SUPPORTING INFORMATION

SYNTHESIS AND CHARACTERIZATION OF 5-MEO-DMT SUCCINATE FOR CLINICAL USE

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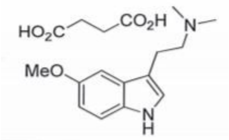
I. Certificate of Analysis

Chemical Formula: $C_{17}H_{24}N_2O_5$ Molecular Weight: 336.38 Freebase Monoisotopic Mass: 218.1419 Lot Number: RCL-0005E-040	Chemical Structure 

Test	Specification		Result	
Appearance	White to off-white solid		Off-white solid	
Identification by ^{1}H NMR (DMSO-d_6)	Conforms to reference spectrum		Conforms to reference spectrum	
Identification by HPLC	Retention time consistent with reference standard		Retention time consistent with reference standard	
Purity by HPLC	≥ 99.0% area		99.8% area	
Impurities by HPLC	5-MeO-DMT N-oxide impurity ≤ 0.50% area Total impurities Report result		5-MeO-DMT N-oxide impurity 0.14% area Total impurities 0.14% area	
Sulphated Ash	≤ 0.5% w/w		0.05% w/w	
Water Content by KF	Report result		0.06% w/w	
Residual Solvents by GC	Acetonitrile	≤ 410 ppm	Acetonitrile	< 12 ppm (LOQ)
	2-Methyl-THF	≤ 720 ppm	2-Methyl-THF	< 1 ppm (LOQ)
	MeOH	≤ 3000 ppm	MeOH	627 ppm
	Acetone	≤ 5000 ppm	Acetone	138 ppm
	Ethanol	≤ 5000 ppm	Ethanol	< 12 ppm (LOQ)
Limit Test for Dimethylaminobutyraldehyde diethyl acetal	Report result		≤ 500 ppm	
Quantification of 4-Methoxyphenylhydrazine HCl	Report result		≤ LOQ (0.05% w/w)	
Elemental Impurities by ICP-MS	Cd	≤ 0.2 ppm	Cd	< 0.080 ppm (LOQ)
	Pb	≤ 0.5 ppm	Pb	< 0.195 ppm (LOQ)
	As	≤ 1.5 ppm	As	< 0.610 ppm (LOQ)
	Hg	≤ 0.3 ppm	Hg	< 0.115 ppm (LOQ)
	Co	≤ 0.5 ppm	Co	< 0.195 ppm (LOQ)
	V	≤ 1 ppm	V	1.46 ppm
	Ni	≤ 2 ppm	Ni	< 0.773 ppm (LOQ)
	Li	≤ 25 ppm	Li	< 9.94 ppm (LOQ)
	Sb	≤ 9 ppm	Sb	< 3.50 ppm (LOQ)
	Cu	≤ 30 ppm	Cu	< 11.8 ppm (LOQ)
Polymorph by XRPD	Conforms to reference diffractogram		Conforms to reference diffractogram	
Succinate Counter Ion by ^{1}H NMR	31.6 – 38.6% w/w		34.1% w/w	

Table S1: 5-MeO-DMT succinate salt Certificate of Analysis.

II. Estimated Solubility of 5-MeO-DMT Succinate Salt

The solubility of 5-MeO-DMT succinate salt was estimated in 16 solvent systems using an aliquot addition method. Aliquots of test solvent were added to an accurately weighed sample (~20 mg) of 5-MeO-DMT at ambient temperature. The aliquot volumes were typically 25-100 µL. Complete dissolution of the test material was determined by visual inspection. If dissolution did not occur after the last aliquot of solvent was added (typically ~50 volumes of solvent), the sample was subjected to two cycles of the following temperature cycling protocol on a Clarity crystallization station:

1. Heat from 20 °C to within 3 °C of solvent boiling point (or 100 °C, whichever was lower) at 0.5 °C/minute.
2. Cool to 20 °C at 0.2 °C/minute.
3. Stirrer speed 600 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. Samples were then held at ambient temperature for ~12 hours to maximize the chance of precipitation. The solubility values for 5-MeO-DMT succinate were expressed as a range and rounded to the nearest whole number (**Table S2**). From this data, solvent systems were grouped in the following manner (see **Table S3**):

1. Solvents: 5-MeO-DMT succinate salt was soluble in ≤ 50 volumes (≥ 20 mg/mL) at ambient temperature.
2. Soluble with heating: 5-MeO-DMT succinate salt was not soluble in 50 volumes at ambient temperature but dissolved at higher temperatures. These solvents could be regarded as possible solvents for cooling crystallizations.
3. Anti-solvents: 5-MeO-DMT succinate salt was not soluble in 50 volumes at all temperatures studied.

The solubility was estimated from these experiments based on the total solvent used to provide complete dissolution. It should be noted that the actual solubility may be greater than that calculated because of the use of solvent aliquots that were too large or due to a slow rate of dissolution. The material was insoluble in most of the solvents tested but exhibited the highest solubility in DMSO, DMF, TFE, water, and aqueous mixtures.

Solvent	Solubility Range (mg/mL)
Acetone	< 20
Acetonitrile	< 20
Dimethyl formamide	136 - 204
Dimethyl sulfoxide	202 - 269
Ethanol	< 20
Ethyl acetate	< 20
Methanol	50 - 67
Methyl tert-butyl ether	< 20
Tetrahydrofuran	< 20
Toluene	< 20
Trifluoroethanol	400 - 800
Water	269 - 404
50:50 Acetone/Water	398 - 796
50:50 Acetonitrile/Water	404 - 808
50:50 THF/Water	275 - 412
50:50 Ethanol/Water	272 - 408

Table S2: Solubility estimates of 5-MeO-DMT succinate salt at 20 °C.

Solvents	**Soluble with Heating**	**Anti-Solvents**
Methanol	Ethanol	Acetone
Trifluoroethanol	-	Acetonitrile
Water	-	Ethyl acetate
-	-	Methyl tert-butyl ether
-	-	Tetrahydrofuran
-	-	Toluene

Table S3: Solvent systems grouped into categories.

III. Characterization of 5-MeO-DMT Succinate Salt

Thermogravimetric/Differential Thermal Analysis (TG/DTA)

Thermogravimetric/Differential Thermal Analysis (TG/DTA) was performed to determine the thermal profile and associated % weight changes of 5-MeO-DMT succinate salt (**Figure S1**). Thermogravimetric analyses were carried out on a Mettler Toledo TGA/DSC1 STARe instrument. The calibration standards were indium and tin. Samples were accurately weighed in an aluminum pan on an analytical balance and inserted into the TG furnace. Under a stream of nitrogen at a rate of 10 °C/minute, the heat flow signal was stabilized for one minute at 30 °C, prior to heating to 300 °C.

A weight loss of ~0.1% was noted from ~30 °C to 132 °C suggesting minimal moisture or solvent content, indicating the 5-MeO-DMT succinate salt to be an anhydrous material. A second weight loss at temperatures greater than 240 °C corresponds to the initiation of decomposition of the material. An endotherm due to the melt was observed with onset temperature of 133 °C.

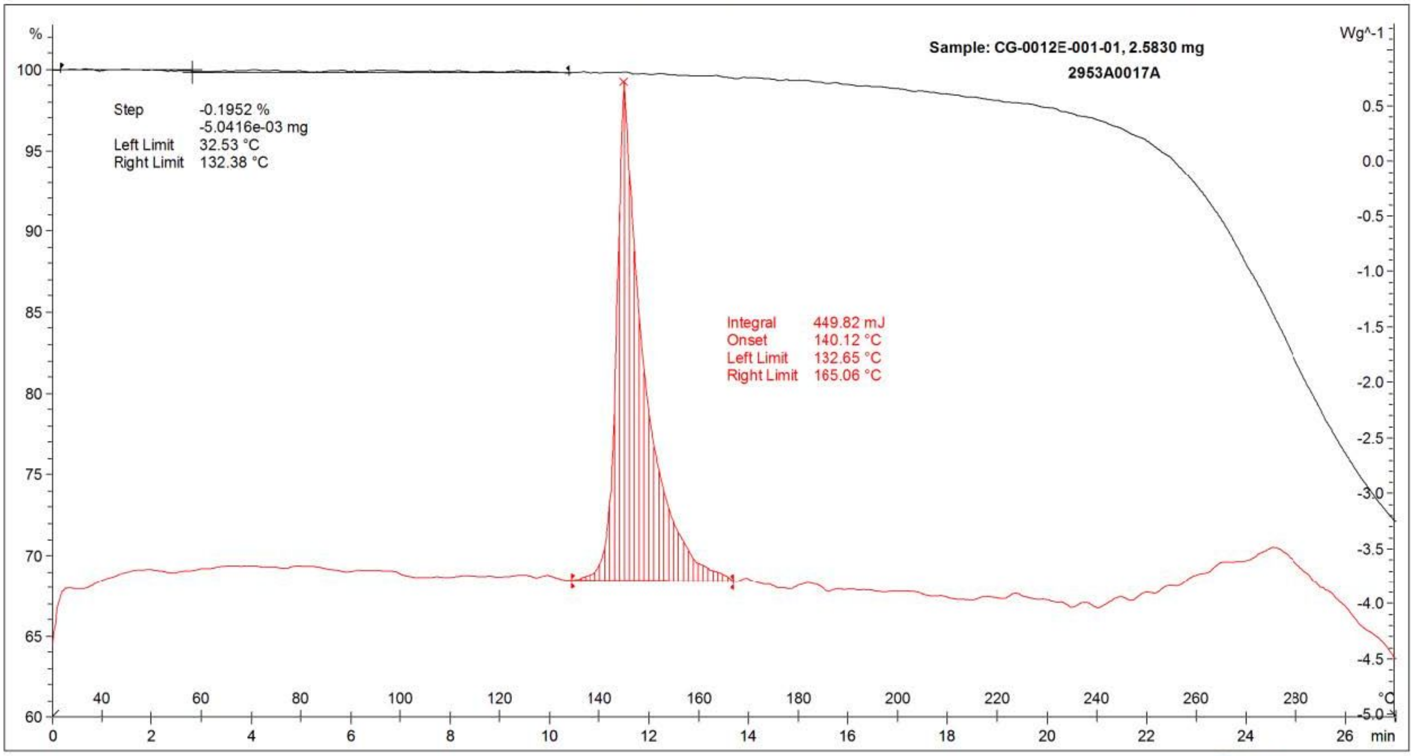


Figure S1: TG/DTA thermogram for 5-MeO-DMT succinate salt analyzed from 30-300 °C at 10 °C per minute.

Differential Scanning Calorimetry (DSC)

DSC analyses were carried out on a Perkin Elmer Jade Differential Scanning Calorimeter. Accurately weighed samples were placed in crimped aluminum pans (i.e. closed but not gas tight). Each sample was heated under nitrogen at a rate of 10 °C/minute to a maximum of 220 °C. Indium metal was used as the calibration standard. Temperatures were reported at the transition onset to the nearest 0.01 degree. Note: where multiple thermal events are observed at similar temperatures, ΔH values are prone to significant error.

The DSC thermogram obtained for 5-MeO-DMT succinate salt at 10 °C/min (**Figure S2**) is concordant with TG/DTA data with onset of melt at 140 °C. As a single thermal event was noted in the DSC (due to the melt), the material is likely to be composed of a single phase and was labeled Form A.

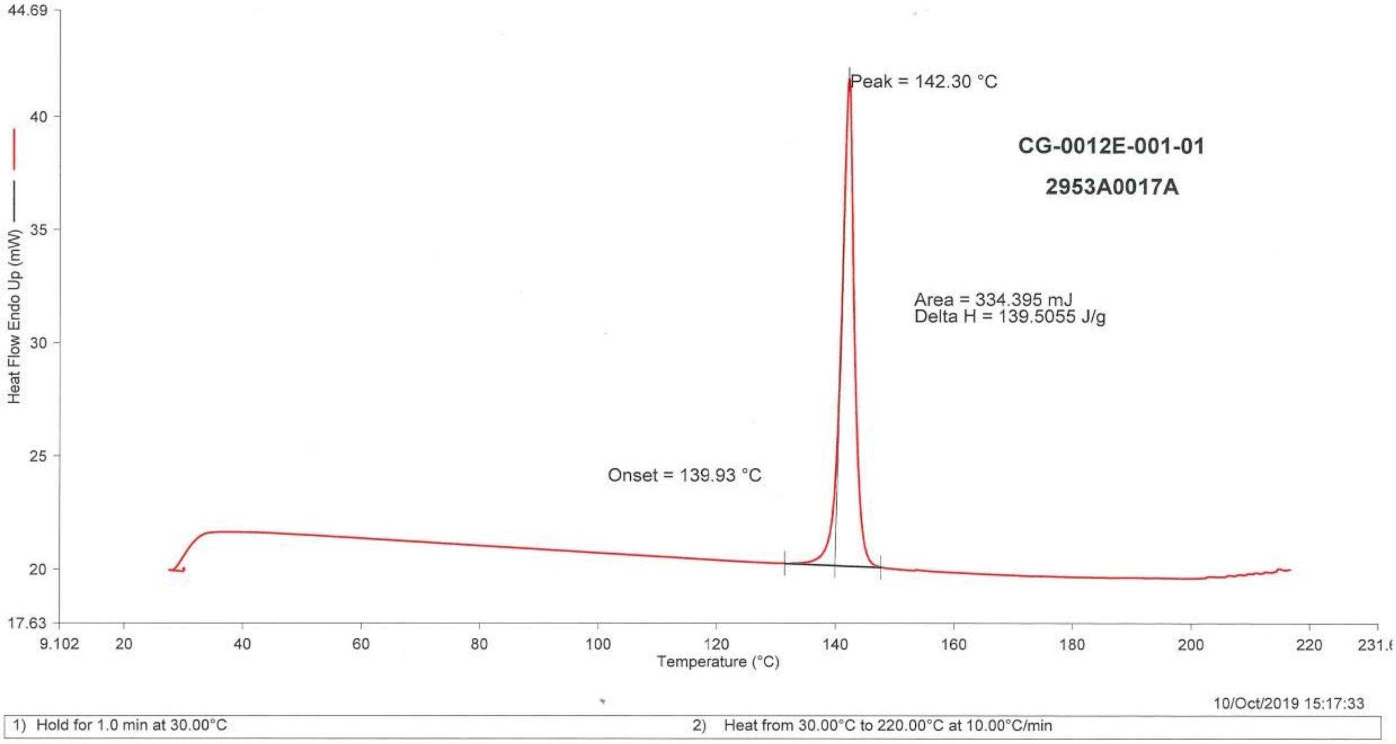


Figure S2: DSC thermogram for 5-MeO-DMT succinate salt analyzed from 30-220 °C at 10 °C per minute.

Hyper Differential Scanning Calorimetry (DSC)

Hyper DSC analyses were carried out on a Perkin Elmer DSC8500 Differential Scanning Calorimeter. Accurately weighed samples were placed in crimped aluminum pans (i.e. closed but not gas tight). Each sample was heated and cooled under nitrogen over two cycles at a rate of 300 °C/minute using a set temperature range of -70 to 200 °C. Indium metal was used as the calibration standard.

Hyper DSC was performed in order to generate amorphous material from fast cooling of molten 5-MeO-DMT succinate salt and determine the temperature of glass transition (T_g) during the re-heat cycle. The thermogram (**Figure S3**) exhibited a thermal event in the reheat cycle attributed to the T_g at 30 °C (half height c_p value).

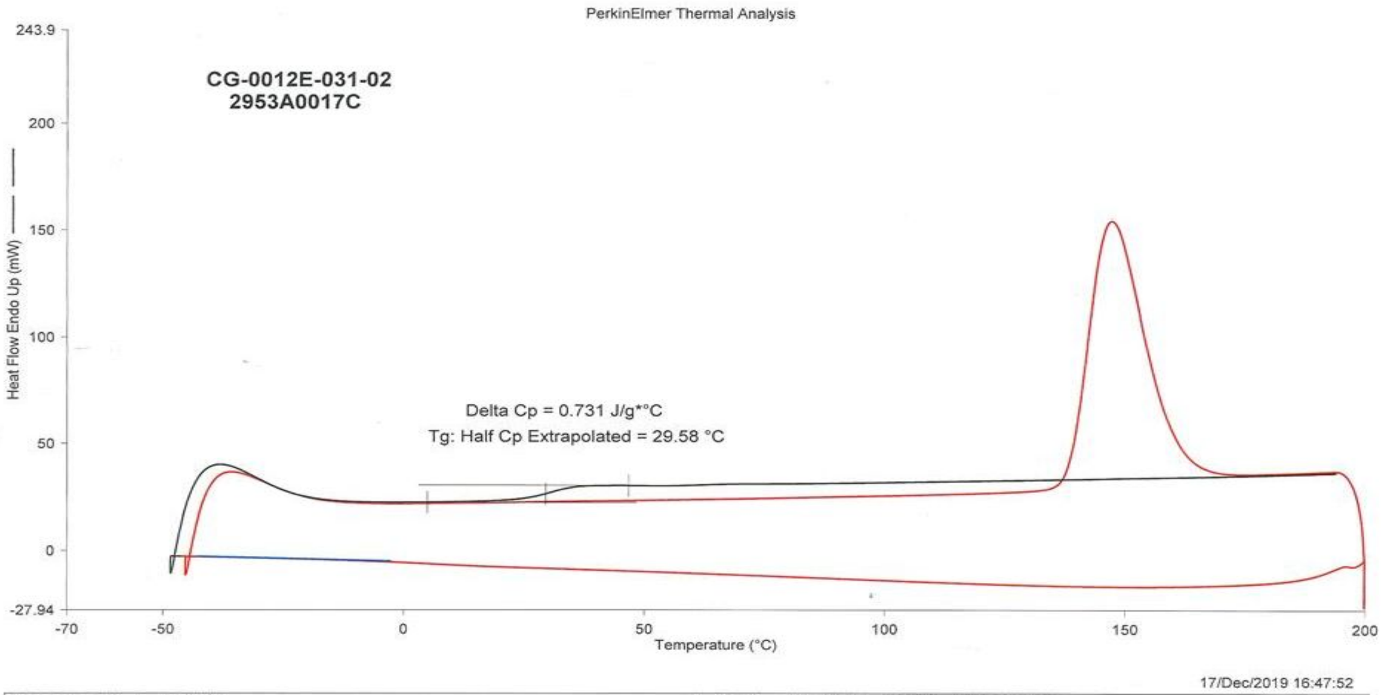


Figure S3: Cyclic hyper DSC thermogram analyzed at 300 °C per minute.

Dynamic Vapor Sorption (DVS)

The hygroscopicity and the sorption properties of 5-MeO-DMT succinate salt were determined using Dynamic Vapor Sorption (DVS). DVS was performed using a SMS DVS Intrinsic Vapor Sorption Balance. Approximately 20 mg of sample was placed into a wire-mesh vapor sorption balance pan, loaded into the vapor sorption balance, and held at 25 °C ± 0.1 °C. The sample was subjected to a step profile from 40-90% RH at 10% increments, followed by desorption from 90-0% RH at 10% increments and a second sorption cycle from 0-40% RH. The equilibrium criterion was set to 98.0% step completion within a minimum of 60 minutes and a maximum of 5 hours for each increment. The weight change during the sorption cycle was monitored, allowing for the hygroscopic nature of the sample to be determined. The data collection interval was in seconds.

The isothermal plot obtained is shown in **Figure S4** and the kinetics plot is displayed in **Figure S5**. The isotherm showed that the material exhibits slow uptake of moisture, generating an increase in weight from 40-70% RH followed by an increase in weight at a higher rate between 80-90% RH. The isotherm showed the total weight gain observed between ambient (i.e. 40% RH) and 80% RH to be 0.1% w/w which indicates that the sample is not hygroscopic, based on the European Pharmacopoeia classification (**Table S4**).

The rate of desorption was comparable with that of sorption, with little or no hysteresis, and all of the adsorbed water was removed by the end of the run. The post DVS XRPD data matched that of the starting material, indicating no detectable solid form changes or changes in crystallinity (**Figure S6**).

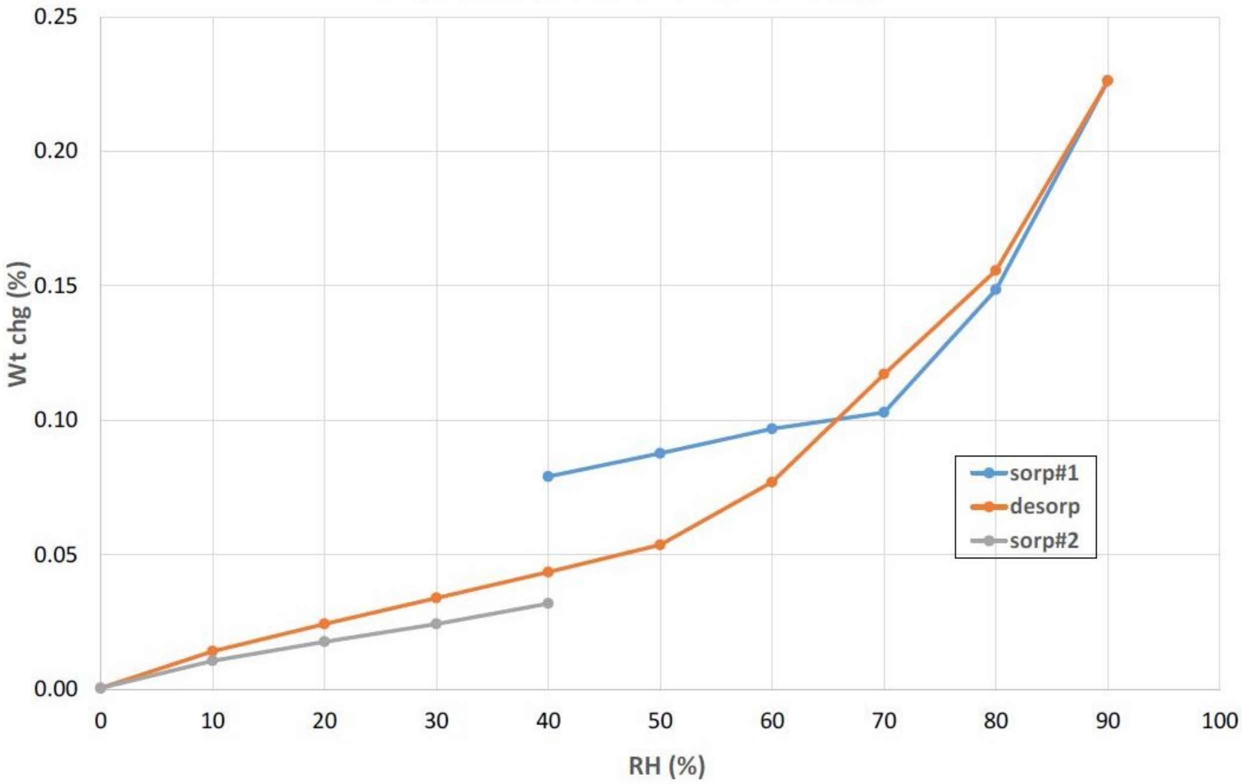


Figure S4: DVS isothermal plot for 5-MeO-DMT succinate salt.

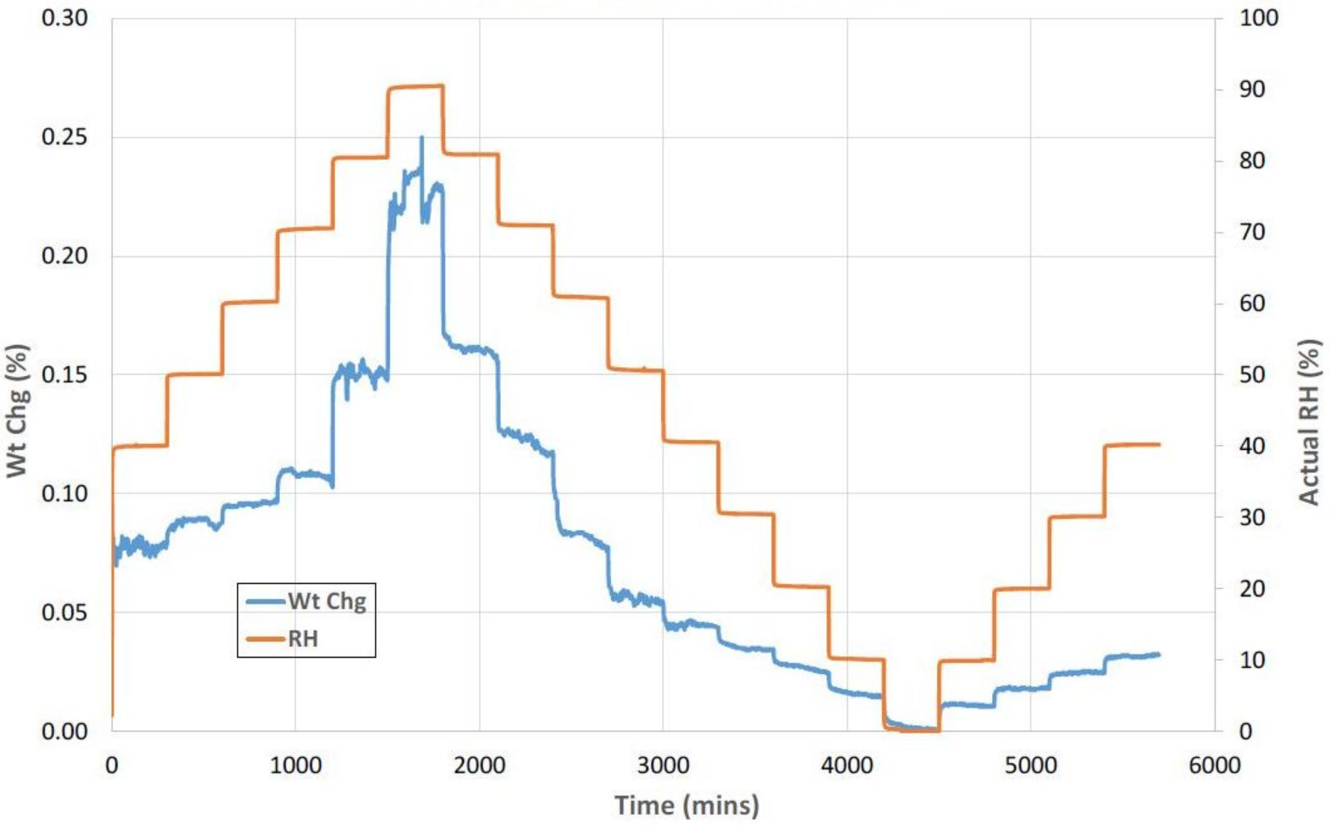


Figure S5: DVS kinetics plot for 5-MeO-DMT succinate salt.

Classification	Weight increase from 40-80% RH (25°C)
Non hygroscopic	< 0.2%
Slightly hygroscopic	≥ 0.2 % and < 2 %
Hygroscopic	≥ 2% and < 15%
Very hygroscopic	≥ 15%
Deliquescent	Sufficient water is absorbed to form a liquid

Table S4: Hygroscopicity Classification[1]

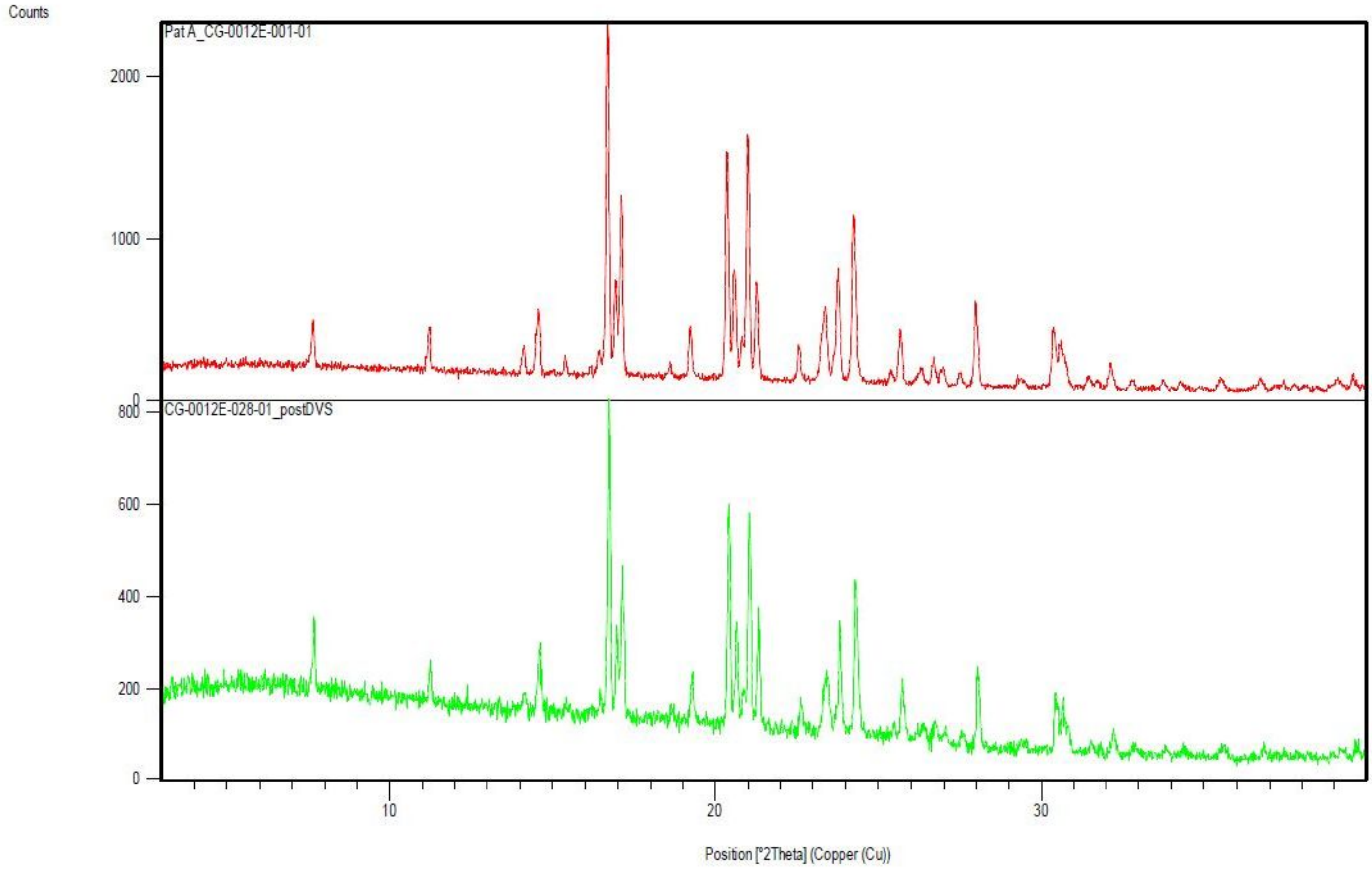


Figure S6: XRPD patterns of 5-MeO-DMT succinate salt (top) and post DVS sample (bottom).

^{1}H and Nuclear Magnetic Resonance spectroscopy (NMR)

NMR analysis was carried out on a Bruker 500MHz instrument in DMSO-d_6. The proton and carbon NMR spectrum of 5-MeO-DMT succinate salt (**Figure S7 and S8**) conformed to the molecular structure. An equimolar stoichiometry of freebase to succinic acid was noted.

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[1] V. Sihorkar et al, Pharmaceutical Dev. & Technol. (2013), 18(2), 348-358

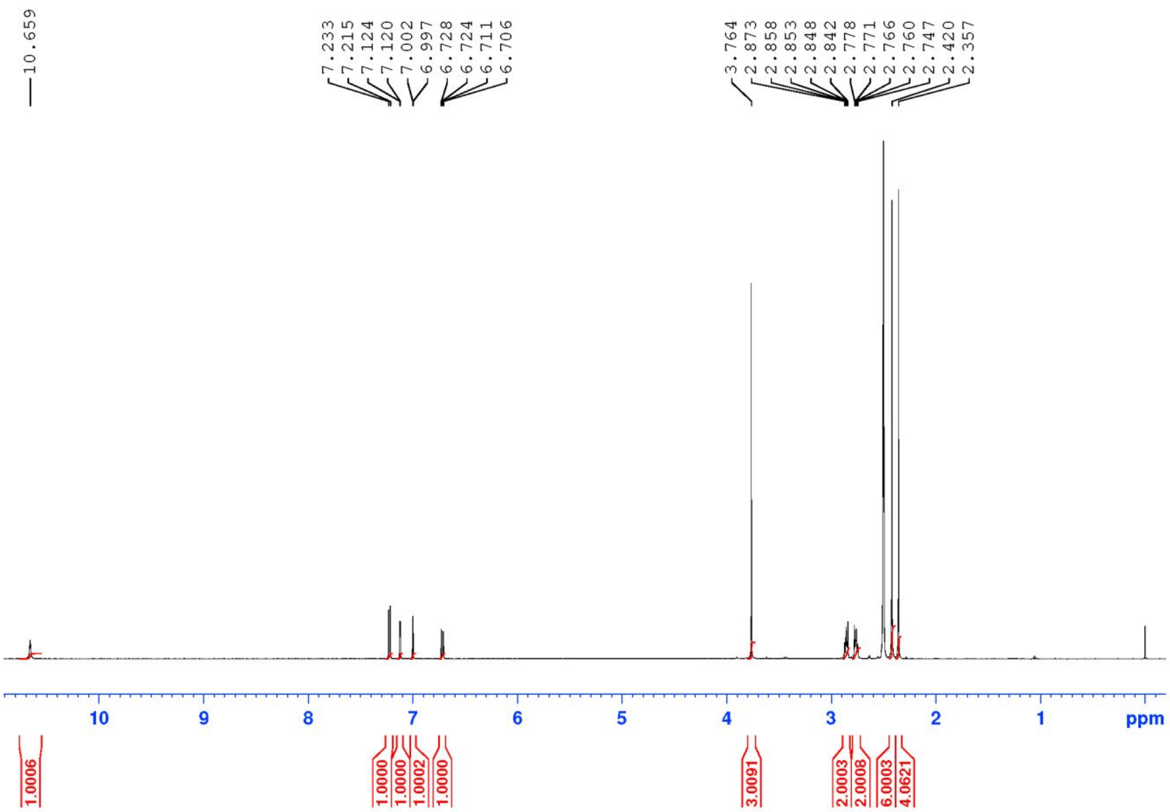

Figure S7: ^{1}H NMR spectrum of 5-MeO-DMT succinate salt in DMSO-d_6.

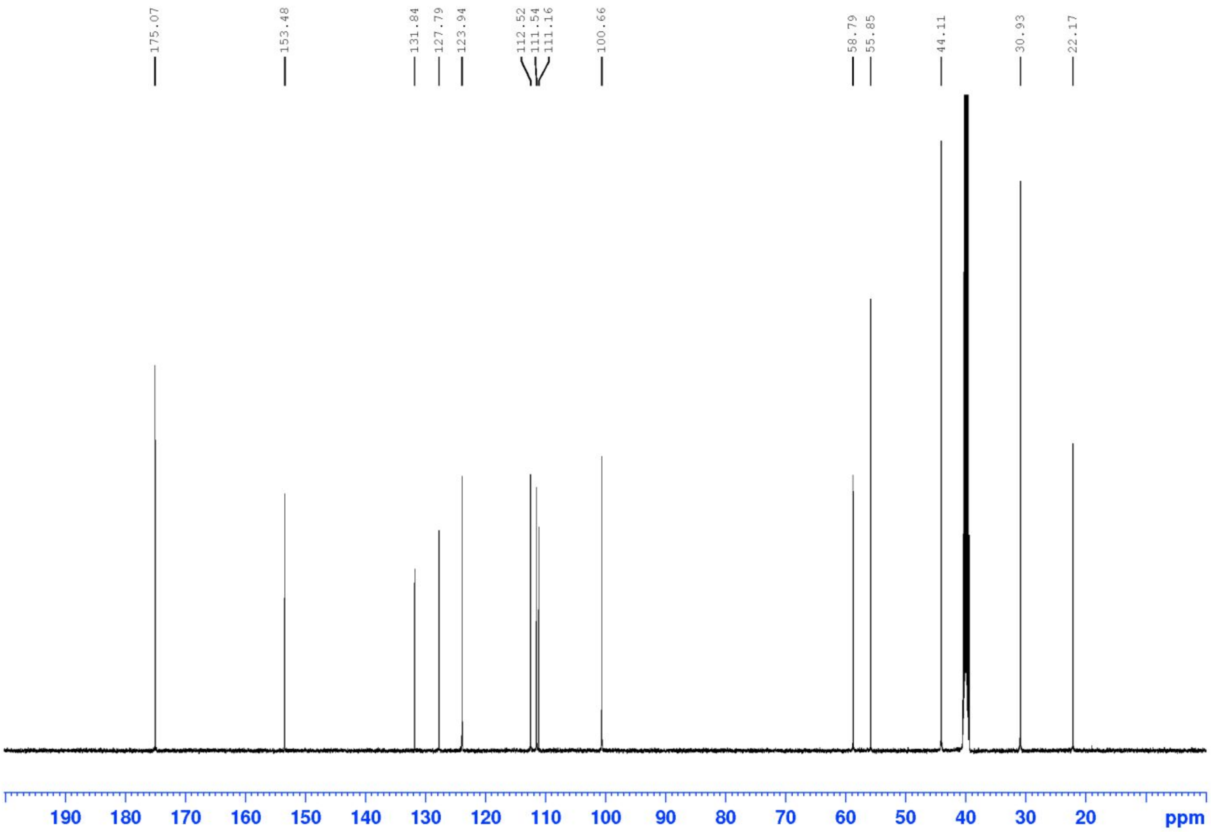

Figure S8: ^{13}C NMR spectrum of 5-MeO-DMT succinate salt in DMSO-d_6.

Optical Microscopy

Microscopy analyses were carried out using an Olympus BX51 stereomicroscope with cross-polarized light and a 1st order red compensator plate. Photomicrographic images were captured using a ColorView IIIu digital camera and SynchronizIR basic V5.0 imaging software with objective lens magnification of x10.

Photomicrographic images of 5-MeO-DMT succinate salt were collected under cross-polarized light (**Figure S9**). The sample was composed of plates with a particle size range of 10-140 µm.

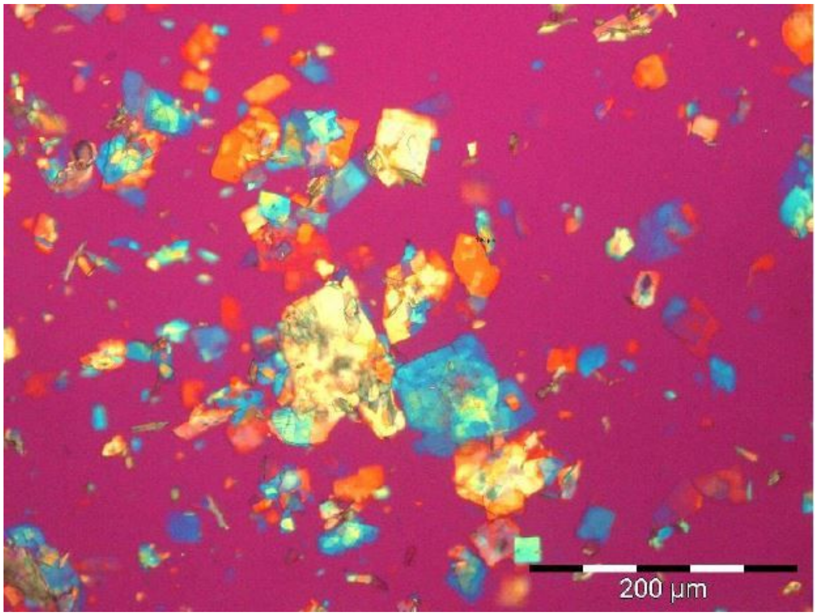

Figure S9: Photomicrographic images of 5-MeO-DMT succinate salt.

IV. Polymorph Screen

Summary and Results

A standard polymorph screen was performed on 5-MeO-DMT succinate salt to identify the form most appropriate for development and GMP manufacture. The approach is to generate solids under a wide and diverse range of nucleation conditions, designed to mimic the process conditions and solvents used during development and formulation.

Approximately 180 experiments were carried out using solvent and non-solvent based techniques. In addition to a supplied sample of 5-MeO-DMT succinate salt designated Form A, ten unique crystalline solids (**Table S5 and Figure S10**) were observed during this study and in order of discovery were labeled Pattern B-K materials.

Form A material exhibited good solid state properties being a crystalline, non-hygroscopic anhydrate and was isolated from the majority of experiments. Given that Pattern K material was only seen from two experiments and Patterns B-J materials are solvated, Form A was selected as the solid form with clear potential for drug development.

Material	Prep Conditions	Comments
Form A	From methanol, acetone, or ethanol	Crystalline anhydrate
Pattern B	In situ salt prep from EtOAc-THF	THF solvate
Pattern C	VS of amorphous with DCE	DCE solvate
Pattern D	VS of amorphous with 2Me-THF	2-MeTHF solvate

Pattern E	VS of amorphous with EtOAc	EtOAc solvate
Pattern F	VS of Form A with MEK	MEK solvate
Pattern G	40 °C slurry of Form A in dioxane	Dioxane solvate
Pattern H	40 °C slurry of Form A in cyclohexane	Cyclohexane solvate
Pattern I	40 °C slurry of Form A in NMP	NMP solvate
Pattern J	5 °C slurry of Form A in toluene	Toluene solvate
Pattern K	Form A slurry in NMP-water (1:1)	Mixture containing probable hydrate or NMP solvate
Amorphous	Melt–quench of Form A	T_g = 30 °C (half-height cp value)

Table S5: Unique solids isolated from the polymorph screen of 5-MeO-DMT succinate salt.

1. VS = vapor stress.
2. All unique solids were composed of equimolar stoichiometry of API and succinic acid

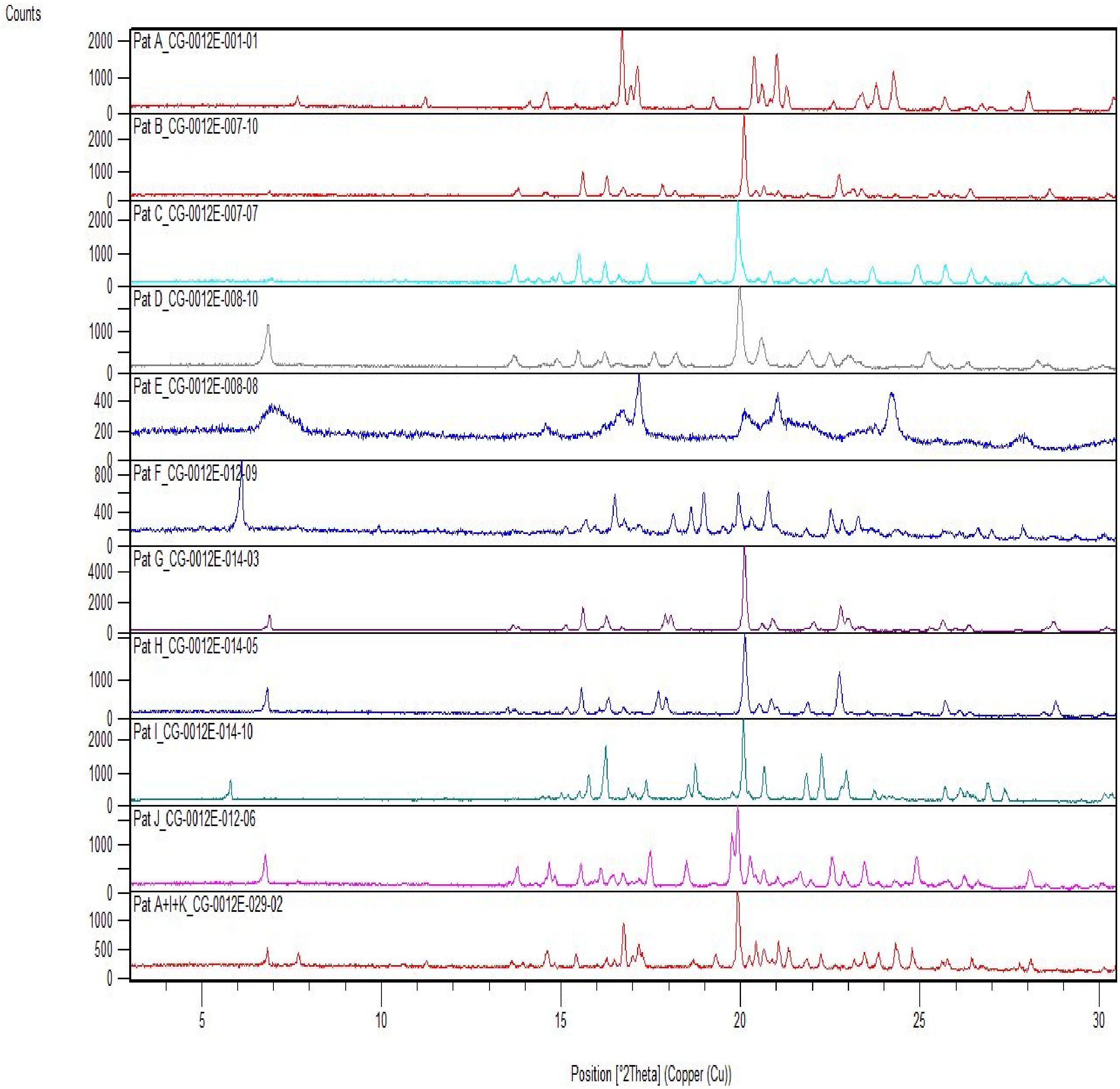

Figure S10: XRPD patterns of each of the unique materials isolated during the polymorph screen.

Characterization of Unique Solids

All solids from the crystallization experiments were analyzed by XRPD and the resulting patterns compared to that exhibited by the supplied sample of 5-MeO-DMT succinate salt. Where sufficient material was available, further analysis (e.g. NMR or TGA) was conducted on solids with novel XRPD patterns to allow tentative assignment of the novel Form as a polymorph, solvate, hydrate, degradant, or mixture thereof (**Table S6**).

The majority of samples analyzed in the screen were composed of Form A anhydrate. Most appear to be solvated and all contain 1 molar eq succinic acid; no hydrates were found in the screen. Some of the solvates exhibited similar XRPD patterns, suggesting that they are composed of similar crystal structures. In particular, the similar XRPD patterns for Pattern G and H materials suggest that they are isostructural solvates with dioxane and cyclohexane.

Two samples isolated from the final few experiments of the polymorph screen were composed of a mixture containing mainly Form A with minor Pattern I and a new form designated Pattern K material. As Pattern K material was only isolated as a minor component of a mixture, it was difficult to characterize but is likely a NMP solvate or hydrate. The mixture containing Pattern K material can be fully converted to Form A material by heating to ~120 °C but may also convert at a lower temperature.

Material	Comments [1,2,3]
Form A	Crystalline anhydrate (supplied material)
Pattern B	TG: 7.0% wt loss from 44-134 °C (0.35 mol eq THF), NMR: 0.44 mol eq THF
Pattern C	TG: 12.7% wt loss from 70-135 °C (0.5 mol eq DCE), NMR: 0.5 mol eq DCE
Pattern D	TG: 10.7% wt loss from 64-135 °C (0.5 mol eq 2MeTHF), NMR: 0.5 mol eq 2MeTHF
Pattern E	TG: 6.1% wt loss from 54-136 °C (0.25 mol eq EtOAc), NMR: 0.33 mol eq EtOAc
Pattern F	TG: 12.1% wt loss from 48-123 °C (0.64 mol eq MEK), NMR: 1 mol eq MEK
Pattern G [4]	TG: 5.7% wt loss from 70-130 °C (0.23 mol eq dioxane), NMR: 1.4 mol eq dioxane
Pattern H	TG: 7.5% wt loss from 70-130 °C (0.32 mol eq cyclohexane), NMR: 0.4 mol eq cyclohexane
Pattern I	TG: 2.1% wt loss from 50-130 °C (0.1 mol eq NMP), 20% wt loss from 50-220 °C (0.85 mol eq), NMR: 1.1 mol eq NMP
Pattern J	TG: 7.6% wt loss from 60-130 °C (0.3 mol eq toluene), NMR: 0.5 mol eq toluene
Pattern K [5]	TG: 5.7% wt loss from 70-124 °C (0.2 mol eq NMP/ 1.1 mol eq water), NMR: 0.25 mol eq NMP
Amorphous	T_g = 30 °C (half-height cp value)

Table S6: Summary of the physical forms of 5-MeO-DMT observed during the polymorph screening experiments.

1. TG = thermogravimetric analysis
2. NMR = proton nuclear magnetic resonance
3. Equimolar stoichiometry of API: succinic acid noted in ^{1}H NMR spectrum of Pattern B-J materials
4. It is unknown why the amounts of solvent present differ between TG and ^{1}HNMR analysis
5. Only isolated as a minor component in a mixture, therefore difficult to evaluate data

V. HPLC Methodology and Chromatograms

HPLC analyses (identification, assay, and impurity content) were carried out on a Waters Alliance e2695 reversed-phase HPLC system with UV/PDA detection. The chromatographic separation was performed using a X Bridge BEH Phenyl column (250 x 4.6 mm, 5 µm) at 58 °C. The mobile phases consisted of 0.2 M phosphate buffer, pH 6 (mobile phase A) and 100% methanol (mobile phase B). The mobile phase was delivered at a flow rate of 1.5 mL/min in gradient mode over a total run time of 35 minutes. Identification of 5-MeO-DMT was performed by injecting 10 µL volume of 0.2 mg/mL sample with the PDA set to 225 nm.

Time (minutes)	Mobile Phase A (%)	Mobile Phase B %
0	95	5
18.75	20	80
27.0	20	80
29.0	95	5
35.0	95	5

Table S7: HPLC Gradient conditions.

Name	Retention Time (minutes)	Relative Retention Time
5-MeO-DMT succinate salt	7.952	N/A
N-oxide impurity (**21**)	8.410	1.059

Table S8: Typical retention times and relative retention times for 5-MeO-DMT and the N-Oxide impurity (**21**).

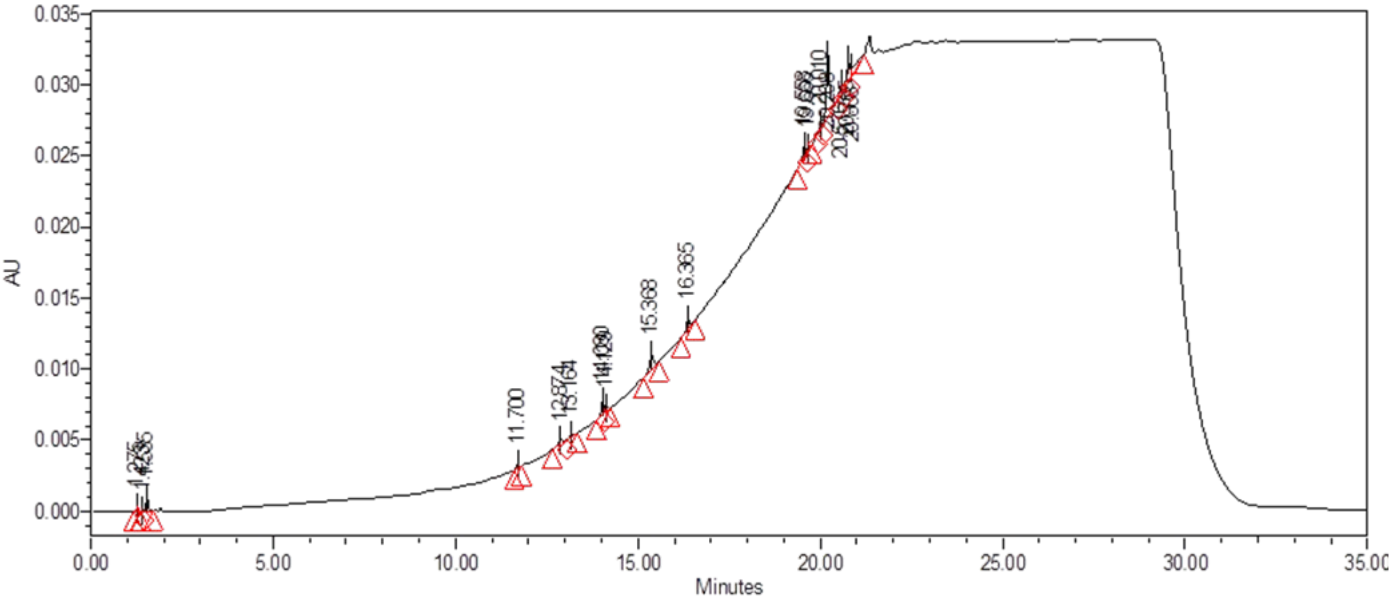

Figure S11: Typical blank diluent injection (95:5 water:methanol) chromatogram output.

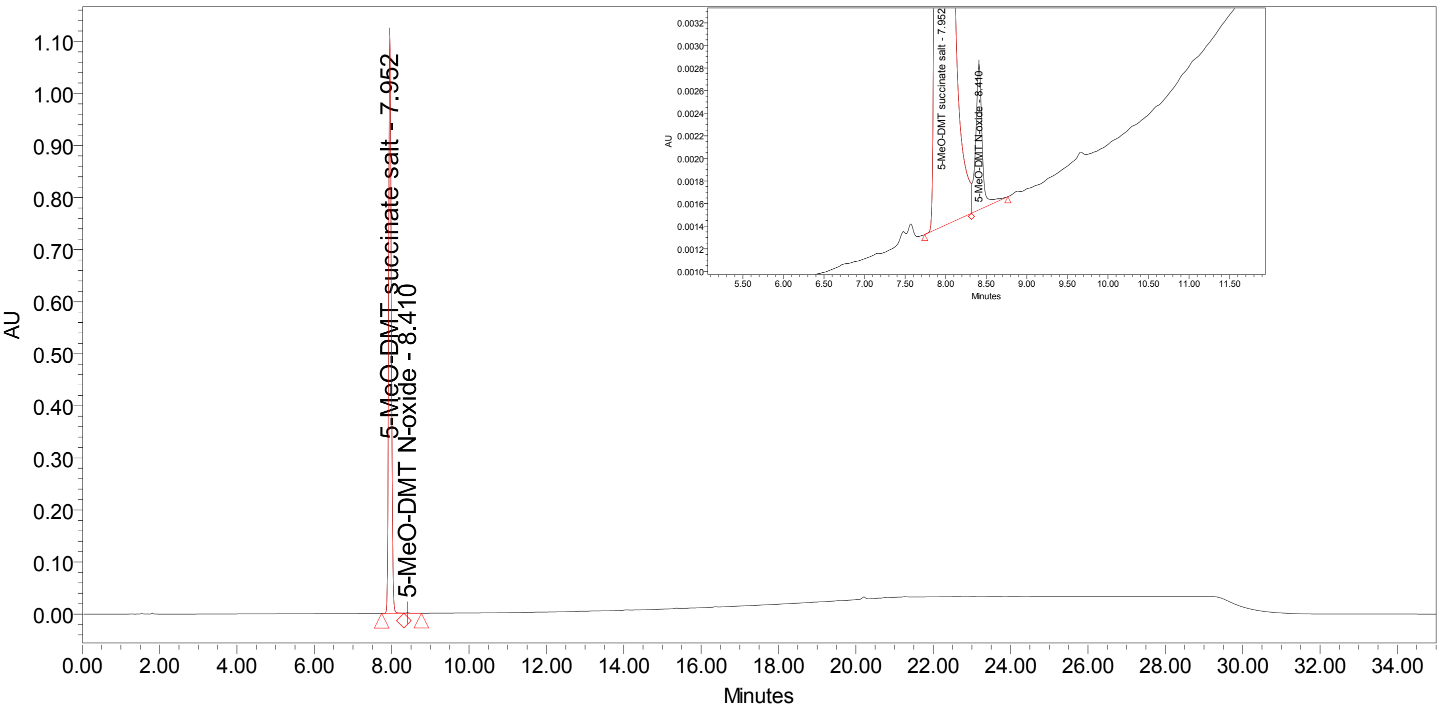

Figure S12: Typical standard/sample chromatogram (expanded).

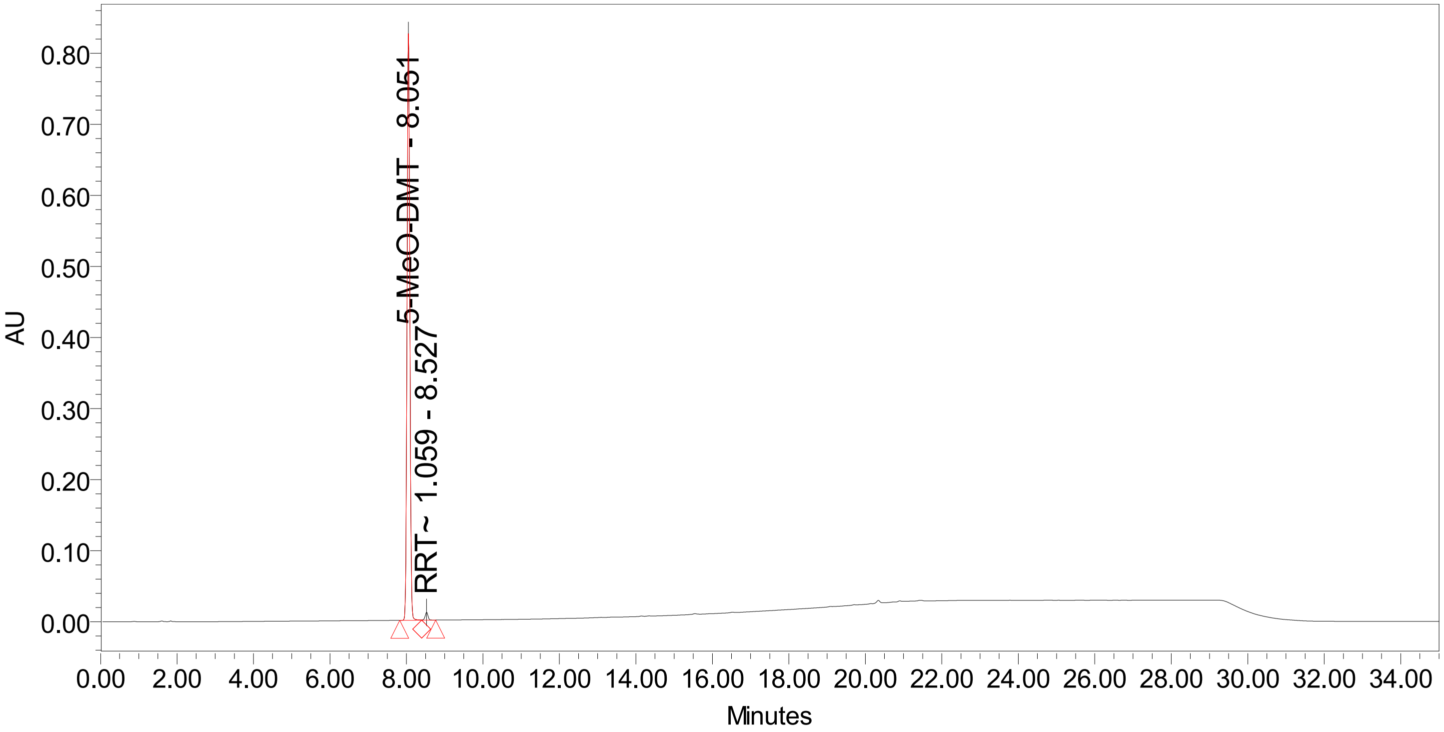

Figure S13: Chromatogram of sample spiked with 1% N-Oxide (**21**).

VI. Impurity Identification and Characterization Data

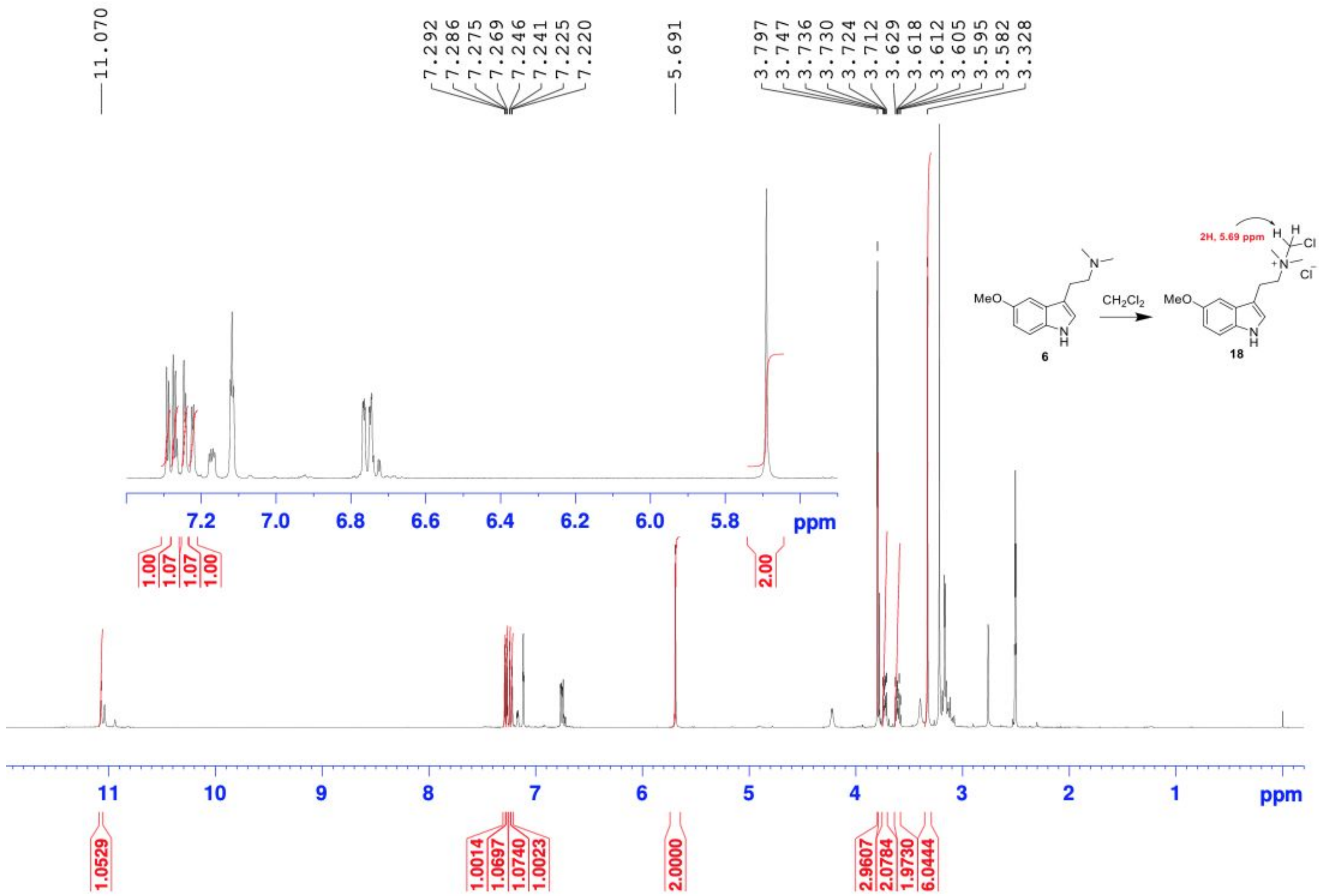

Figure S14: ^{1}H-NMR for crude oil, DCM adduct (**18**).

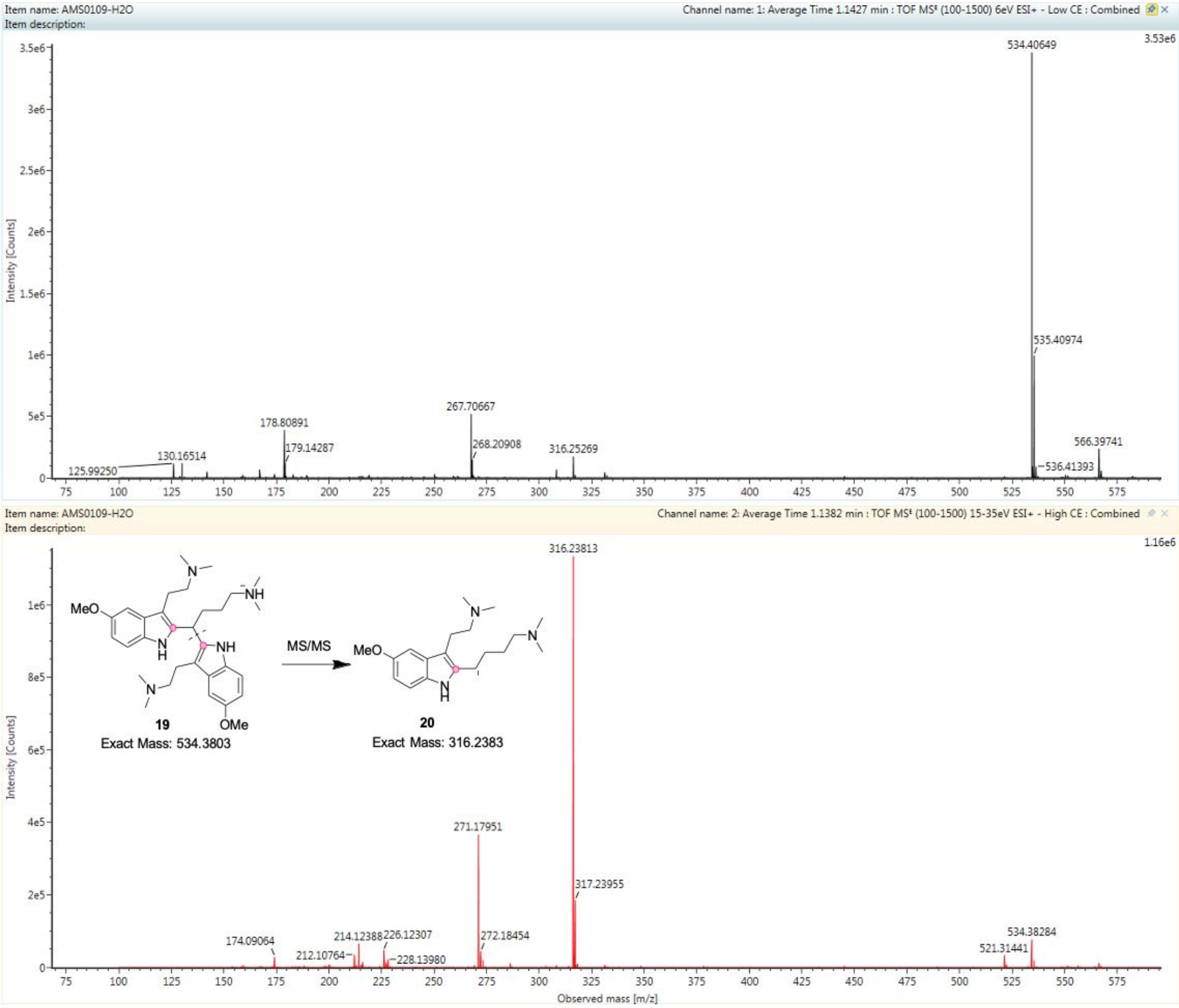

Figure S15: High resolution mass spectra of putative high-level dimer byproduct (**19**). Top spectra low CID showing parent ion at m/z 534.4064 and bottom high CID showing m/z 316.2381 fragment.

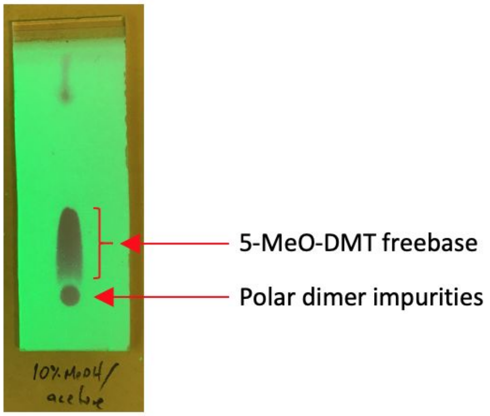

Figure S16: Thin layer chromatography analysis of post-workup reaction mixture with 10% MeOH/acetone mobile phase. Polar dimer impurities remain adhered to baseline and supported the use of a silica pad filtration for purification of the crude reaction mixture.

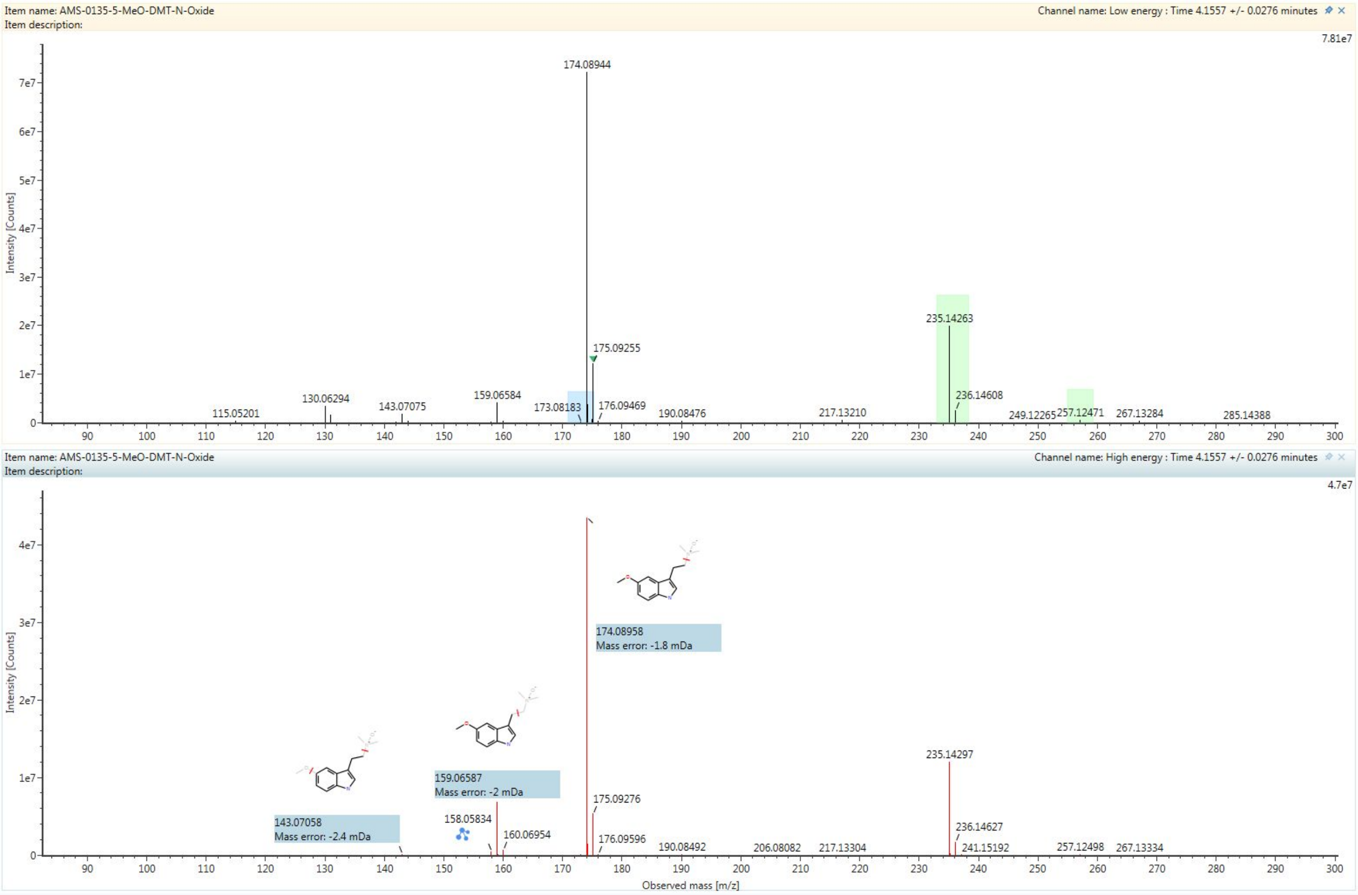

Figure S17: High resolution mass spectra for N-oxide (**21**).

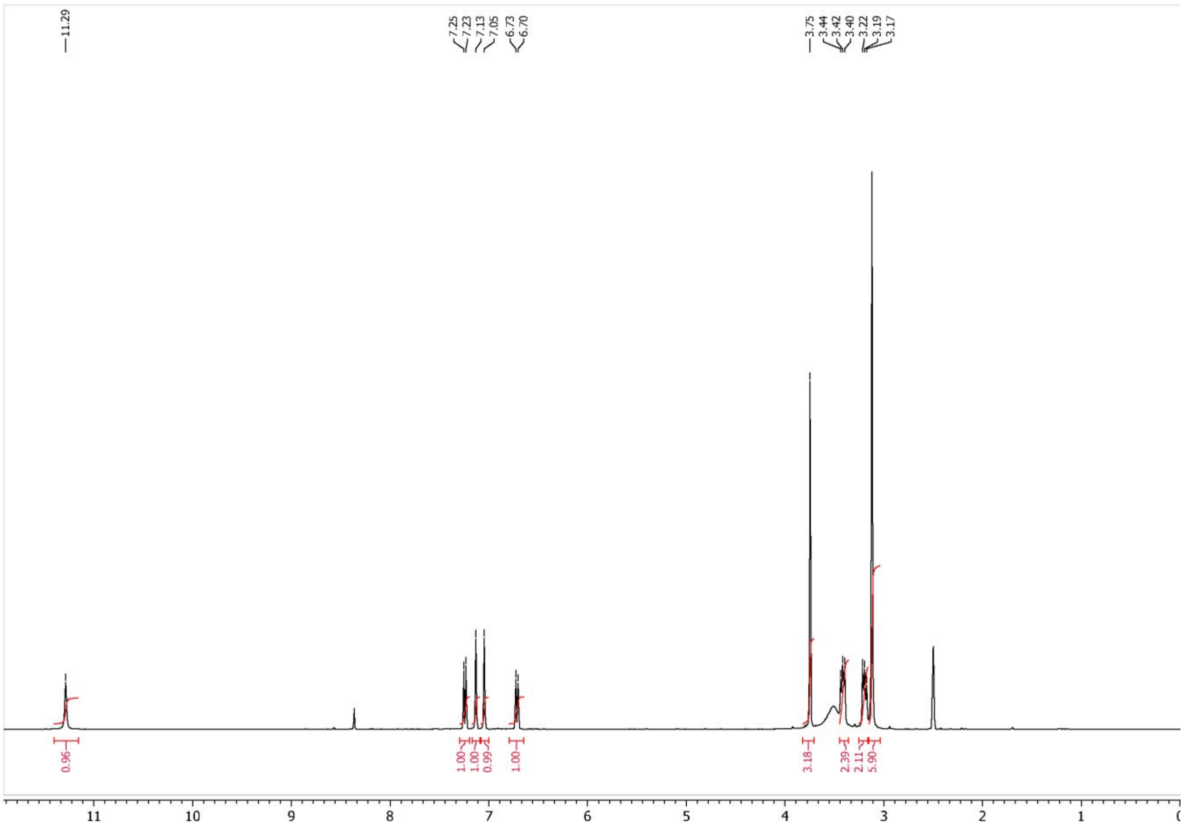

Figure S18: ^{1}H NMR spectrum of N-oxide (**21**) in DMSO-d$_6$.

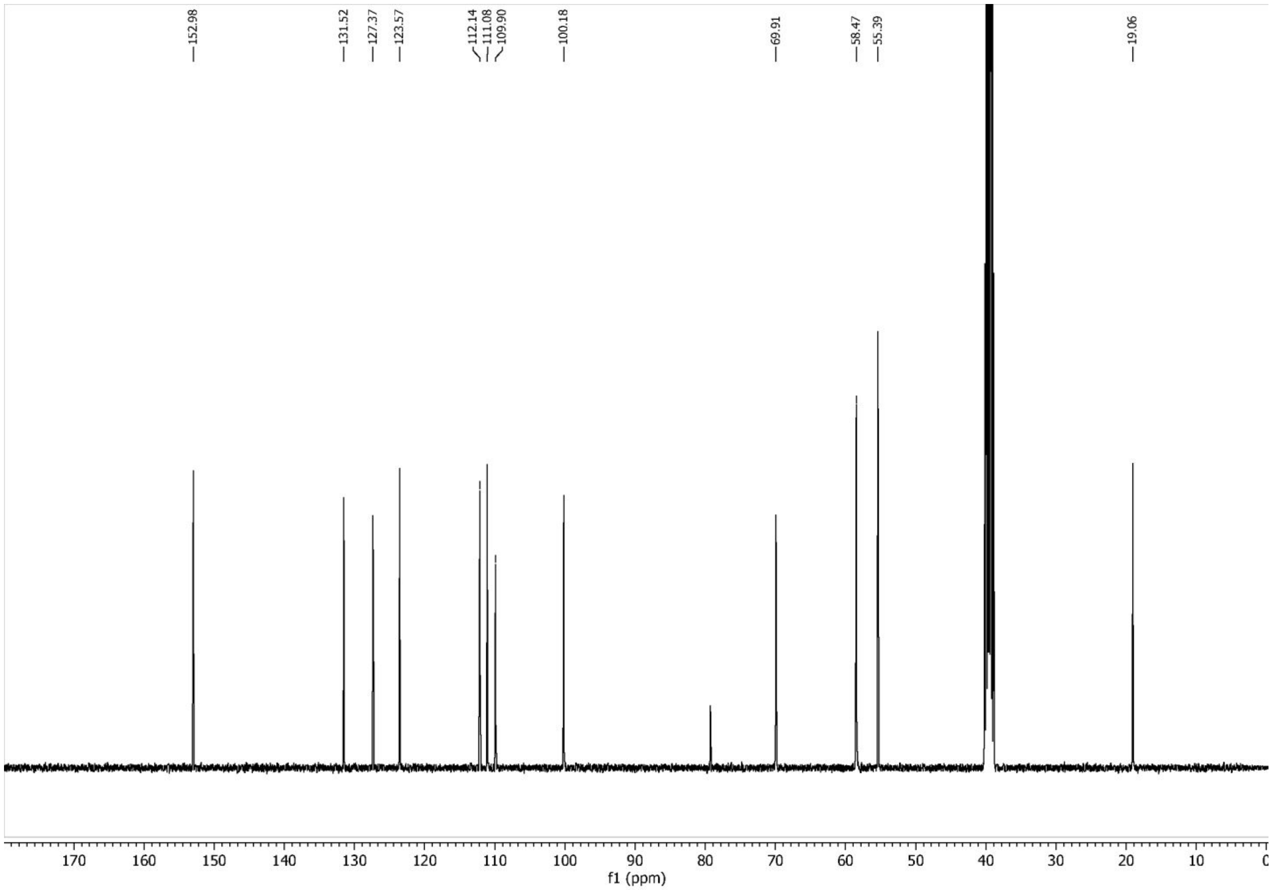

Figure S19: ^{13}C NMR spectrum of N-oxide (**21**) in DMSO-d$_6$.