Electronic Supplementary Information

Pharmaceutical Solvate Formation for the Incorporation of the Antimicrobial Agent Hydrogen Peroxide

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SI 1. Experimental Methods

Caution: *Hydrogen peroxide is a strong oxidizing reagent. Proper safety practices and equipment were used to prevent undesired explosion or fire. Be aware that the potential for severe injury exists if these materials are handled improperly.*[1]

Materials. Miconazole, 99% was obtained from Alfa Aesar. Ethanol was obtained from Fisher Scientific. 50% Hydrogen peroxide was obtained from Sigma-Aldrich. 98% Hydrogen peroxide was obtained from PeroxyChem LLC. Triphenylphosphine, 99% was obtained from Acros Organics. Talc powder was obtained from Fisher Scientific. These reagents were used as received. Nitric acid was obtained from Fisher Scientific and diluted to a 1M solution. Acetonitrile was obtained from Fisher Scientific and was passed through an activated alumina column before use.

Candida glabrata (ATCC® 66032™) was obtained freeze-dried from ATCC. The sample were rehydrated according to ATCC protocol.[2] Briefly, the freeze-dried samples were resuspended in sterile water and incubated at 25 °C for 2 hours. A 50 μL aliquot of the suspension was spread on a prewarmed YPD agar plate, and the plates were incubated at 25 °C for 3 days.

Crystallization. Crystals of the miconazole hemihydrate were grown from ethanol solutions (350 mg/mL) heated to 65 °C to dissolve all solids. Solutions were passed through a syringe filter (1.5 mL) into a 4 mL vial with the interior walls etched with a diamond-tipped glass cutter. Water (1.5 mL) is added and the vials left to sit overnight, sealed at room temperature, allowing colorless needles to grow.

Crystals of the miconazole nitrate salt were grown from ethanol solutions (40 mg/mL). The vial is sonicated until all solids are dissolved. Nitric acid (1M, 2 mL) was added and the vial allowed to sit overnight, allowing colorless needles to grow.

Crystals of the peroxide solvated samples were grown from ethanol solutions (400-420 mg/mL) heated to 65 °C to dissolve all solids. Solutions were passed through a syringe filter into a 4 mL vial and either 50 or 98% peroxide was added. For 50% peroxide, 0.5 mL miconazole solution and 0.5 mL hydrogen peroxide were mixed, and the vials left open to evaporate overnight, forming colorless, blocky crystals. For 98% peroxide in open atmosphere, 0.6 mL miconazole solution and 0.2 mL hydrogen peroxide were mixed, and the vials left open to evaporate. After 12

hours, a gel was present in the vials, and this was stirred with a glass pipette and left open to evaporate. After 12 hours, colorless blocky crystals formed. For 98% peroxide in controlled atmosphere, 0.6 mL miconazole solution and 0.2 mL hydrogen peroxide were mixed, and the vials left uncapped in a desiccator containing Drierite® ($CaSO_4$ and $CoCl_2$ for indicator) to absorb water. After 12 hours, clear blocky crystals were present.

Drug formulations for fungal testing were prepared by sieving miconazole samples to a particle size of 90-120 μm. Talc powder was sieved to remove clumps. Miconazole solid forms (4 mg) and talc (196 mg) were added to a 20 mL vial, and shaken vigorously for 10 minutes to fully mix the components. The formulations (50 mg) were added directly to the preincubated agar plates as described below.

Raman Spectroscopy. Raman spectra of miconazole samples were collected using a Renishaw inVia Raman Microscope equipped with a Leica microscope, 633 nm laser, 1800 lines/mm grating, 50 μm slit, and a RenCam CCD detector. Spectra were collected in extended scan mode with a range of 300-1700 cm^{-1} and then analyzed using the WiRE 3.4 software package (Renishaw). Calibration was performed using a silicon standard in static mode.

Raman spectra of miconazole hemi-HP in water were collected using a Kaiser Optical Systems Raman Rxn Microprobe equipped with a Multi-Rxn non-contact optic, 785 nm laser, and a multichannel CCD detector. Spectra were collected with a range of 150-3425 cm^{-1} and then analyzed using the HoloGRAMS 4.1 software package (Kaiser). Calibration was performed using a HoloLab Calibration Accessory and a cyclohexane standard.

Powder X-Ray Diffraction (PXRD). Powder X-ray diffraction (PXRD) data of miconazole samples were collected at ambient temperature on a PANalytical Empyrean diffractometer in Bragg-Brentano geometry using Cu-Kα radiation (λ = 1.54187 Å), operating at 45 kV and 40 mA. The incident beam was equipped with a Bragg-Brentano^{HD} X-ray optic using fixed slits/soller slits. The detector was a silicon-based linear position sensitive X'Celerator Scientific operating in 1-D scanning mode. Data were collected from 5 to 50° 2θ using a step size of 0.008° and a count time of at least 10 s per step. Powder patterns were processed using Jade 8 XRD Pattern Processing, Identification & Quantification analysis software (Materials Data, Inc).[3] All powder patterns were compared to their respective simulated powder patterns from single crystal X-ray diffraction structures and were found to agree with the predicted patterns.

Single Crystal Structure Determination. Single-crystal X-ray diffraction data of miconazole samples were collected using a Rigaku XtaLAB Synergy-S diffractometer equipped with a low temperature device and a PhotonJet-S microfocus Cu source ($\lambda = 1.54187$ Å) operating at 50 kV and 1 mA and a HyPix-6000HE (hybrid photon counting (HPC) detector placed 34.00 mm from the sample, operated at 100(1) K and/or 293(2) K. Samples were mounted onto polyimide MicroMounts (MiTeGen, LLC) with Paratone-N oil. The data were processed with CrysAlisPro v38.46 (Rigaku Oxford Diffraction) and corrected for absorption. The structures were solved in OLEX2[4] using SHELXTL[5] and refined using SHELXL.[6] All non-hydrogen atoms were refined anisotropically with hydrogen atoms placed in a combination of refined and idealized positions. Single crystals of miconazole nitrate display splintering and ultimate decomposition under ambient conditions, which is accelerated by thermally shocking specimens, preventing data collection at low temperature for this sample. At room temperature, miconazole nitrate does not diffract beyond 0.95 Å.

Nuclear Magnetic Resonance (NMR). ^{31}P NMR measurements were carried out on Varian vnmrs 500 spectrometer operating at 202 MHz at room temperature, using 1 scan with a 25 sec. relaxation delay. For the chemical test in Figure S8, a standard solution of TPP (229.16 mg, 0.87 mmol) in dry ACN (10 mL) was prepared. The miconazole HP sample (11.49 mg [0.13 mmol HP]) was dissolved in 1 mL of this standard TPP solution and allowed to shake for 30 min to react. For the chemical test in Figure S9, a standard solution of TPP (163.92 mg, 0.63 mmol) in dry ACN (10 mL) was prepared. The miconazole HP sample (17.90 mg [0.02 mmol HP]) was dissolved in 1 mL of this standard TPP solution and allowed to shake for 30 min to react. The ratio of TPP to TPPO for each spectrum was calculated by integration of the peaks using ACD/Labs V.12.01, 1D NMR Processor: Academic Edition, 2010.

The expected ratio of ^{31}P peaks can be calculated from the percentage of HP in the hemi-HP miconazole sample (3.93% HP) following the reaction below where x<y:

$$x \text{ mol } H_2O_2 + y \text{ mol TPP} \longrightarrow x \text{ mol TPPO} + (y-x) \text{ mol TPP}$$

Thermogravimetric Analysis (TGA). Thermograms of miconazole samples were recorded on a TA Instruments Q50 TGA. All experiments were run in platinum TGA sample pans under a nitrogen purge of 50 mL/min with a heating rate of 10 °C/min, while covering the

temperature range of 35 °C to 350 °C. The instrument was calibrated using the Curie points of alumel and nickel standards. Thermograms were analyzed using TA Universal Analysis 2000, V 4.5A.

Karl Fisher Titration. The relative amounts of water of miconazole samples, expressed as a percent (where 1 mg/g = 0.1%), were determined via coulometric Karl Fisher titration using a Mettler Toledo C20 Coulometric KF Titrator.

Dynamic Vapor Sorption. Moisture sorption and desorption isotherms were generated at 25 °C using a TA Instruments Q5000 SA. The Q5000 is equipped with a thermobalance and an autosampler. The instrument was calibrated using sodium bromide deliquescence. All experiments were carried out with metal-coated quartz pans. The procedure involved 10% steps of relative humidity (RH) between 5-95% RH. Equilibrium was assumed to be established when there was a weight change of no more than 0.01% over a period of 5 min., with a maximum dwell time of 90 min. Isotherms were analyzed using TA Universal Analysis 2000, V 4.5A.

Solubility Testing. Solubility measurements were taken using the Pion Rainbow Dynamic Dissolution Monitor® system. Standard solutions of known concentrations of miconazole were used to create calibration curves. All values collected during dissolution were compared to these calibration curves. For solubility measurements, excess solid of each form was added to empty vials. Solution media (PCB buffer, pH 4) was added to each vial (15 mL), stirred at 200 rpm, and UV-Vis probes with a path length of 10 mm were submerged in the solution. A time dependent concentration curve was observed in situ using the AuPRO software (Version 5.1.1.0). The lambda maximum for the absorbance of miconazole is located at 268 nm in PCB buffer. The solubility for each form is determined based on the maximum equilibrium concentration reached in solution.

Fungal Cell Culture. A single colony of *C. glabrata* was resuspended in 5 mL of YPD media, and the culture was grown overnight at 30 °C, 250 RPM. The following morning, 200 µL of the seed culture was used to inoculate 5 mL of YPD media. The culture was grown at 30 °C, 250 RPM until an OD660 of 1.0 was reached. 200 µL of the culture was spread evenly onto prewarmed YPD agar plates. The plates were incubated at 30 °C for 1 h to allow any residual liquid to evaporate, then the drug formulation and vehicle control were added as a single line down the center of the plate. Incubation of the plates at 30 °C was continued for 2 days, and growth inhibition was assessed at 24 h and 48 h.

SI 2. Raman spectroscopy of miconazole crystal forms

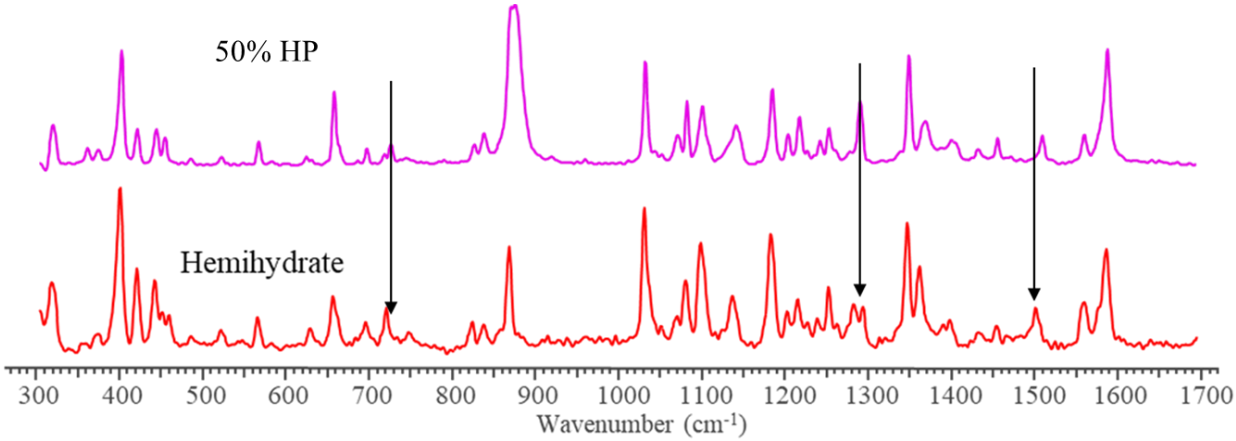

Figure S1. Raman spectra of the hemihydrate form of miconazole (bottom) in comparison to the sample obtained from crystallization with 50% HP (top). Differences are highlighted with arrows.

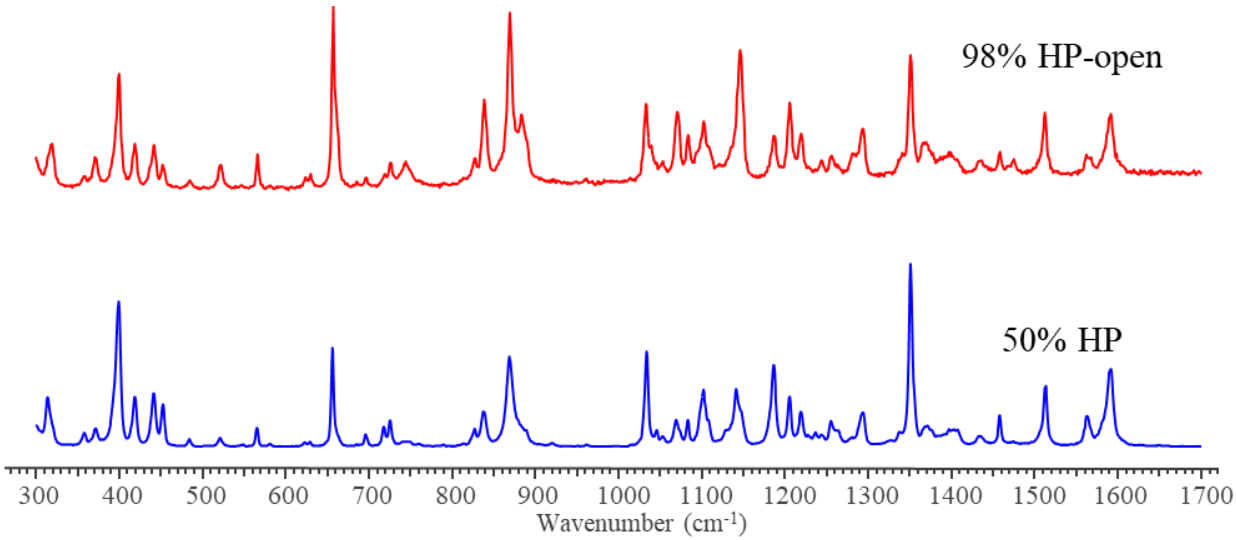

Figure S2. Raman spectra of miconazole samples obtained from crystallization with 50% HP (bottom) and with 98% HP in open atmosphere (top).

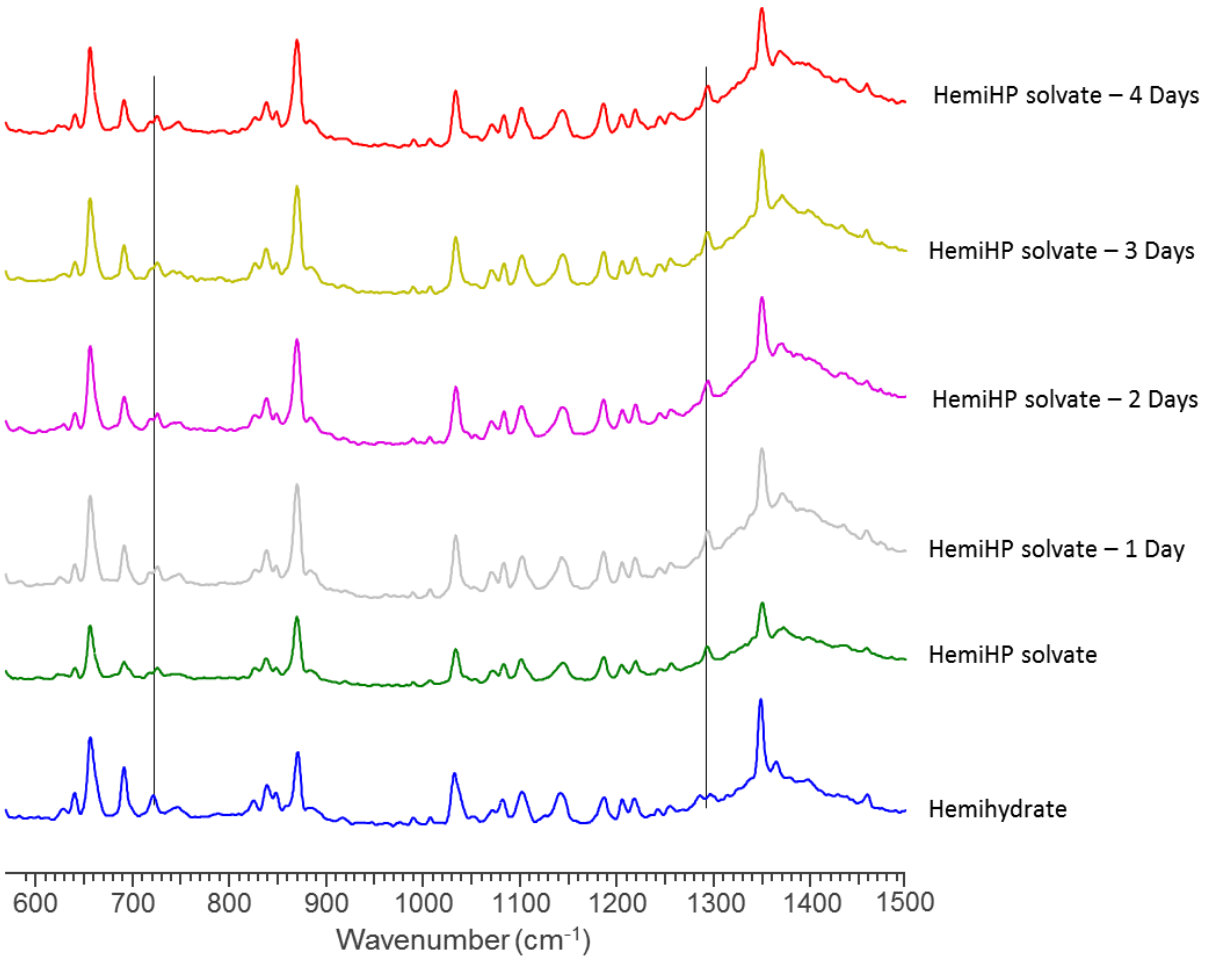

Figure S3. Raman spectra of miconazole hemi-HP solvate sample slurried in water for 4 days showing no changes from the hemi-HP to the hemihydrate.

SI 3. Powder X-Ray diffraction of miconazole crystal forms

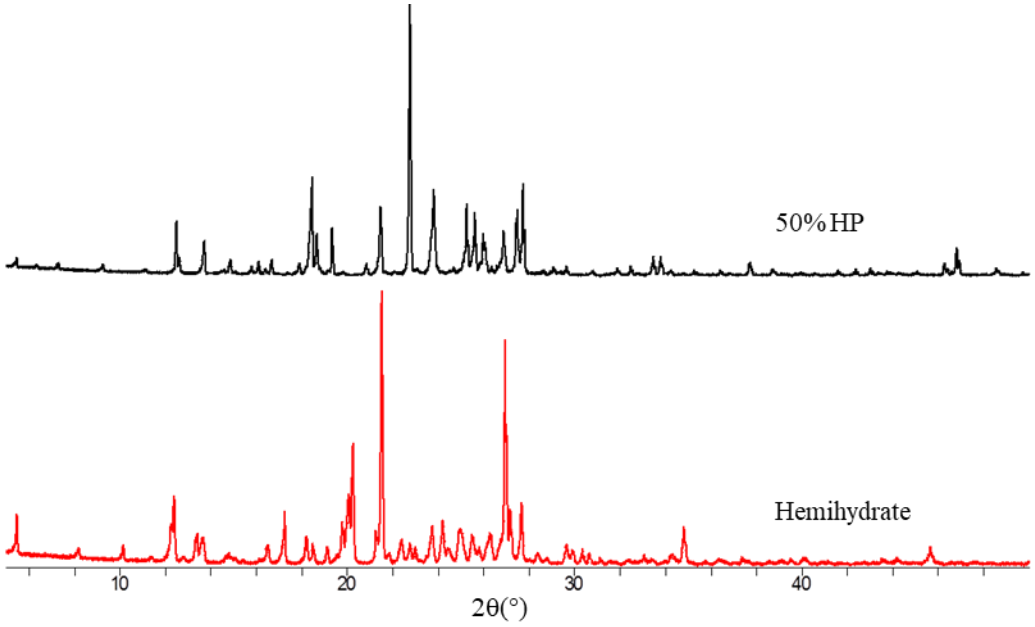

Figure S4. PXRD patterns for the hemihydrate form of miconazole (bottom) in comparison to the sample obtained from crystallization with 50% HP (top).

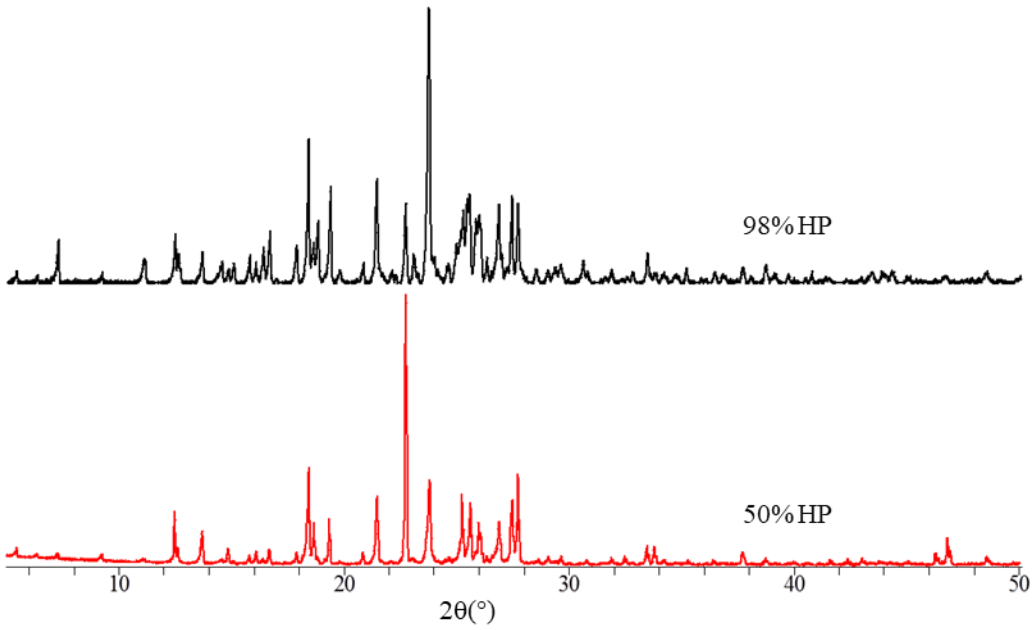

Figure S5. PXRD patterns of miconazole samples obtained from crystallization with 50% HP (bottom) and with 98% HP in open atmosphere (top).

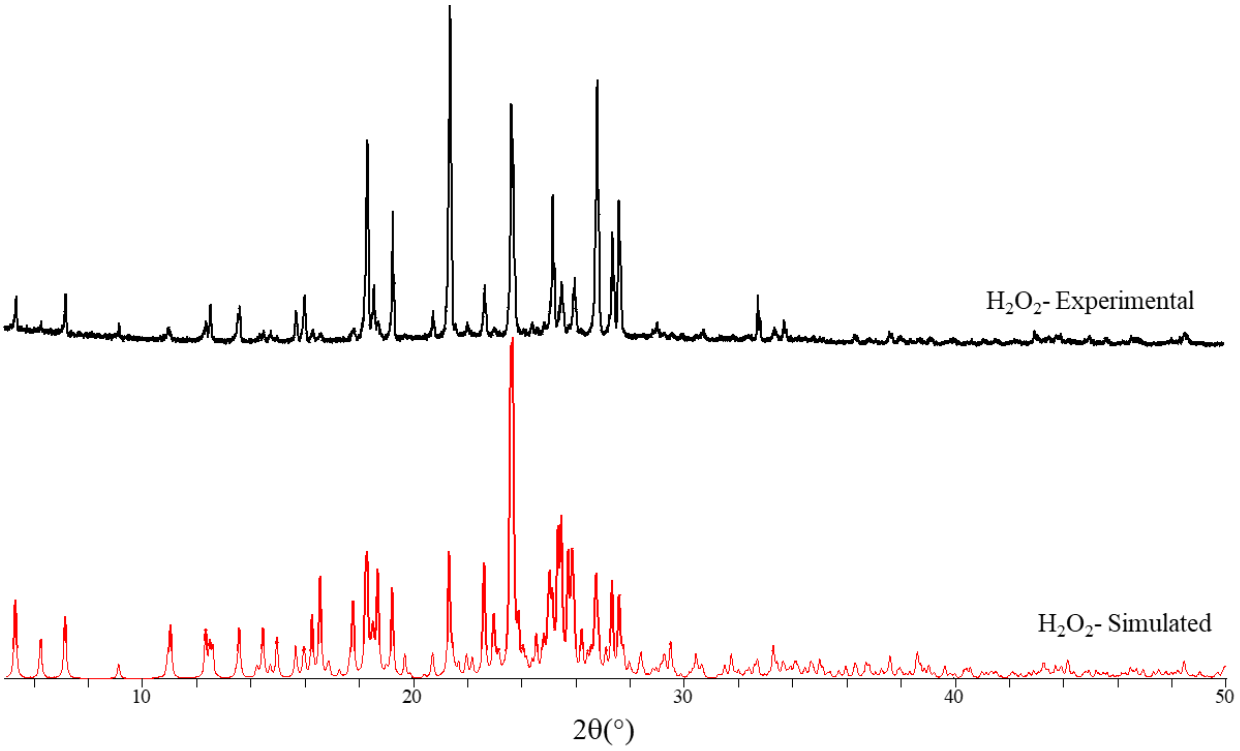

Figure S6. Simulated room temperature PXRD pattern and experimental PXRD pattern for the hemi-HP solvate of miconazole.

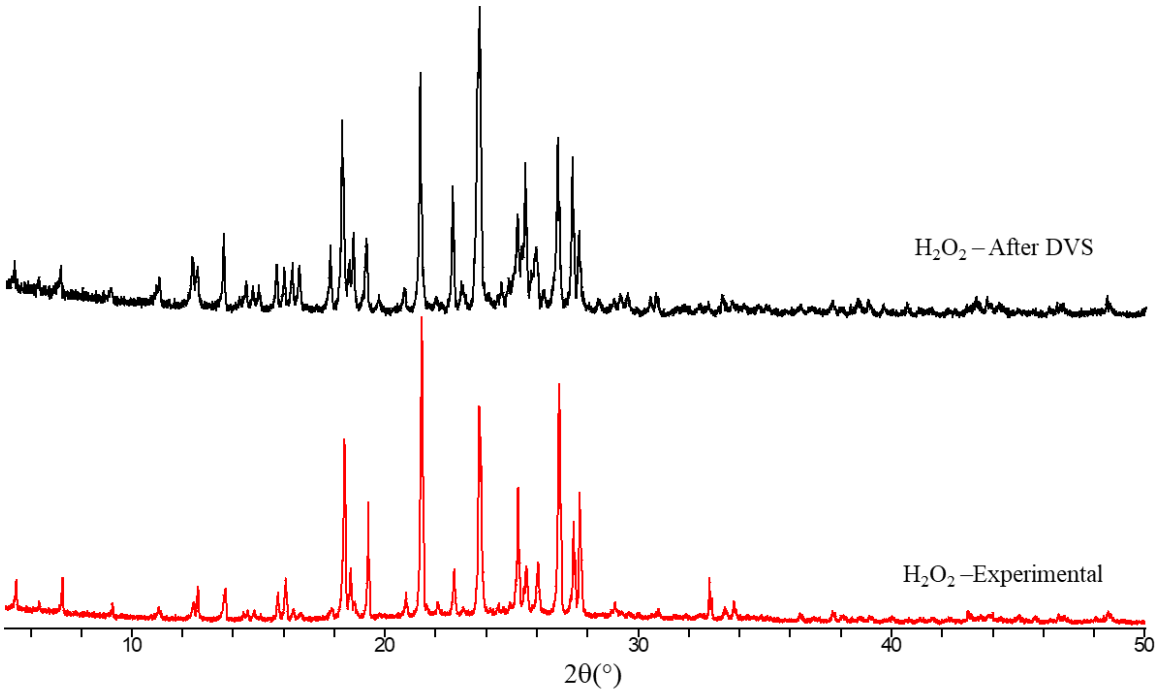

Figure S7. PXRD pattern of miconazole hemi-H_2O_2 after dynamic vapor sorption measurement compared to PXRD pattern of fresh sample at RT.

SI 4. Table of crystallographic information for miconazole crystals

Table S1. Crystallographic parameters for miconazole crystals.

	Hemi-H_2O_2	Hemihydrate	Nitrate Salt
CCDC #			
Chemical formula	$C_{18}H_{14}Cl_4N_2O$, ½ (H_2O_2)	$C_{18}H_{14}Cl_4N_2O$, ½ (H_2O)	$C_{18}H_{14}Cl_4N_2O$, HNO_3
Formula Weight (g/mol)	433.14	425.14	479.14
Crystal System	Triclinic	Monoclinic	Orthorhombic
Space group	P -1	P 2_1/n	Pca2_1
a (Å) =	7.9439(2)	7.95530(10)	7.6784(7)
b (Å) =	14.5250(3)	32.5473(4)	15.3357(13)
c (Å) =	16.8592(4)	14.6180(2)	35.305(4)
α (°) =	75.966(2)	90	90
β (°) =	85.836(2)	91.3620(10)	90
γ (°) =	87.403(2)	90	90
V ($Å^3$) =	1881.48	3783.87	4157.29
Z' =	0	0	0
Z =	2	4	4
Temperature (K) =	100	100	293
Reflections measured	7,663	7,728	4,357
R factor (%)	5.83	4.19	7.35

SI 5. Nuclear Magnetic Resonance (NMR) of miconazole hemi-HP solvate and TPP reaction

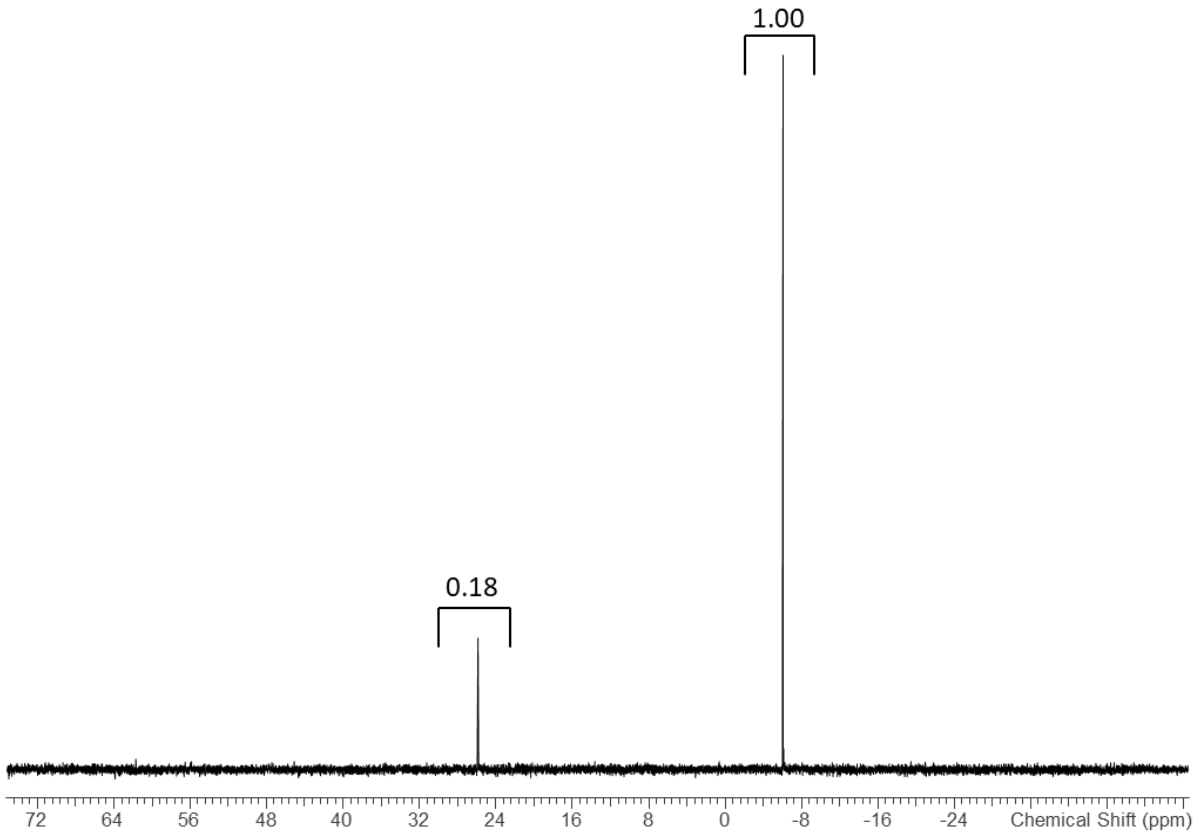

Figure S8. ^{31}P NMR spectrum of solution reaction between TPP and hemi-HP solvate of miconazole. Numbers above the peaks indicate integration values. The expected ratio of peaks for a hemi-HP solvate for this sample is 0.18/1.

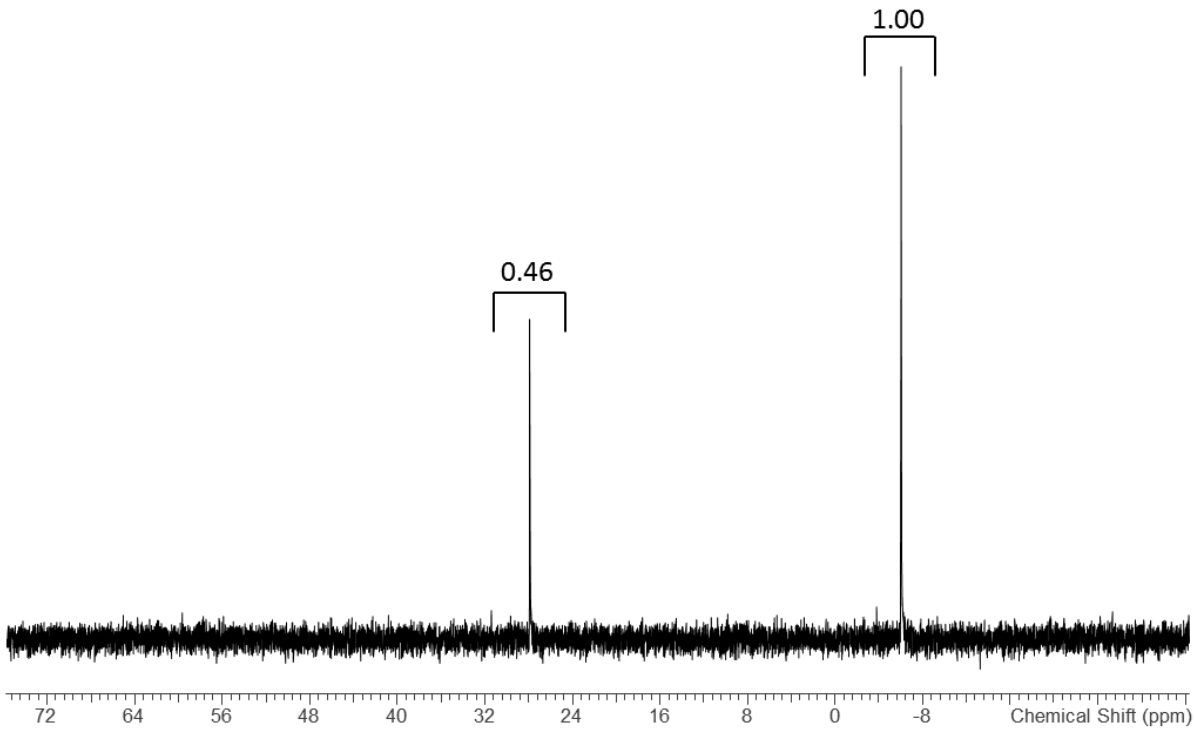

Figure S9. ^{31}P NMR spectrum of solution reaction between TPP and hemi-HP solvate of miconazole after vigorously shaking in water for 6 hours. Numbers above the peaks indicate integration values. The expected ratio of peaks for a hemi-HP solvate for this sample is 0.49/1. The difference between the expected and observed ratios equals 0.00007 mmol HP for this sample.

SI 6. Thermogravimetric Analysis (TGA) of miconazole forms

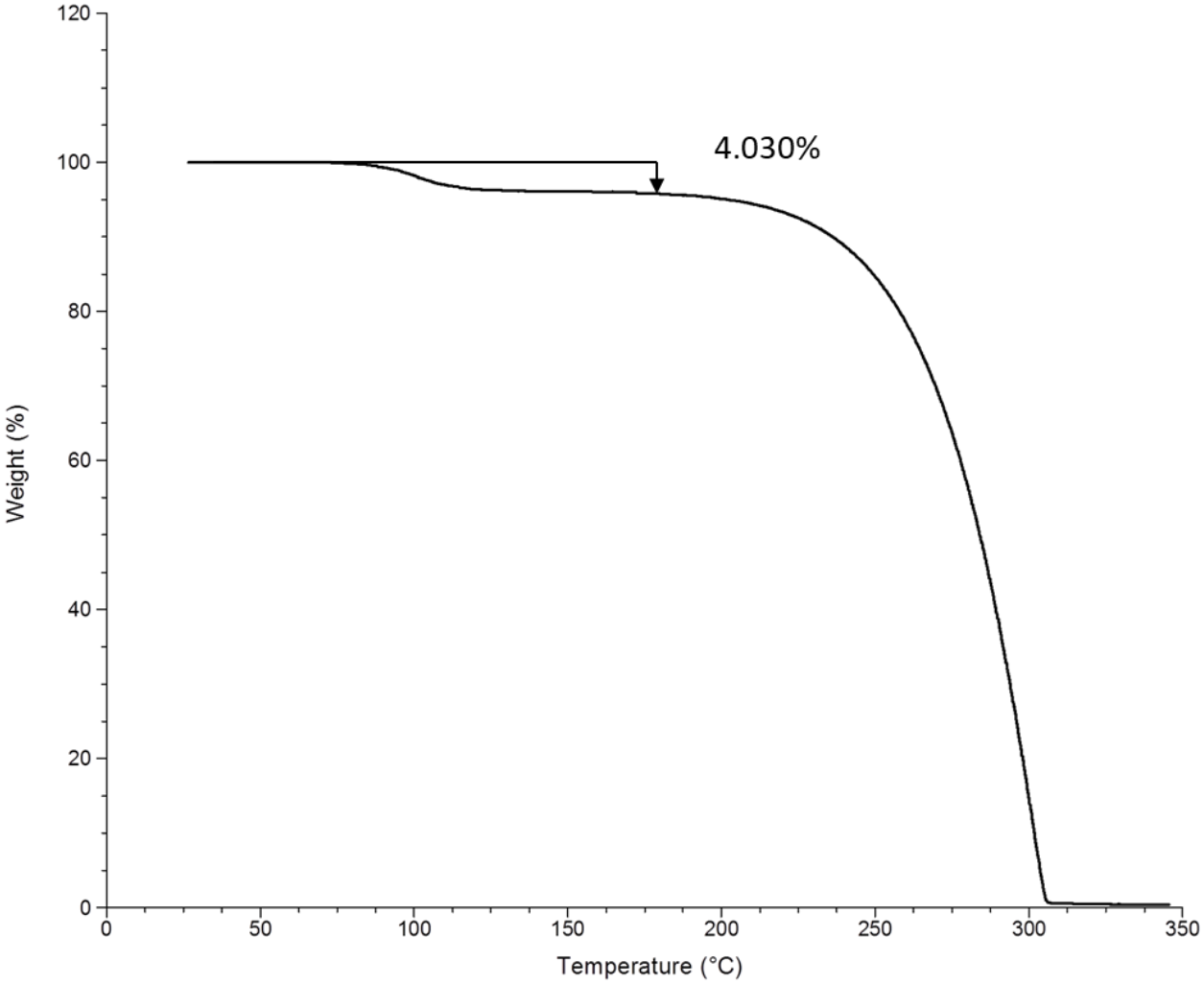

Figure S10. Thermogram of miconazole hemi-HP solvate 1 week after crystal harvest.

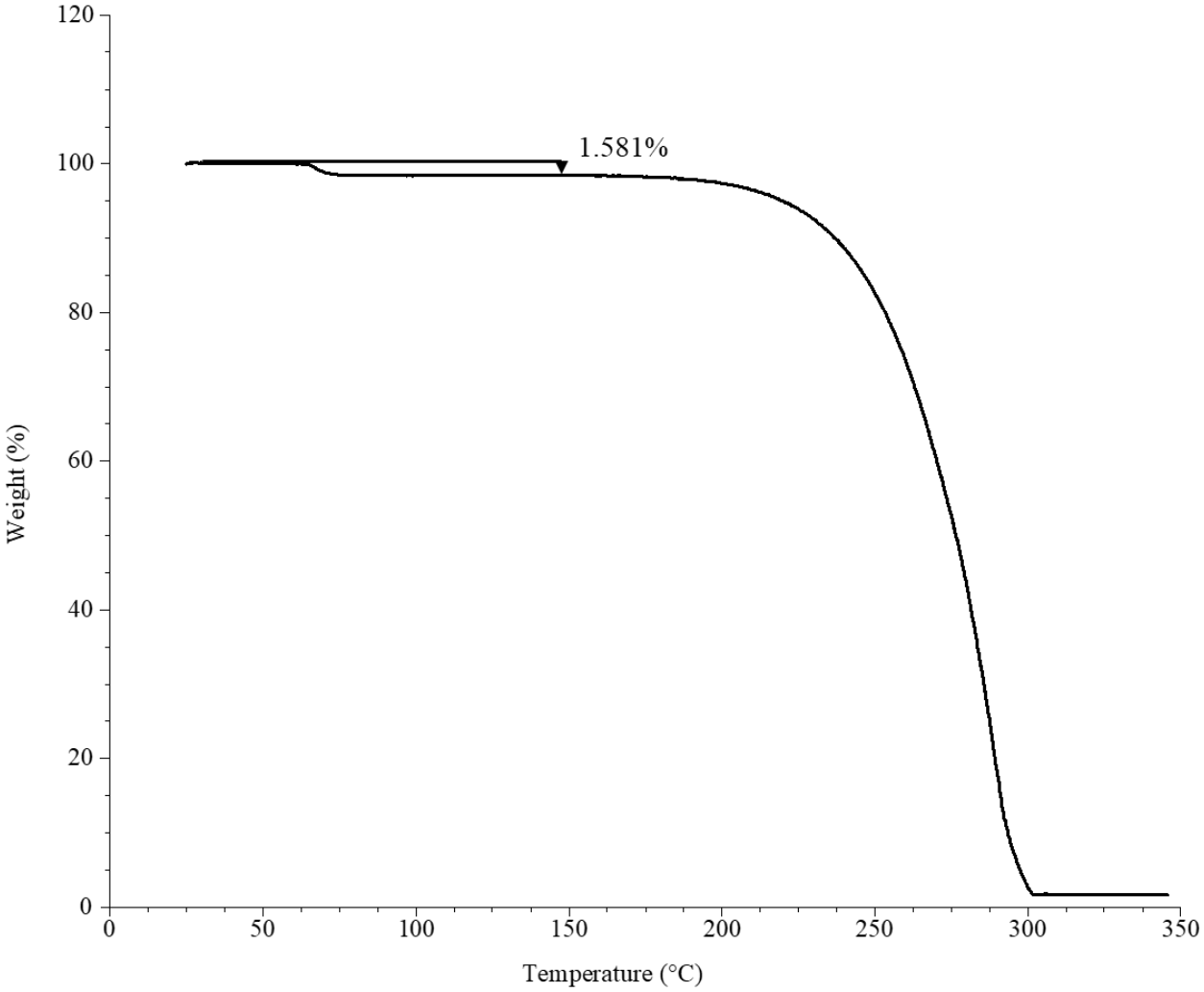

Figure S11. Thermogram of the hemihydrate form of miconazole.

SI 7. Dynamic Vapor Sorption measurement

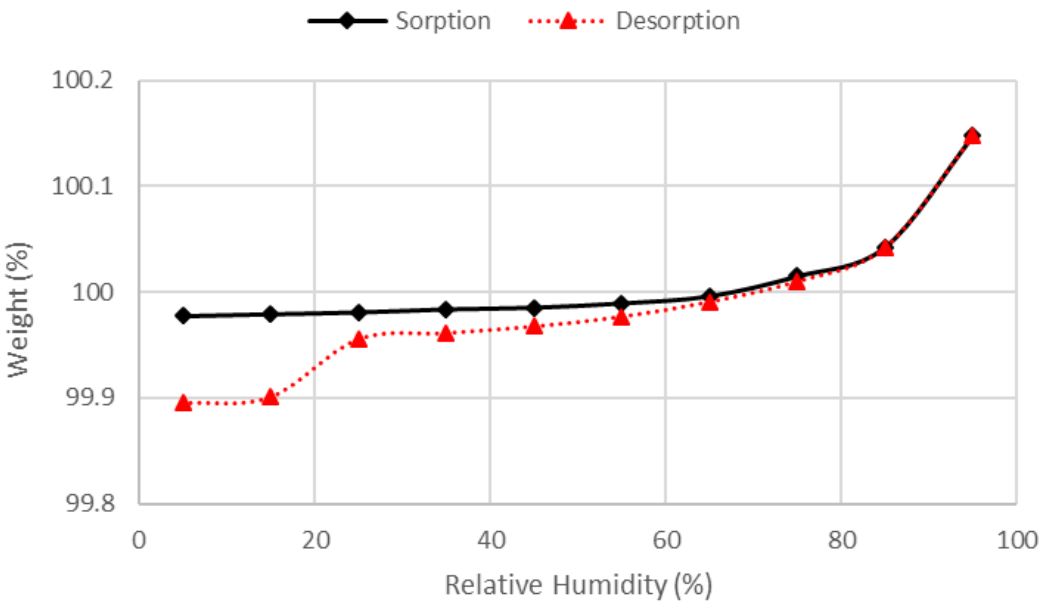

Figure S12. Plot of the change in weight percent as a function of relative humidity of the hemi-HP solvate of miconazole.

SI 8. Solubility testing of miconazole forms

Table S2. Equilibrium Solubility Measured for Miconazole Forms (all values in µg/mL)

Trial	Hemi-HP solvate	Hemihydrate	Nitrate Salt	Anhydrate
1	241	175	602	265
2	199	165	512	197
3	285	167	700	183
4	221	139	814	205
5	244	184	1038	182
6	235	148	397	196
7	189	136	770	184
Average	230.5714	159.1429	690.4286	201.7143
Std. Dev.	31.8531	18.3977	211.4457	29.2216

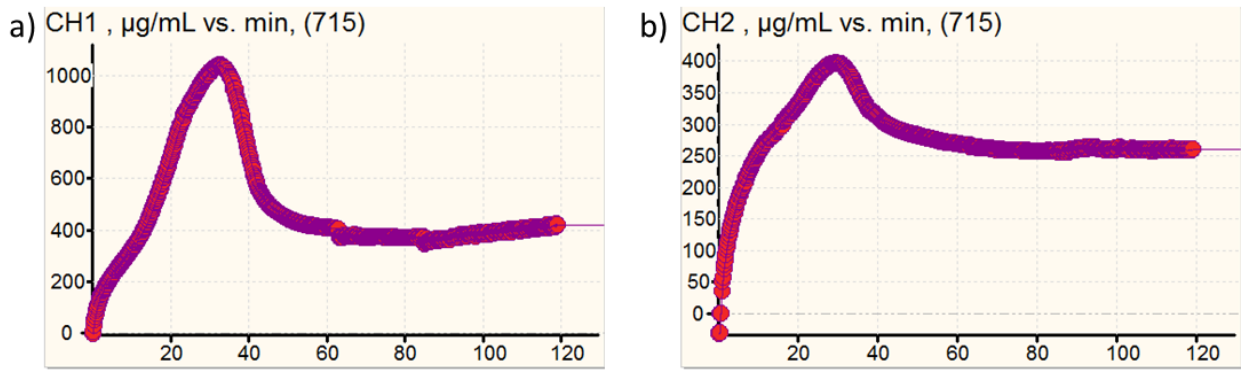

Figure S13. Example dissolution graphs for miconazole nitrate, corresponding to (a) trial 5 and (b) trial 6 of Table S2, showing the spring and parachute effect observed for this solid form. The equilibrium solubility for the nitrate salt cannot be determined using the chosen method because supersaturation of miconazole occurs quickly and reprecipication of miconazole hemihydrate at varying times causes drastically different maximum solubilities to be observed from trial to trial.

SI 9. Stability testing of miconazole hemi-HP solvate

Inclusion of hydrogen peroxide into the crystal structure of an API has the potential to accelerate degradation through an oxidative pathway. The susceptibility of an API will depend both on its chemical structure and the crystal packing. In the case of miconazole hemi-HP solvate, storage of the material at room temperature for 60 days led to negligible degradation as judged by proton NMR spectroscopy and UPLC analysis.

SI 10. References

(1) Shanley, E. S., Greenspan, F. P. Ind. Eng. Chem. 1947, 39, 1536-1543.

(2) American Type Culture Collection. How to Revive Cultures. https://www.atcc.org/en/How_to_Revive_Cultures.aspx#fungi (accessed October 15, 2017).

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(4) Dolomanov, O. V., Bourhis, L. J., Gildea, R. J., Howard, J. A. K. & Puschmann, H. *OLEX2*: a complete structure solution, refinement and analysis program. *J. Appl. Cryst.* **42**, 339-341 (2009).

(5) Sheldrick, G. M. *SHELXT* – Integrated space-group and crystal-structure determination. *Acta Cryst. A* **71**, 3-8 (2015).

(6) Sheldrick, G. M. Crystal structure refinement with *SHELXL*. *Acta Cryst. C* **71**, 3-8 (2015).