SUPPORTING INFORMATION

Psilocybin: Characterization of Metastable Zone Width (MSZW), Control of Anhydrous Polymorph and Particle Size Distribution (PSD)

Robert B. Kargbo,* 1 Alexander M. Sherwood, 1 Poncho Meisenheimer, 1 Kelsey Lenoch, 1 Solomon Abebe, 2

1 Usona Institute, 2780 Woods Hollow Road, Madison, WI 53711
2 Almac Sciences, 20 Seagoe Industrial Estate, Craigavon, BT63 5QD, United Kingdom

Table of contents

1 Experimental techniques .................................................................................................S2
1.1 X-ray Powder Diffraction (XRPD) ...........................................................................S2
1.2 Thermogravimetric Differential Thermal Analysis (TG/DTA) ...............................S2
1.3 1H / 13C Nuclear Magnetic Resonance spectroscopy (NMR) ...............................S2
1.4 Optical and hot-stage microscopy ...........................................................................S2
1.5 UPLC .......................................................................................................................S2
1.6 Particle size distribution (PSD) ............................................................................S2
2 Hydrolysis Investigation .............................................................................................S3
2.1 Metastable zone width (MSZW) estimation ..............................................................S5
3 Crystallisation process development ..........................................................................S7
3.1 Drying Studies .........................................................................................................S8
3.2 Effect of powder bed density on drying ....................................................................S10
3.3 Design of Experiments (DoE) ................................................................................S14
3.4 Acetone displacement wash ..................................................................................S22
3.5 Acetone slurries to prevent pattern H formation ....................................................S24
3.6 Process Scale-up .....................................................................................................S26
3.7 Particle size analysis by LLD for scale-up batches ..................................................S40
4 Conclusions ................................................................................................................S44
1 Experimental techniques

1.1 X-ray Powder Diffraction (XRPD)

XRPD analyses were performed using a Panalytical Empyrean diffractometer equipped with a Cu X-ray tube and a PIXcel 1D-Medipix3 detector system. The samples were analysed at ambient temperature in transmission mode and held between low density PVC films. The Almac default XRPD program was used (range 4-40°2θ, step size 0.01313°, counting time 92sec, ~20min run time). Samples were spun at 60rpm during data collection. XRPD patterns were sorted, manipulated using HighScore Plus v4.9 software.

1.2 Thermogravimetric Differential Thermal Analysis (TG/DTA)

Thermogravimetric analyses were carried out on a Mettler Toledo TGA/DSC1 STARe. The calibration standards were indium and tin. Samples were placed in an aluminium sample pan, inserted into the TG furnace and accurately weighed. Under a stream of nitrogen at a rate of 10°C/minute, the heat flow signal was stabilised for one minute at 30°C, prior to heating to 300°C.

1.3 \(^1\)H / \(^{13}\)C Nuclear Magnetic Resonance spectroscopy (NMR)

NMR analysis was carried out on a Bruker 500MHz instrument in DMSO-d\(_6\). Instrumental parameters are listed on the relevant spectrum plots.

1.4 Optical and hot-stage microscopy

Digital microscopy images were acquired using a Keyence VHX-1000E at x500 magnification with cross-polarised light using 3D focus for maximum number of in-focus particles. A grid of 4 x 4 images were acquired using these parameters and stitched together in software to produce a single image.

1.5 Particle size distribution (PSD)

Particle size analysis (PSA) was carried out using a wet dispersion method with parameters outlined in Table S1. on a Malvern Master Sizer 3000.

| Parameter                           | Value                                      |
|-------------------------------------|--------------------------------------------|
| Sample Unit                         | Hydro MV                                   |
| Particle Type                       | Non-spherical particle mode                |
| Scattering Model                    | Mie                                        |
| Particle refractive index (red & blue light) | 1.644                                      |
| Particle absorption index (red & blue light) | 0.01                                       |
| Dispersant Name                     | Heptane                                    |
| Dispersant Refractive Index         | 1.390                                      |
| Background measurements (red & blue light) | 10 sec                                     |
| Sample Measurements (red & blue light) | 10 sec                                     |
| Number of Measurements              | 3 measurement cycles per sample            |
| Delay between Measurements          | 0 sec                                      |
| % Obscuration                       | Low limit: 10%, High limit: 15%            |
| Stirrer Speed                       | 2250 rpm                                   |
| Analysis Model & Sensitivity        | General Purpose & Normal                   |
2 Hydrolysis Investigation

Psilocybin hydrolysis back to psilocin was shown previously and it was thought prudent to understand the conditions that facilitate such hydrolysis prior to attempting crystallization procedure. To determine the rate of degradation at various process temperatures in aqueous solution, experiments were set up at temperatures of 60 °C, 65 °C, 70 °C and 75 °C. Aliquots were taken at 0, 1, 2, 4, and 8 hours for each experiment as shown in Table S2. The samples were analysed by UPLC and the % purity data for API and the psilocin impurity profile can be seen in Figure S1 these are also shown graphically in Figure S2. These experiments suggest the rate of hydrolysis can be approximated at a constant rate, ranging from 0.14% /hr at 60 °C to 0.64% /hr at 75 °C.

| Time (hr) | % Area |        |        |        |        |        |        |        |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|
|          | API (Psilocybin) | 75 °C | 70 °C | 65 °C | 60 °C | 75 °C | 70°C | 65 °C | 60 °C |
| 0        | 99.1   | 99.11  | 99.14  | 99.31  | 0.09  | 0.08  | 0.05  | 0.03  |
| 1        | 98.3   | 98.68  | 98.94  | 99.08  | 0.9   | 0.53  | 0.26  | 0.12  |
| 2        | 97.23  | 98.04  | 98.61  | 98.95  | 1.95  | 1.17  | 0.6   | 0.27  |
| 4        | 95.46  | 97.29  | 97.97  | 98.6   | 3.77  | 2.02  | 1.24  | 0.61  |
| 8        | 93.98  | 95.08  | 97.01  | 98.05  | 5.19  | 4.15  | 2.2   | 1.15  |

Figure S1 - Area % from UPLC for API peak and Psilocin impurity peak

Figure S2 – Contour plot for temperature vs time. Contour bands correspond to respective API % purity levels.
Trend analysis of the data that compare the effect of time and temperature suggests that time is the critical parameter which is reflected in Equation S1, where time has a parameter weighting almost 3 times higher than temperature. This indicates that a higher dissolution temperature, up to a maximum of 75 °C, would be preferable as it would reduce the time above the hydrolysis threshold. The API purity data from these 4 experiments were modelled using JMP 15 statistical software for visualization and prediction purposes.

shows a 2D contour plot which depicts the relationship between time and solution temperature, which was as expected and shows a positive interaction. It shows degradation increasing when either parameter is increased, at much higher rate at higher temperature than at lower temperature. This relationship is mathematically described in Equation S1, with the correlation analysis shown in . This model also shows good correlation between actual and predicted values, with an $R^2$ of 0.97 and low root mean squared error (RMSE) of 0.27 % area.
Equation S1 - Fitted parameters equation for prediction of API purity

\[ \text{Predicted Purity (\%) = } 108.05 - 0.13292 \times T_{\text{process}} - 0.39306 \times \text{Time} - 0.033945((T_{\text{process}} - 67.5) \times (\text{Time} - 3)) \]

2.1 Metastable zone width (MSZW) estimation

Another method which was examined to reduce the extent of hydrolysis was to use a lower seeding temperature. This involved decreasing the starting concentration, which was examined in experiment PL-0005E-001. In this experiment, samples of psilocybin were prepared at various concentrations in water and subjected to the following conditions in a Clarity Unit, which is equipped with an infrared transmission detector:

- Heat from 20°C to 75 °C at 0.5°C/min
- Hold for 10 mins
- Cool to 20°C at 0.2°C/min
- Stirrer speed 800 rpm

From the infrared (IR) transmission data of the sample vials, dissolution and precipitation events were recorded as the point of complete transmission of IR and the onset of turbidity by IR respectively. Experiments were also carried out at a fast-cooling rate (1°C/min) to determine the effect on the MSZW. These experiments showed only slightly lower \( T_{\text{cryst}} \) and much higher variability of \( T_{\text{Cryst}} \).

The estimated \( T_{\text{diss}}, T_{\text{cryst}}, \) and MSZW can be seen in

Figure S
Figure S3 - Metastable zone width data for psilocybin. Solid blue line: Measured MSZW, dotted green line: Estimated $T_{\text{cryst}}$ profile, dotted red line: Estimated $T_{\text{diss}}$ profile.

and the data is tabulated in Table S3.

Figure S3 - Metastable zone width data for psilocybin. Solid blue line: Measured MSZW, dotted green line: Estimated $T_{\text{cryst}}$ profile, dotted red line: Estimated $T_{\text{diss}}$ profile.
This data indicated that a slight increase in process volume could slightly reduce the dissolution temperature but could also give a larger reduction in seed temperature. To determine the effect of the increase in volume on product yield, equilibrium solubility estimations were carried out at 20 °C and 5 °C by slurring for 24 hours in water at each temperature. Concentration was determined by UPLC and solid was also isolated from these solubility slurries and analysed by XRPD to determine if there were any changes in form at the lower temperature. Both samples were determined to be Form B and the solubility at each temperature can be seen in Table S4 along with solubility estimates, as measured by aliquot addition, at higher temperatures. Aliquot addition was used as opposed to equilibrium solubility for these higher temperatures as hydrolysis of psilocybin would obscure the measurements for equilibrium solubility. The combined solubility measurements are shown in Figure S.  

| Experiment | Concentration (mg/ml) | Volumes (V) | $T_{\text{diss}}$ (°C) | $T_{\text{cryst}}$ (°C) | Comments |
|------------|-----------------------|-------------|------------------------|------------------------|----------|
| 1          | 83.3                  | 12          | 75                     | 72                     | 5 min dissolution hold. Dissolved after ~10 mins (after cooling had already begun) |
| 2          | 83.3                  | 12          | 75                     | 69                     | Re-run of previous sample with 10 mins dissolution hold. |
| 3          | 71.6                  | 14          | 74.3                   | 65.4                   | N/A |
| 4          | 62.6                  | 16          | 71.8                   | 61.9                   | N/A |
| 5          | 55.6                  | 18          | 71.1                   | 59.7                   | N/A |
| 6          | 71.6                  | 14          | 74.8                   | 62.5                   | Re-run of a previous sample with 1 °C/min cooling rate |
| 7          | 62.6                  | 16          | 72.4                   | 57.2                   | Re-run of a previous sample with 1 °C/min cooling rate |
| 8          | 55.6                  | 18          | 72                     | 57.8                   | Re-run of a previous sample with 1 °C/min cooling rate |

**Table S4 – Psilocybin Solubility determination**

| Entry | Solvent system | Temperature (°C) | Solubility (mg/mL) | Comment |
|-------|----------------|------------------|---------------------|---------|
| 1     | Water          | 75               | 84.6 - 84.6         | Determined by aliquot addition |
| 2     | Water          | 70               | 56.1 - 63.1         | Determined by aliquot addition |
| 3     | Water          | 65               | 38.3 - 41.6         | Determined by aliquot addition |
| 4     | Water          | 60               | 29.3 - 31.2         | Determined by aliquot addition |
| 5     | Water          | 20               | 10.45               | Equilibrium Solubility, Form B |
| 6     | Water          | 5                | 8.02                | Equilibrium Solubility, Form B |
3 Crystallisation process development – The crystallization experiment was carried out at 5 g scale, in a HEL Polyblock reactor system, according to the procedure in Table S5. Based on the work carried out in the MSZW experiment, there were several changes applied to the received process prior to starting this experiment including increasing the process volume from 12V to 14V, adding seed material as a slurry instead of directly adding solid seed, and the removal of an 8 hours hold between steps 5 and 6 as shown in Table S5. Figure S shows a plot of the seed temperature vs the final temperature and the ideal temperature-solubility profile for cooling.

Table S5 – Procedure for PL-0005E-004

| Step | Description |
|------|-------------|
| 1    | Charge psilocybin (5 g) to water (14V) at 75 °C |
| 2    | Hold 10 mins or until dissolution |
| 3    | Cool to 69 °C |
| 4    | Add seed material in 0.1V water |
| 5    | Cool to 40 °C at 10 °C/hr |
| 6    | Cool to 5 °C at 5 °C/hr |
| 7    | Hold for 9.5 hrs |
| 8    | Filter reactor contents |
| 9    | Charge water (1V) wash |
| 10   | Charge water (2V) wash |
| 11   | Portion wet cake for drying experiments |
| 12   | Dry at respective drying conditions for up to 90 hrs (Section 3.1) |

Reaction temperature and turbidity showed a sharp rise in turbidity can be seen at seed addition (~45 mins), after which turbidity slowly increases during the 10 min aging time before a sharper increase as the cooling ramp begins. At approx. 64 °C, a much more rapid precipitation occurred. A longer seed aging time may help prevent this increase in precipitation.
rate by consuming supersaturation further which should favour crystal growth, however, yield loss to hydrolysis would also increase.

Figure S5 – Temperature-solubility profile for PL-0005E-004. Orange circles: Seed temperature and final temperature, green circle: Concentration at seeding temperature, red dotted line: Ideal temperature-solubility profile for cooling.

3.1 Drying Studies

In order to investigate the drying process of Form B wet cake to Form A, generated following crystallization experiment PL-0005E-004, experiments were conducted at selected temperatures using samples contained both in an open vial and sealed in Polypropylene (PP)/Nylon liner to compare different drying conditions. The respective drying conditions and the outcome of the solid form can be seen in Table S6. In all cases Form A was obtained in the drying condition listed but in some cases pattern H was observed at trace levels. It is apparent that both increased drying time and drying temperature lead to an increased proportion of pattern H. Individual patterns for each sample can be seen in Figure S6, Figure S7, and Figure S8. The anomalous result at 90 hours at 40°C in the PP/Nylon liner indicates possible inhomogeneity. Furthermore, pattern H was observed in samples dried at 35-40 °C that had a cake bed thickness of approx. 25 mm, while samples dried under the same conditions but with a cake bed thickness of approx. 5 mm (such as in Figure S9) contained only Form A. However, even at this lower bed thickness drying at 35-40 °C was still unpredictable with respect to pattern H formation (Table S6). As seen in Figure S6-S8, drying at 20-25 °C consistently produced Form A, with the caveat of requiring a much longer drying time in comparison to 35-40 °C. Cake bed thickness was again shown to be important in the drying process at 20-25 °C, although at this temperature, a larger cake bed depth caused slower conversion from Form B to Form A but still showed no pattern H formation, indicating a lower risk process albeit with the disadvantage of longer processing times. Furthermore, pattern H was observed in samples dried at 35-40 °C that had a cake bed thickness of approx. 25 mm, while samples dried under the same conditions but with a cake bed thickness of approx. 5 mm contained only Form A. However, even at this lower bed thickness drying at 35-40 °C was still unpredictable with respect to pattern H formation. Drying at 20-25 °C consistently produced Form A, with the caveat of requiring a much longer drying time in comparison to 35-40 °C. Cake bed thickness was again shown to be important in the drying process at 20-25 °C, although at
this temperature, a larger cake bed depth caused slower conversion from Form B to Form A but still showed no pattern H formation, indicating a lower risk process albeit with the disadvantage of longer processing times.

Table S6 - XRPD results for the drying experiments of Form B at 40 – 55 °C

| Container   | Temperature (°C) | XRPD | Drying Time (hr) | XRPD Pattern |
|-------------|------------------|------|------------------|---------------|
|             |                  |      | 0 (WC)           |               |
| Open Vial   | 40               | B    | 16               | - ✓ - ✓ + ✓   |
|             | 45               | B    | 25               | + ✓ + ✓ ++ ✓  |
|             | 55               | B    | 90               | +++ ✓ +++ ✓ +  |
| PP/Nylon Liner | 40     | B    | 16               | - ✓ + ✓ - ✓   |
|             | 45               | B    | 25               | - ✓ + ✓ + ✓   |
|             | 55               | B    | 90               | - ✓ + ✓ + ✓   |

- No pattern H observed, + indicates relative intensity of pattern H peak, ✓ major component

Figure S6 – XRPD data for Drying of Form B up 16 hours

Figure S7 - XRPD data for drying of Form B up to 25 hours
3.2 Effect of powder bed density on drying

As many of the solids in the small-scale drying experiments displayed Form A only, scale appeared to be a significant factor in the formation of pattern H during drying. Therefore, it was thought that the bulk density and/or solid depth of the material during drying could be facilitating conditions which are conducive to pattern H formation.

In order to test this hypothesis, two crystallisation experiments PL-0005E-007 and PL-0005E-008 were carried out according to Table S7. The process volume was further increased from the previous experiment to facilitate the addition of a polish filtration to the crystallisation process. Prior to drying, isolated solid from PL-0005E-007 was densely packed into a PP/Nylon liner with a cake bed thickness of approx. 25 mm, whilst material generated from experiment PL-0005E-008 was spread out in a borosilicate glass dish to a bed thickness of approx. 5 mm, and placed into a PP/Nylon liner (see Figure S9).

**Table S7 - Process outline for Experiments PL-0005E-007 and PL-0005E-008**

| Step | Description |
|------|-------------|

Figure S8 – XRPD data for Drying of Form B for up to 90 hours
1. Charge psilocybin (3 g) to water (15.5 V, 46.5 ml) at 73 °C
2. Cool to 70 °C
3. Hold 20 mins
4. Add 0.5 V, 1.5 ml mimic wash
5. Cool to 67 °C
6. Add 0.5% w/w (wrt input) seed material in 0.1 V, 0.3 ml water
7. Hold 10 mins
8. Cool to 5 °C at 10 °C/hr
9. Hold for 11 hrs
10. Filter reactor contents
11. Charge water (1 V, 3 ml) wash
12. Charge water (2 V, 6 ml) wash
13. Charge 2 x Acetone (3 V, 9 ml) wash
14. Dry at 35-40 °C under vacuum with N₂ bleed

Figure S9 - Drying container for PL-0005E-008 in open and close drying conditions

After the drying process of Form B, XRPD was carried out on samples taken at two points for PL-0005E-007-01 i.e., at the core and edge of the powder mass. XRPD patterns can be seen in Figure S10, showing complete conversion of Form B to Form A after only 6 hours, but also showing trace amounts of pattern H. Samples were taken from the outer edge of the powder mass, as well as from the central core of the powder mass. Both samples showed the same pattern indicating the conditions for pattern H formation are not localised within in the powder mass and, at least at this scale, are consistent throughout.

Figure 3 - XRPD of PL-0005E-007. Top: full pattern, Bottom: zoomed in on peak indicative of pattern H
On the dried PL-0005E-008, XRPD was also carried out. XRPD patterns are shown in Figure 3 with reference XRPD patterns. Results showed complete conversion from Form B to form A, again after 6 hours, however, with this experiment there was no trace of pattern H. Sample taken after 25 hrs also showed Form A with no trace of pattern H suggesting the cake bed thickness is a critical parameter. A separate sample (PL-0005E-008-01) was dried at ambient temperature under vacuum with no N\textsubscript{2} flow which again showed only Form A after 36 hours.
Thermogravimetric analysis (TGA, Figure S) exhibited weight loss of 0.55\% w/w, well within the desired specification for water content.

Microscopy of PL-0005E-007 and PL-0005E-008 can be seen in Figure Error! Reference source not found.. PL-0005E-007 showed what appeared to be lath-like crystals with a relatively small proportion of fines. Samples were taken at several stages during PL-0005E-008 experiment, before isolation, after isolation and at different stages of filtration. A sample of the slurry before isolation showed long, thin needles. Samples images indicate that during filtration shapes and size of the particles have changed due to the breakage of needles during isolation. Subsequent samples show a gradual decrease of particle sizes as a result of successive vacuum filtrations cycles. The initial samples (PL-0005E-008-[02-03]) were still quite wet so the particles appear to have agglomerated, however, this is only aggregation which was easily dispersed by agitating the cover slip. The particle size in PL-0005E-008-06 (wet cake) is larger than for PL-0005E-007, however, this is not representative due to the weaker vacuum used during isolation.
3.3 Design of Experiments (DoE)

The planned set of DoE experiments were designed to determine the effect of both seed temperature and seed loading on the PSD of the recrystallisation product. The experiments were all carried out according to Table S8, the only change made from the process used in the previous two experiments was to extend the aging time at step 7, between the seed addition and the start of the cooling ramp. The purpose of this approach was to consume a larger proportion of supersaturation, prior to cooling, in an effort to obtain higher degree of control in subsequent crystallisation favouring particle growth over nucleation. The parameters for steps 5 and 6 (seed temperature and loading respectively) can be seen in Table S9.
1. Charge psilocybin (3 g) to water (15.5V, 46.5 ml) at 73 °C
2. Cool to 70 °C
3. Hold 20 mins
4. Add 0.5V, 1.5 ml mimic wash
5. Cool to seed temperature
6. Add seed material in 0.1V, 0.3 ml water
7. Hold 30 mins
8. Cool to 5 °C at 10 °C/hr
9. Hold for 11 hrs
10. Filter reactor contents
11. Charge water (1V, 3ml) wash
12. Charge water (2V, 6ml) wash
13. Charge 2 x Acetone (3V, 9ml) wash
14. Pull dry on sinter for 5 mins
15. Dry at 35-40 °C under vacuum with N₂ bleed

Table S9 - Experimental parameters for each of the DoE runs

| Experiment number | Seed Temperature (°C) | Seed Loading (% w/w) |
|-------------------|------------------------|----------------------|
| PL-0005E-010-01   | 70                     | 0.1                  |
| PL-0005E-010-02   | 70                     | 0.5                  |
| PL-0005E-010-03   | 70                     | 1                    |
| PL-0005E-010-04   | 67                     | 0.1                  |
| PL-0005E-010-05   | 67                     | 0.5                  |
| PL-0005E-010-06   | 67                     | 1                    |
| PL-0005E-010-07   | 64                     | 0.1                  |
| PL-0005E-010-08   | 64                     | 0.5                  |
| PL-0005E-010-09   | 64                     | 1                    |
| PL-0005E-010-10   | 67                     | 0.5                  |

The drying process was also kept the same as PL-0005E-008, with material being spread to a bed depth of approx. 5mm. Four batches showed trace amounts of pattern H, indicating this method does not consistently achieve pure Form A.

As PL-0005E-010-02 and PL-0005E-010-03 both showed trace amounts of pattern H after drying, an aliquot was taken from each of the subsequent batches and dried at ambient temperature under vacuum to monitor the reliability of this method in addition to drying at 35-40 °C. XRPD also showed traces of pattern H in PL-0005E-010-07 and PL-0005E-010-09 however, the samples from these batches which were dried at ambient temperature showed only Form A. Figures S14-S17 depict various drying conditions that did inform the conditions that produce Form A and minimize the formation of Form H.
Figure S14 - XRPD of PL-0005E-010 for 35-40 °C drying. Bottom overlay on PL-0005E-010-02 and PL-0005E-010-03 which had shown trace pattern H

Figure S15 - XRPD for PL-0005E-010-[04-06] Form B dried at ambient temperature
Figure 4 - XRPD of PL-0005E-010 for 35-40 °C drying. Bottom overlay on PL-0005E-010-07 and PL-0005E-010-09 which had shown trace H
Figure S17 - XRPD for PL-0005E-010-[07-10] dried at ambient temperature
Microscopy images are shown in Figure and show the general trend in particle size according to which seeding parameters were used can be seen, with the average calculated equivalent circle diameter shown in Table S10.

**Figure S18 - PLM for various seed loading conditions for PL-0005E-010**
PL-0005E-010-01 (seeding temp. 70 °C & seed load 0.1 %)

PL-0005E-010-02 (seeding temp. 70 °C & seed load 0.5 %)

PL-0005E-010-03 (seeding temp. 70 °C & seed load 1 %)

PL-0005E-010-04 (seeding temp. 67 °C & seed load 0.1 %)

PL-0005E-010-05 seeding temp. 67 °C & seed load 0.5%

PL-0005E-010-06 seeding temp. 67 °C & seed load 1%
PSA was initially carried out by LLD, however, the method was found to be breaking the particles during measurements and was therefore found to be unsuitable for analysis. As a result, particle size was measured by calculating the diameter of an equivalent circle (by area) for approx. 1000 particles per sample and using the average of these measurements. These results are displayed in Table S10 and are presented as a contour plot in Figure S generated using JMP 15 statistical software. It should be noted that the outlier, PL-0005E-010-10, was removed from Figure S. These data indicate particle size positively related to temperature and negatively related to seed loading. The effect of seed loading was lessened as the seed temperature decreased, possibly due to the fact that the crystallisation likely became nucleation dominated at lower seeding temperatures due to a higher supersaturation ratio.

| Experiment number | Seed Temperature (°C) | Seed Loading (% w/w) | Diameter of equivalent circle (µm) |
|-------------------|-----------------------|----------------------|-----------------------------------|
| PL-0005E-010-01   | 70                    | 0.1                  | 23.2                              |
| PL-0005E-010-02   | 70                    | 0.5                  | 20.1                              |
| PL-0005E-010-03   | 70                    | 1                    | 18.2                              |
| PL-0005E-010-04   | 67                    | 0.1                  | 19.6                              |
| PL-0005E-010-05   | 67                    | 0.5                  | 18.7                              |
| PL-0005E-010-06   | 67                    | 1                    | 17.6                              |
3.4 Acetone displacement wash

In order to reduce drying time and temperature, an acetone cake wash was added to the isolation protocol to displace as much residual water as possible with acetone which should dry faster at the lower temperatures needed. Previous work during the polymorph screen indicated there were no acetone solvates and a slurry of Form A in acetone showed no change after 3 days.

To test this, ~2g of Form A was slurried for 3 hours in water and was confirmed to have converted to Form B. This slurry was then isolated on a sinter and washed with approx. 3 V water. At this stage the cake had formed a wet paste which retained water readily (this had previously been observed by the chemistry team). Half of the filter cake was removed, and this was further split into 2 portions, one to dry with an N₂ bleed in the oven and one without. A sample was also removed at this point and slurried in acetone overnight (PL-0005E-006) to check for any form changes, however this remained Form B. The remaining filter cake was levelled and washed with 2 x 3 V acetone. After the first acetone wash, the cake was a powder with no sign of the previous paste behaviour, after the second acetone wash, the cake was more freely flowing which suggested a drier powder. The powders were placed into liners, then dried at 35-40 °C at ambient pressure overnight to increase humidity, then vacuum and N₂, where applicable, were applied. The experiment was also repeated with vacuum and N₂ being applied immediately. Results can be seen in Table S11 and individual XRPD patterns can be seen in...
Figure S5 and Figure S6. All results showed Form A, suggesting at this scale than neither acetone wash, N\textsubscript{2} bleed, nor timing of vacuum application have any effect on pattern H formation. The acetone wash was kept in the isolation protocol due to the reduction in residual moisture and subsequent reduction in drying time.

| Sample              | Acetone wash | N\textsubscript{2} Bleed | XRPD | Comment                                      |
|---------------------|--------------|---------------------------|------|----------------------------------------------|
| PL-0005E-005-01     | X            | X                         | A    | Overnight hold at drying temp but ambient pressure |
| PL-0005E-005-02     | X            | ✓                         | A    | Overnight hold at drying temp but ambient pressure |
| PL-0005E-005-03     | ✓            | X                         | A    | Overnight hold at drying temp but ambient pressure |
| PL-0005E-005-04     | ✓            | ✓                         | A    | Overnight hold at drying temp but ambient pressure |
| PL-0005E-005-05     | N/A          | N/A                       | B    | weight cake sample taken after acetone wash   |
| PL-0005E-005-06     | X            | X                         | A    | Vacuum applied immediately                    |
| PL-0005E-005-07     | X            | ✓                         | A    | Vacuum and N\textsubscript{2} applied immediately |
| PL-0005E-005-08     | ✓            | X                         | A    | Vacuum applied immediately                    |
| PL-0005E-005-09     | ✓            | ✓                         | A    | Vacuum and N\textsubscript{2} applied immediately |

Figure S5 - XRPD data of Form B after acetone wash and drying up to 21 hours (PL-0005E-005-[01-04])

Figure S6 - XRPD data of Form B after acetone wash and drying up to 25 hours (PL-0005E-005-[01-04])
3.5 Acetone slurries to prevent pattern H formation

Due to the inconsistency of the drying process in delivering pure Form A, and the fact that the temperature which is considered ‘safe’ from pattern H formation has been steadily decreasing throughout, therefore an alternative method for obtaining Form A was investigated. This involved the use of acetone slurries to convert any other forms to Form A. Two approaches were taken:

- **PL-0005E-011-01**: Slurry of Form A material, with pattern H impurity, in 10V acetone for 2 days followed by 2 hours drying under vacuum at ambient temperature.
- **PL-0005E-011-02**: Slurry of Form B material in 40V acetone for 2 days followed by 2 hours drying under vacuum at ambient temperature.

The rationale behind PL-0005E-011-01 was that if Form A was the most stable form in acetone at this temperature, as suggested by previous work with the Physical Sciences Polymorph Screening team, any remaining pattern H should convert to Form A in suspension. XRPD showed only Form A (although with baseline aberration at 17.6 ° 2θ indicating a potential extra peak). This could possibly be used as a failure loop in the event of trace pattern H. However, further study is required understand the complete interconversion of Form A to Form H and vice versa, which seems to be always isolated as a mixture of both forms.

Experiment PL-0005E-011-02 was designed to simplify the drying process. The current drying process involves both the removal of water from the crystal lattice and the removal of residual moisture/solvent. The difficulties with pattern H appear to stem from the drying of Form B to Form A, indicated by the previous screening work which appears to show up to, and including, 80 °C to be safe against pattern H formation when drying pure Form A. To prevent this, it is possible to use a slurry with a low enough water activity to dehydrate the Form B material to Form A, which could then be dried solely for residual solvent. Being a trihydrate, Form B has a high water content (~15%) and when paired with the sharp water activity curve (Figure S7), this means that a large volume of acetone would be required to counteract this water content and provide a liquor of low enough water activity to dehydrate to Form A. XRPD shows this method was successful in converting to Form A, however, did show a broad peak at 18° 2θ (Figure S23).
Figure S7 - Water activity in acetone as a function of volume fraction

Figure S8 - XRPD of PL-0005E-011-01 and PL-0005E-011-02
3.6 Process Scale-up

In order to assess the impact of scale up in a jacketed reactor environment, studies were conducted at 10.75-12g scale using a Radley's 250ml reactor vessel equipped with temperature probe and a process analytical technology (PAT) instrument (BlazeMetrics 900). The PAT instrument is equipped with high dynamic range turbidity and microscopy as well as Blaze advanced chord length (A-CLD) particle tracking system (see Figure S9).

![Figure S9: Image of empty Radley's reactor apparatus with PAT probe](image-url)
The first process (JM-0033E-001-01) was conducted using conditions most favourable to larger particle size, based on initial observations from the DoE investigation. Seed temperature of 70°C and seed loading of 0.1%w/w were applied (Error! Reference source not found.). In each experiment the input material was used as seed. The stir rate used (330 rpm) calculated using the Dynochem model based on constant power per unit mass in relation to the stir rate employed during DoE experiments.

Table S12: Process outline for JM-0033E-001-01

| Step | Description |
|------|-------------|
| 1    | Charge psilocybin (10.75g) (WGF-00017E-038) to 250mL Radleys reactor $T_j = 70^\circ$C |
| 2    | Add water (15.5V, 166.625ml) heated to ~73 °C. Following addition to the reactor $T_{process}$ was = 61°C. |
| 3    | Heated to $T_{process}$ 73 °C over ~30mins. Stir rate = 330rpm |
| 4    | Held for ~10mins, add water (0.5V, 5.375 ml) as mimic wash |
| 5    | Cool to $T_{process}$ 70 °C over ~15 mins |
| 6    | Add seed material (0.1%w/w, 10.8mg) in water (0.1V, 1.075 ml) |
| 7    | Hold 30 mins |
| 8    | Cool to 5 °C at 10 °C/hr |
| 9    | Held for 12.9 hrs |
| 10   | Filter reactor contents -50kPa (500mBar) required to filter. Time to dry land = ~3 minutes, further ~0.5 mins to deliquor. |
| 11   | Charge water (1V, 10.75ml) wash |
| 12   | Charge water (2V, 21.5ml) wash |
| Step | Description |
|------|-------------|
| 13   | Charge 2 x Acetone (3V, 32.25ml) wash |
| 14   | Pulled dry on sinter for 15 mins |
| 15   | Wet cake weight = 11.87g. Solid placed in 60mL amber jar, solid level near the top. |
| 16   | Dry at RT (oven set at 20-25 °C) under vacuum with nitrogen bleed. Pack cake deeply, depth = 5cm. Sample after 36 hrs at top, middle and bottom of cake by XRPD. Further samples were taken at same cake locations after total drying time of 102, 122 and 145 hours. Still Form B present in each sample with high proportion in bottom and middle. Batch was spread to depth of approximately 1cm and dried for further 45 hours. XRPD analysis indicated Form A only. Yield= 88.7% |

Images and data collected using PAT are shown in Figure S10. As shown in Figure S10 after seeding and during the hold period prior to cooling an increase in particle count was not observed showing neither crystal growth nor nucleation present. A sharp rise in particle count for 40-100 µm (amber colour trend line) population observed after about 30 minutes after cooling began, about 45 minutes after the cooling started (at ~62.5°C) a significant increase in turbidity and particle counts were observed. The images indicated rapid change in number of particles between approximately 67°C-66.5°C and conversion to needle habit. Subsequently, a drop in rate of crystallisation was observed, indicated by the turbidity and particle count rising slowly and step decrease of 40-100µm (amber colour trend line) counts until near final temperature of 5°C. During the aging period of ~13 hours, the particle counts remained fairly consistent as depicted by PAT, however, when observed closely, the image taken just before isolation indicates an increase in proportion of smaller particles <100 µm in length were present, suggesting that attrition of crystals had occurred to some extent in aging process.

**Figure S10: PAT images at selected stages in experiment JM-0033E-001-01**

![PAT images at selected stages in experiment JM-0033E-001-01](image-url)
Filtration of the batch was initially attempted using the same vacuum level employed in the DOE studies (-30kPa) but this was insufficient and -50kPa was applied. However, filtration was not very rapid (~3 minutes to reach dry land/top of cake).

Drying of the isolated solid was performed at ambient temperature (20-25°C) to avoid Form H formation. The cake was added to a glass jar at a relatively large depth of 5 cm. In order to assess effect of cake depth on drying and form conversion, samples for XRPD analysis were taken from the top, middle and bottom of cake at selected intervals to a total of 145 hours drying time. Results are shown in Figure S12. This data indicates that cake depth has a very significant impact on efficacy of drying and conversion to anhydrate Form A. The results reveal that after 36 hours drying, a relatively high proportion of Form B remained in the bottom and middle of the cake and no significant further conversion to A occurred up to 145 hours. Even samples taken from the top of the cake, still displayed trace Form B after 145 hours, suggesting that the presence of large depth of cake underneath isolated particles has a negative impact on completion of dehydration to A. In order to achieve complete conversion, the solid was spread to a depth of ~1cm and dried for a further 45 hours. Subsequent XRPD analysis indicated presence of Form A only. Yield obtained (88.7%) was comparable to that obtained in DOE studies.

Images of dried solid taken by polarised light microscopy (PLM) (Figure S11) displayed significant change in particle size and morphology to that of observed by PAT prior to isolation.
The crystals were of reduced size and length and were not of consistent needle shape but of broader habit, indicating severe particle breakage to have occurred during filtration.

Figure S11: PLM images of isolated material from experiment JM-0033E-001-01

Figure S12: XRPD of solid isolated from experiment JM-0033E-001-01, following various durations of drying (H=hrs). T=top of cake, M=middle, B=bottom
In order to determine if the resultant particle size could be increased further, a slower cooling rate of 3°C/hour was used in the next experiment (JM-0033E-002-01). A description of the process is shown in Table S13. To maximise the impact of the seeding step, a lower seed temperature of 67°C and higher seed loading of 0.5% w/w were employed. This process entailed use of a batch of GMP input material which displayed noticeably different dissolution behaviour, complete dissolution occurring at around 70°C.

Images and data collected using PAT are shown in Figure S. Following seed addition, a small increase in turbidity and particle count were observed by PAT (Figure S). The image plane of the PAT instrument was changed and optimised during the course of initial cooling, in order to achieve better visualisation of particles and therefore enhanced analytical response.

A more significant rise in crystallisation rate was observed around 65°C with rod-shaped crystals apparent (see Figure S). However, rapid transition to particles of needle habit had occurred by ~64°C. The PAT indicated a slower rate of crystallisation by turbidity and particle count than that of the previous experiment (JM-0033E-001-01) which has used a faster cooling rate. In this experiment, the turbidity in displayed a more gradual gradient up to the final temperature. A decrease in count of larger particles of 125-400 µm during cooling (Figure S) suggested occurrence of breakage.

After short aging time of 1.2 hrs, the batch was isolated via filtration at -50kPa vacuum. Filtration was rapid (~20 seconds) and following application of washing regime, the wet cake was packed to depth of 5cm in a glass jar prior to drying at ambient temperature. A small sample was also removed and spread thinly (approx. 1-3mm) in order to confirm that complete conversion to Form A could be achieved with a minimal cake depth. XRPD results obtained (Figure S3014).
Figure S3014: XRPD of solid isolated from experiment JM-0033E-002-01, following various durations of drying (H=hrs). T=top of cake, M=middle, B=bottom

Images and data collected using in situ PAT are shown in Figure . As with experiment JM-0033E-001-01 which also used low seed loading of 0.1%w/w, no particle growth or nucleation was observed by PAT during equilibration period prior to cooling. Between ~64-62.5°C transition from the rod habit of the seed material to larger needles was observed from images taken by PAT probe (Figure ). This was accompanied by rapid rise in number of particles of 40-100µm. The patterns of particle count and turbidity during subsequent cooling appeared to be generally more gradual than those of experiment JM-0033E-001-01 and JM-0033E-002-01. PAT data suggested that less attrition of crystals had occurred during cooling, possibly as a result of the reduced stir rate. The batch filtered rapidly at similar rate to the preceding process.

For the bulk material were similar to that of the previous batch, as after 110 hours, a significant proportion of Form B hydrate remained in each sample. However, the thinly spread portion of cake exhibited complete conversion to A within 66 hours.

In order to achieve complete conversion of the bulk batch, the solid was spread to a depth of ~1.0 cm and dried for a further 45 hours. Subsequent XRPD analysis indicated presence of Form A only. Yield obtained (85.7%) was comparable to that obtained in DOE studies.

Table S13: Process outline for JM-0033E-002-01

| Step | Description |
|------|-------------|
| 1    | Charge psilocybin (12.0g) (D000407 Q000004391) to 250mL Radleys reactor vessel Tj=70°C |
| 2    | Add water (15.5V, 186ml) heated at ~73 °C. When added to the reactor Tprocess was ~60°C. |
| 3    | Heated to Tprocess =72 °C. Dissolved at ~70°C. Stir at 330rpm |
| 4    | Add 0.5V, 6.0 ml mimic wash |
| 5    | Cool to Tprocess 67 °C over ~20 mins |
| 6    | Add seed material (0.5%w/w, 60mg) in 0.1V, 1.2 ml water |
| Step | Description |
|------|-------------|
| 7    | Hold 30 mins |
| 8    | Cool to 5 °C at 3°C/hr |
| 9    | Held for 1.2 hrs. Slurry sample removed for PLM analysis. |
| 10   | Filter reactor contents -50kPa (500mBar) required to filter. Time to dry and = ~20s, further ~0.5 mins to deliquor. |
| 11   | Charge water (1V, 12ml) wash |
| 12   | Charge water (2V, 24ml) wash |
| 13   | Charge 2 x Acetone (3V, 36ml) wash |
| 14   | Pulled dry on sinter for 15 mins |
| 15   | Wet cake weight = 12.62g. Solid placed in 60mL amber jar, solid level near the top. |
| 16   | Sample removed to be spread thinly (0.17g) Remaining solid (weighed as = 12.46g)- cake depth =5cm |

Dry at RT (oven set at 20-25 °C) under vacuum with nitrogen bleed. Pack cake deeply, depth=5cm. Samples taken at top, middle and bottom of cake by XRPD. Further samples were taken at same cake locations after total drying time of 66, 88 and 110 hours. A small sample was also removed and spread thinly (approx. 1-3mm) and dried for 66 hours after which complete conversion to Form A was observed by XRPD. Form B still present in each sample from the bulk batch with high proportion in bottom and middle. Batch was spread to depth of approximately 1cm and dried for a further 45 hours. XRPD indicated Form A only. Yield= 85.7%

Figure S28: PAT images at selected stages in experiment JM-0033E-002-01

Seeded at 67°C

~65°C
PLM images of the solid taken prior to isolation again displayed significantly larger crystals than that of the isolated material (Error! Not a valid bookmark self-reference.) indicating severe breakage to have occurred during filtration and drying of sample obtained is shown in Figure S30.

Figure 13: PLM images of solids from experiment JM-0033E-002-01
Images and data collected using in situ PAT are shown in Figure . As with experiment JM-0033E-001-01 which also used low seed loading of 0.1%w/w, no particle growth or nucleation was observed by PAT during equilibration period prior to cooling. Between ~64-62.5°C transition from the rod habit of the seed material to larger needles was observed from images taken by PAT probe (Figure ). This was accompanied by rapid rise in number of particles of 40-100µm. The patterns of particle count and turbidity during subsequent cooling appeared to be generally more gradual than those of experiment JM-0033E-001-01 and JM-0033E-002-01. PAT data suggested that less attrition of crystals had occurred during cooling, possibly as a result of the reduced stir rate. The batch filtered rapidly at similar rate to the preceding process.

Comparison of PLM images of a slurry sample taken prior to isolation with the dried material (Figure S3) confirmed severe particle breakage to have occurred during isolation. Due to the results observed from previous experiments using large cake depth, the resultant wet cake was spread to a depth of approximately 1cm and samples taken for XPRD analysis after 23 and 41 hours (Figure 15). After 23 hours a small proportion of Form B remained, however at 41 hours Form A only was observed. The final yield obtained of 79.6% was lower than those normally achieved (>85%), suggesting that a longer aging time may be required to maximise recovery of solid
Figure S32: PLM of solids from experiment JM-0033E-003-01
The third experiment (JM-0033E-003-01) entailed the same slower cooling rate, but with a lower seed loading (0.1%w/w). A lower stir rate (250rpm) was also employed in order to assess impact of change in mixing hydrodynamic. A description of the process is shown in Table S14.

**Table S14: Process outline for JM-0033E-003-01**

| Step | Description |
|------|-------------|
| 1    | Charge psilocybin (12.0g) (WGF-0017E-036) to 250mL Radleys reactor Tj=70°C |
| 2    | Add water (15.5V, 186ml) heated at ~73 °C. When added to the reactor Tprocess was =59 °C |
| 3    | Heated to Tprocess = 72.5°C. Dissolved at 72.5 °C. Stir at 330rpm |
| 4    | Add 0.5V, 6.0 ml mimic wash |
| Step | Description |
|------|-------------|
| 5    | Cool to Tprocess 67 °C over ~20 mins. Stir rate= 250rpm |
| 6    | Add seed material (0.1%w/w, 12mg) in 0.1V, 1.2 ml water |
| 7    | Hold 30 mins |
| 8    | Cool to 5 °C at 3°C/hr |
| 9    | Held for ~1.5hrs. Slurry sample removed for PLM analysis. |
| 10   | Filter reactor contents -50kPa (500mBar) required to filter. Time to dry and = ~20s, further ~0.5 mins to deliquor. |
| 11   | Charge water (1V, 12ml) wash |
| 12   | Charge water (2V, 24ml) wash |
| 13   | Charge 2 x Acetone (3V, 36ml) wash |
| 14   | Pulled dry on sinter for 15 mins |
| 15   | Wet cake weight = 11.7 g. Solid placed in a circular drying dish, solid depth 1cm. |
| 16   | Dry at RT (oven set at 20-25 °C) (cake depth~1cm) under vacuum with nitrogen bleed. Samples taken for XRPD analysis after 23 and 41 hours. Weight of solid after completion of conversion to Form A = 9.56g (79.6% yield) |

A summary comparison of the three batches of 10 to 12 g scale process and trend diagrams from in situ PAT are shown in Table S15. Well controlled crystal growth driven crystallisation process displayed by JM-0033E-003-01 process trends. Particle size measurement by diameter of equivalent circle of the PLM images using Keyence software (Table S15) indicates that the experiments using the slower cooling rate (JM-0033E-002-01 and JM-0033E-003-01) have, as expected, generated larger particle size in comparison to the process which employed the same cooling rate (JM-0033E-001-01) used in the DoE studies. These results are also supported by PSD measured by LLD as shown in Table S15. It appears a slow stirring speed helps to generate large crystals (JM-0033E-001-01) although this batch was aged for short time compared to previous batches which probably reduced attrition compromising yield. PSD by LLD has been provided as explained in Section 0.
| Batch Ref      | Scale (g) | Cooling rate (°C/hr) | Seed load (%w/w of input) | Seed temp (°C) | Stir Rate (RPM) | Particles image pre isolation | Particles image post isolation | PSD by LLD D10,D50 & D90 (µm) | Diameter of equivalent circle (µm) | Yield (%) |
|----------------|-----------|----------------------|---------------------------|----------------|-----------------|-------------------------------|-------------------------------|-----------------------------|--------------------------------|-----------|
| JM-0033E-001-01 | 10.75     | 10                   | 0.1                       | 70             | 330             |                               |                               | 1235           | 85                               | 50.8       | 88.7     |
| JM-0033E-002-01 | 12        | 3                    | 0.5                       | 67             | 330             |                               |                               | 1138           | 92                               | 60.4       | 85       |
| JM-0033E-003-01 | 12        | 3                    | 0.1                       | 67             | 250             |                               |                               | 1748           | 121                              | 63.4       | 79.6     |
Utilizing process outlined in Table S14, 121.8 g of psilocybin (WGF-0017E-040) was charged into the Radleys reactor and subjected to the water crystallization. As seen in Figure S34, PLM images indicate crystals at 5 °C, ageing and after isolation.

Figure S34: PLM images taken from the WGF-0017E-040-batch of the slurry before, after ageing and after isolation

Figure S35 shows different drying conditions of WGF-0017E-040. When the sample was dried in an oven with inbuilt nitrogen bleed system complete conversion to the desired Form A (105.3 g, 86.5% recovery) as analyzed by XRPD.

Figure S35: XRPD data from WGF-0017E-040-sampled throughout drying

3.7 Particle size analysis by LLD for scale-up batches

Three batches (JM-0033E-001-01, JM-0033E-002-01 and JM-0033E-003-01) were analysed after modifying the PSD analysis method. Three preparations were used for each batch.

For all batches, samples were directly added to Malvern Mastersizer 3000 measurement cell. It was observed that particle size was decreasing over time, suggesting particles are fragile and breaking. Stable obscuration was obtained with first three runs, hence the first three runs are taken representative of the bulk particle size. However, for JM-0033E-002-01, there were difficulties with obtaining obscuration within range during the measurements. This could be due to the different input material having a different solubility in the dispersion solvent, heptane, which correlates with a different solubility seen during processing.

Figure  to  show the microscopic images of the sample taken before PSA and the typical distribution plots for each batch. The results indicate that the combination of slow cooling and low stirring speed provides large particles D90 of 121µm.
Figure S36 - Microscopic pictures, typical distribution curve and numerical results for 3 independent sample preparations for JM-0033E-001-01

| Record Number | Sample Name     | D<sub>x</sub> (10) (µm) | D<sub>x</sub> (50) (µm) | D<sub>x</sub> (90) (µm) |
|---------------|----------------|--------------------------|--------------------------|--------------------------|
| 2             | JM-33E-001-01 Prep1 | 12.367                   | 35.651                   | 100.108                  |
| 3             | JM-33E-001-01 Prep1 | 11.902                   | 33.880                   | 81.948                   |
| 4             | JM-33E-001-01 Prep1 | 11.813                   | 33.794                   | 81.695                   |
| Mean          |                | 12.027                   | 34.392                   | 87.917                   |
| 1xStd Dev     |                | 0.208                    | 1.016                    | 10.559                   |
| 1xRSD (%)     |                | 2.474                    | 2.953                    | 12.010                   |

| Record Number | Sample Name     | D<sub>x</sub> (10) (µm) | D<sub>x</sub> (50) (µm) | D<sub>x</sub> (90) (µm) |
|---------------|----------------|--------------------------|--------------------------|--------------------------|
| 12            | JM-33E-001-01 Prep2 | 12.804                   | 36.060                   | 88.541                   |
| 13            | JM-33E-001-01 Prep2 | 12.613                   | 35.662                   | 86.761                   |
| 14            | JM-33E-001-01 Prep2 | 12.455                   | 35.369                   | 85.003                   |
| Mean          |                | 12.624                   | 35.697                   | 86.769                   |
| 1xStd Dev     |                | 0.175                    | 0.347                    | 1.769                    |
| 1xRSD (%)     |                | 1.385                    | 0.972                    | 2.039                    |

| Record Number | Sample Name     | D<sub>x</sub> (10) (µm) | D<sub>x</sub> (50) (µm) | D<sub>x</sub> (90) (µm) |
|---------------|----------------|--------------------------|--------------------------|--------------------------|
| 22            | JM-33E-001-01 Prep3 | 11.810                   | 33.899                   | 81.499                   |
| 23            | JM-33E-001-01 Prep3 | 11.608                   | 33.659                   | 80.444                   |
| 24            | JM-33E-001-01 Prep3 | 11.619                   | 33.535                   | 79.960                   |
| Mean          |                | 11.706                   | 33.697                   | 80.501                   |
| 1xStd Dev     |                | 0.096                    | 0.185                    | 0.970                    |
| 1xRSD (%)     |                | 0.823                    | 0.549                    | 1.205                    |

Figure S37 - Microscopic pictures, typical distribution curve and numerical results for 3 independent sample preparations for JM-0033E-002-01
| Record Number | Sample Name                  | Dx (10) (µm) | Dx (50) (µm) | Dx (90) (µm) |
|---------------|------------------------------|--------------|--------------|--------------|
| 2             | JM-33E-002-01 Prep1          | 12.037       | 39.491       | 98.826       |
| 3             | JM-33E-002-01 Prep1          | 11.114       | 38.121       | 96.174       |
| 4             | JM-33E-002-01 Prep1          | 10.557       | 38.230       | 106.311      |
| Mean          |                              | 11.259       | 38.614       | 100.437      |
| 1xStd Dev     |                              | 0.743        | 0.761        | 5.257        |
| 1xRSD (%)     |                              | 6.599        | 1.971        | 5.234        |
| 11            | JM-33E-002-01 Prep2          | 12.593       | 38.683       | 90.265       |
| 12            | JM-33E-002-01 Prep2          | 11.900       | 37.829       | 87.586       |
| 13            | JM-33E-002-01 Prep2          | 11.449       | 37.144       | 86.047       |
| Mean          |                              | 11.981       | 37.885       | 87.986       |
| 1xStd Dev     |                              | 0.576        | 0.771        | 2.134        |
| 1xRSD (%)     |                              | 4.808        | 2.035        | 2.426        |
| 21            | JM-33E-002-01 Prep3          | 11.391       | 36.256       | 91.143       |
| 22            | JM-33E-002-01 Prep3          | 10.336       | 37.389       | 88.437       |
| 23            | JM-33E-002-01 Prep3          | 9.531        | 37.056       | 87.259       |
| Mean          |                              | 10.419       | 37.580       | 88.946       |
| 1xStd Dev     |                              | 0.933        | 0.642        | 1.992        |
| 1xRSD (%)     |                              | 8.952        | 1.708        | 2.239        |
Figure S38 - Microscopic pictures, typical distribution curve and numerical results for 3 independent sample preparations for JM-0033E-003-01

| Record Number | Sample Name          | Dx (10) (µm) | Dx (50) (µm) | Dx (90) (µm) |
|---------------|----------------------|--------------|--------------|--------------|
| 32            | JM-33E-003-01 Prep1  | 17.533       | 48.549       | 122.630      |
| 33            | JM-33E-003-01 Prep1  | 17.317       | 47.883       | 120.646      |
| 34            | JM-33E-003-01 Prep1  | 17.138       | 47.217       | 118.303      |
| Mean          |                      | 17.329       | 47.883       | 120.526      |
| 1×Std Dev     |                      | 0.198        | 0.666        | 2.166        |
| 1×RSD (%)     |                      | 1.143        | 1.391        | 1.797        |

| Record Number | Sample Name          | Dx (10) (µm) | Dx (50) (µm) | Dx (90) (µm) |
|---------------|----------------------|--------------|--------------|--------------|
| 33            | JM-33E-003-01 Prep2  | 16.247       | 47.188       | 123.469      |
| 34            | JM-33E-003-01 Prep2  | 15.913       | 46.456       | 120.281      |
| 35            | JM-33E-003-01 Prep2  | 15.641       | 45.697       | 116.368      |
| Mean          |                      | 15.934       | 46.447       | 120.039      |
| 1×Std Dev     |                      | 0.304        | 0.745        | 3.557        |
| 1×RSD (%)     |                      | 1.905        | 1.604        | 2.963        |

| Record Number | Sample Name          | Dx (10) (µm) | Dx (50) (µm) | Dx (90) (µm) |
|---------------|----------------------|--------------|--------------|--------------|
| 42            | JM-33E-003-01 Prep3  | 17.617       | 49.622       | 125.907      |
| 43            | JM-33E-003-01 Prep3  | 17.403       | 48.312       | 121.121      |
| 44            | JM-33E-003-01 Prep3  | 17.175       | 47.627       | 119.248      |
| Mean          |                      | 17.465       | 48.521       | 122.092      |
| 1×Std Dev     |                      | 0.325        | 1.014        | 3.434        |
| 1×RSD (%)     |                      | 1.864        | 2.089        | 2.813        |
4 Conclusions

- Hydrolysis rate of psilocybin was found to increase with increases in both time and temperature. This degradation was shown to be linear over time at most temperatures, with only 75 °C showing deviation from linearity over the studied period.

- The predictive model built combined both time and temperature to enable a degradation estimate for any combination of time and temperature within the bounds of the design space. Degradation increased when either parameter increased, at much higher rate at high temperature than at lower temperature.

- Decreasing the process concentration from 83.3 mg/ml (12 Vol) to 62.5 mg/ml (16 Vol) provided a much wider MSZW which enabled the inclusion of polish filtration to this crystallisation step. This was also useful in reducing the extent of hydrolysis due to the decreased seeding temperature.

- DoE study showed that seed temperature and seed loading to have an influence in final particle size, showing positive and negative correlation respectively. However, at lower seeding temperatures, the impact of seed loading was reduced, this could be likely due to nucleation becoming dominant at the increased supersaturation caused by the lower temperature. However, cooling rate seems to significantly affect the final particle size. Experiments carried out with a low cooling rate of 3 °C/min have given large particle size consistently throughout the experiments. In addition, It appears slow cooling combined with low stirring speed provides large particles D90 of 121µm.

- The Form B crystals formed during the crystallisation were acicular in shape and were shown to be relatively fragile during isolation, with particle size decreasing at each successive step in the isolation protocol.

- Initial drying studies showed a greater proportion of pattern H formation as both drying time and drying temperature increased. There was also a much greater proportion of pattern H seen in the samples dried in a vial.

- As longer drying time was a risk factor for pattern H formation, acetone displacement washes were included. The acetone displacement wash shown to reduce the residual water in the filter cake and therefore reduce drying time.

- Powder bed thickness was found to be an important factor in pattern H formation, with pattern H being seen in samples with a bed thickness of approx. 25 mm, but Form A only in samples which were spread out to approx. 5 mm. This was also thought to be the reason for samples in vials generating more pattern H material than those placed into a PP/Nylon liners due to the material being more spread out in the liners. The drying process was still found to be inconsistent at 35-40 °C, even with a reduced powder bed depth, so drying at 20-25 °C was also tested and shown to consistently produce Form A without pattern H formation, however, it has increased the drying time significantly.

- Initial small-scale experiments showed an acetone reslurry could convert both trace pattern H impurities and Form B, to pattern A.

- During scale up, cake bed depth during drying was again shown to be important for drying time. The large cake bed depth had no effect on formation of pattern H at this reduced temperature however, the material was unable to fully dehydrate from Form B to Form A after 145 hours. Subsequent cake bed depth reduction to 1 cm allowed full conversion to Form A after a further 45 hours indicating bed depth to be critical to the conversion rate.
• Form B to Form A conversion rate was found to be significantly increased when using N₂ flow through the entire cake bed on a filter drier under vacuum.

• LLD was found to be unsuitable for GMP PSD determination due to the fragility of the particles and subsequent breakage under even the lowest energy mixing during analysis. The best method for particle size determination for this material would be light microscopy.