroscopy

Figure 5: Birefringence, PXRD and Raman spectroscopy data from evaporation crystallization conditions.

Birefringence PXRD Raman spectroscopy

Figure 6: Birefringence, PXRD and Raman Spectroscopy data from cooling crystallization conditions.



Birefringence PXRD Raman spectroscopy

Figure 7: Birefringence, PXRD and Raman Spectroscopy data from precipitation crystallization conditions.

Using Spectra Studio, PXRD patterns from the slurry, precipitation, cooling and evaporation experiments were grouped together and sorted statistically based on similarity using a Pearson's correlation threshold of 85%. Spectra were then compared, analyzed manually and categorized as amorphous, similar to API, potential new forms and questionable. Raman spectra from the slurry, precipitation, evaporation and cooling experiments were analyzed in a similar manner using Spectra Studio. Wells that were identified as "potential new forms" by both PXRD and Raman analysis were scaled up for further characterization. Wells identified as being a potential new form by either Raman or PXRD analysis (but not both) were considered ambiguous and were also considered as candidates for scale-up and further characterization.

Explore a wide range of crystallization conditions and methods to discover new forms

Analysis of the PXRD and Raman results identified the following as potential new forms from the various crystallization methods:

- Slurry: wells A12, B12, D7 and F9
- Evaporation: wells A6, D1 and D7
- Cooling: well B6

To ascertain whether these wells contained new forms, the experiments were scaled up using 50 mg of API and characterized by birefringence imaging, PXRD, Raman spectroscopy, differential scanning calorimetry, thermal gravimetric analysis and dynamic vapor sorption. From these results, a variety of solvates and one metastable form were identified.

The hydrogenation of *trans*-Cinnamic acid to Hydrocinnamic acid reaction was chosen to benchmark the OSR's performance (Figure 4). Two different types of 5% Rh on Alumina were selected to determine their effects on reaction rates. The design also included four replications for each catalyst support to assess consistency across different reactors. The Rh catalysts evaluated were either on Matrix support (Catalyst 1; Aldrich 212857) or Degussa type support (Catalyst 2; Aldrich 663468). They were both run in the OSR at 40 °C and 50 PSI while stirring overhead at 500 rpm, and time point samples were taken at predefined intervals. The run design is shown in Table 1.

The freeslate system configured for preformulation is an end-to-end solution for automated polymorph screening. The system allows a single scientist to perform hundreds of experiments in parallel with less material than conventional methods for the broad exploration of polymorph space. In this example, Unchained Labs explored 384 different crystallization conditions with approximately one gram of API. The entire polymorph screen was performed by a single scientist and took five days including scale-up activities. Using the freeslate system, process development and materials scientists can systematically explore a wide range of solvents, conditions and crystallization methods to discover potential new crystal forms during development.

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