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(45) Date of B Publication 22.07.2020

(54) Title of the Invention: Crystaline psilocybin polymorph A, a formulation and uses thereof together with a method of preparation

(51) INT CL: ***C07B 63/00*** (2006.01) ***A61K 31/661*** (2006.01) ***A61P 25/00*** (2006.01) ***A61P 25/24*** (2006.01)
C07F 9/572 (2006.01)

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(22) Date of Filing: **28.06.2018**

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(43) Date of A Publication **18.09.2019**

(56) Documents Cited:
GB 0912714 A **US 3075992 A**
Experientia, Vol. 14, 1958, (Hofmann, A. et al), "Psilocybin, a hallucinogen from Psilocybe mexicana", pages 107-9, ISSN: 0014-4754
Synthesis, No. 6, 1999, (Nichols, David E. et al), pages 935-938, ISSN: 0039-7881

(58) Field of Search:
As for published application 2572023 A viz:
Other: **CAS-ONLINE**
updated as appropriate

Additional Fields
INT CL **C07B, C07D, C07F**
Other: **WPI, EPODOC**

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GB 2572023 C2

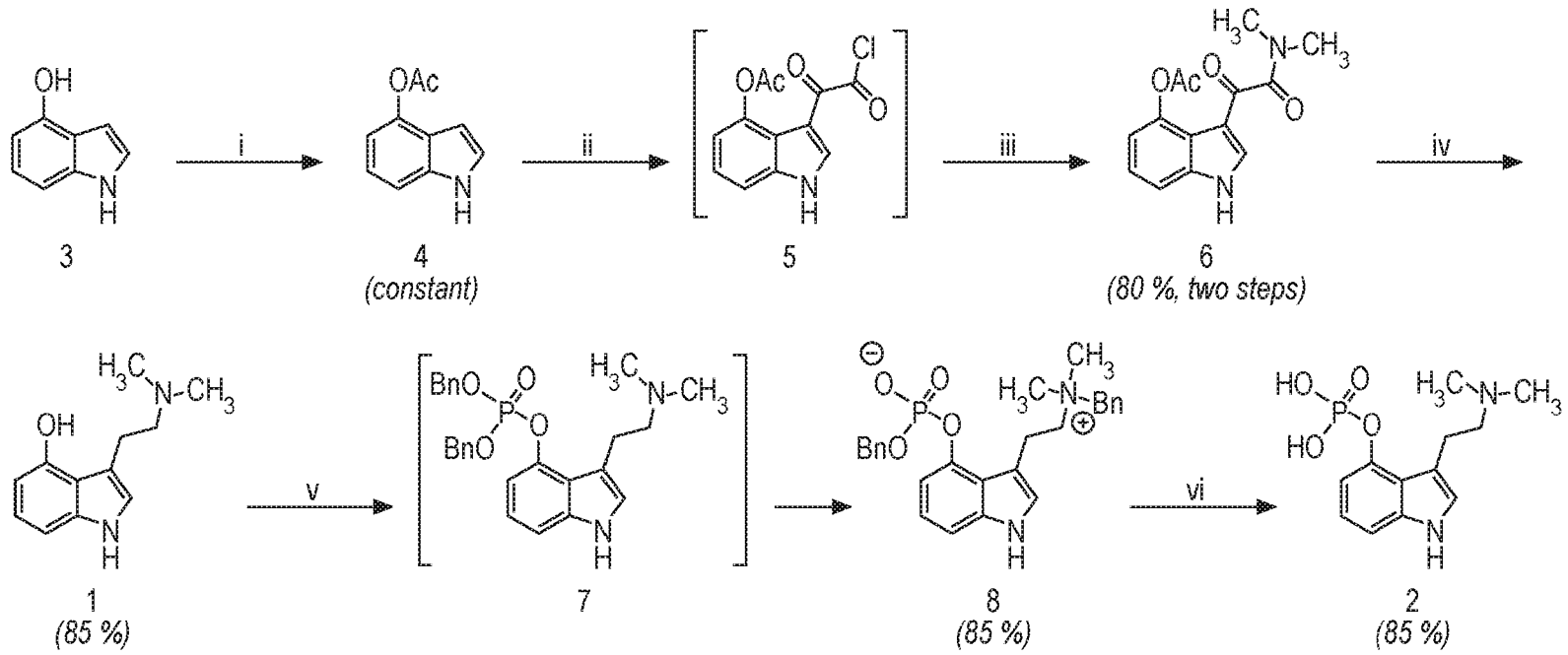
The Patents Act 1977

Specification No. GB (UK) 2572023 C

The following amendment was allowed under Section 27 on 24 January 2022.

The amendments shown in the form of replaced pages are made under Section 27 of the Patents Act 1977 on 24 January 2022.

Intellectual Property Office
7 September 2022



Reagent and conditions: (i) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt; (ii) $(COCl)_2$, ether, 0 °C, *n*-hexane, then –20 °C; (iii) $(CH_3)_2NH$, THF; (iv) $LiAlH_4$, THF, △; (v) $I(BnO)_2POI_2O$, *n*-BuLi, THF, –78 °C to 0 °C; (vi) H_2, Pd/C, MeOH, rt.

FIG. 1

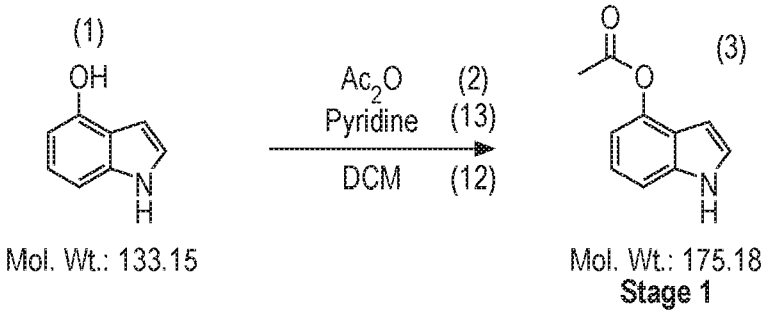

(1)
OH
N
H
Mol. Wt.: 133.15
Ac_2O (2)
Pyridine (13)
DCM (12)
O
O
(3)
N
H
Mol. Wt.: 175.18
Stage 1
Wash with citric acid (14) and $NaHCO_3$ (15)
Dry with $MgSO_4$ (16)
Partition with heptane (17)

FIG. 2

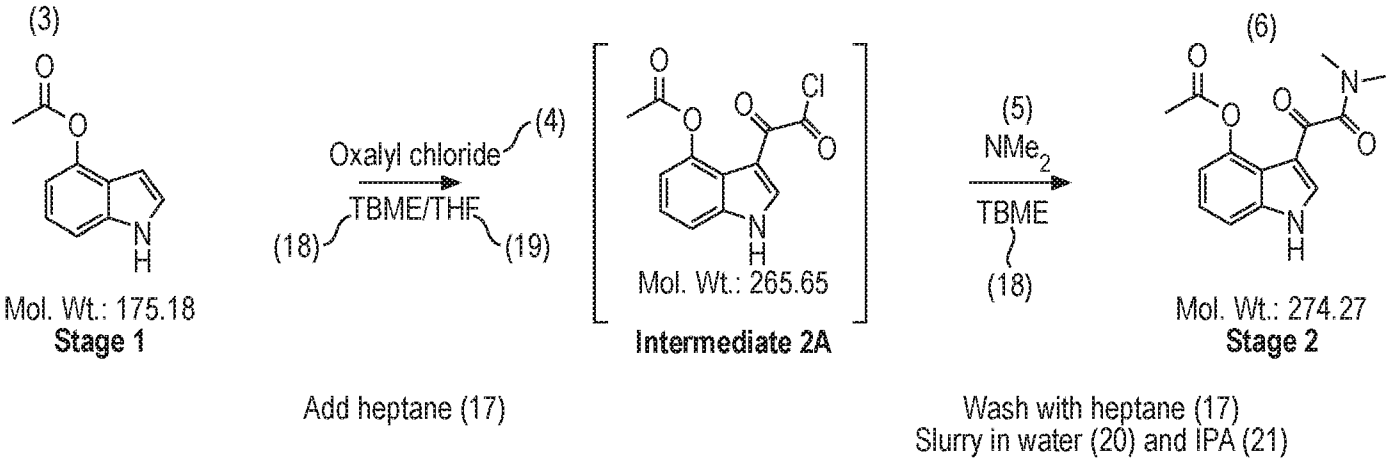

(3)
Mol. Wt.: 175.18
Stage 1
Oxalyl chloride
(4)
TBME/THF
(18)
(19)
Cl
Mol. Wt.: 265.65
Intermediate 2A
(5)
NMe2
TBME
(18)
(6)
Mol. Wt.: 274.27
Stage 2
Add heptane (17)
Wash with heptane (17)
Slurry in water (20) and IPA (21)

FIG. 3

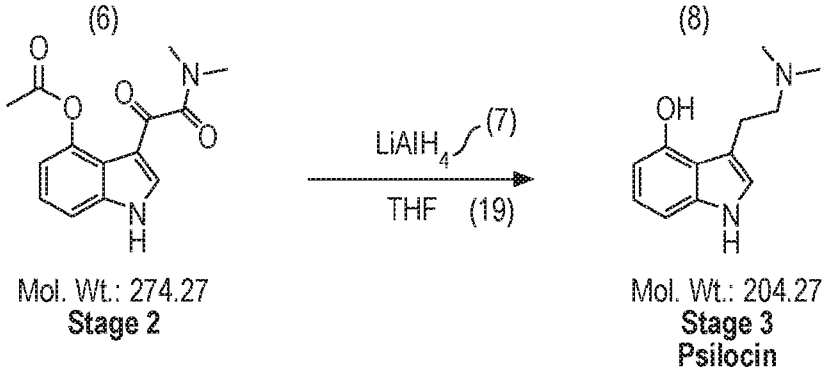


FIG. 4

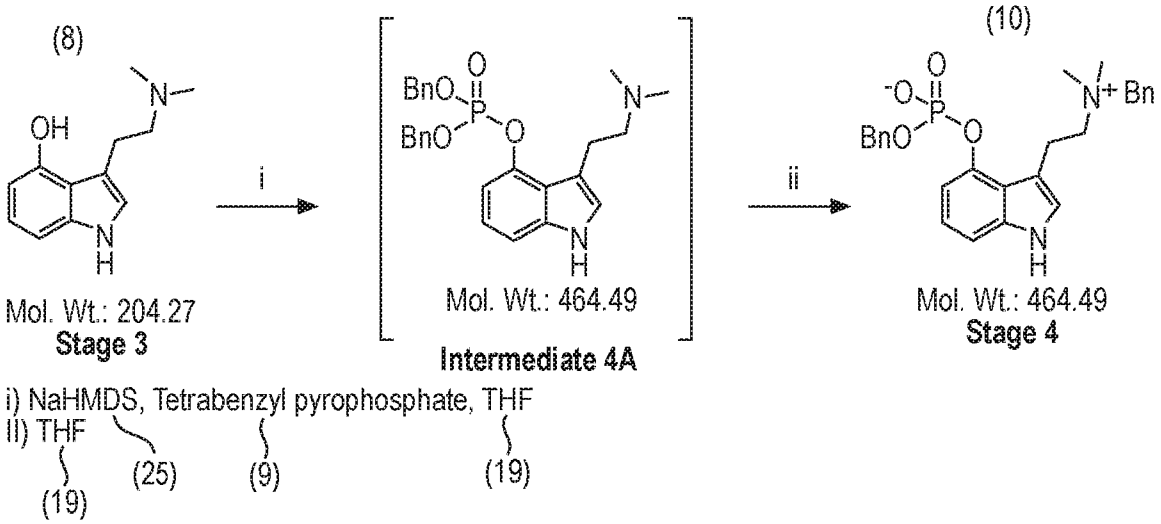


FIG. 5

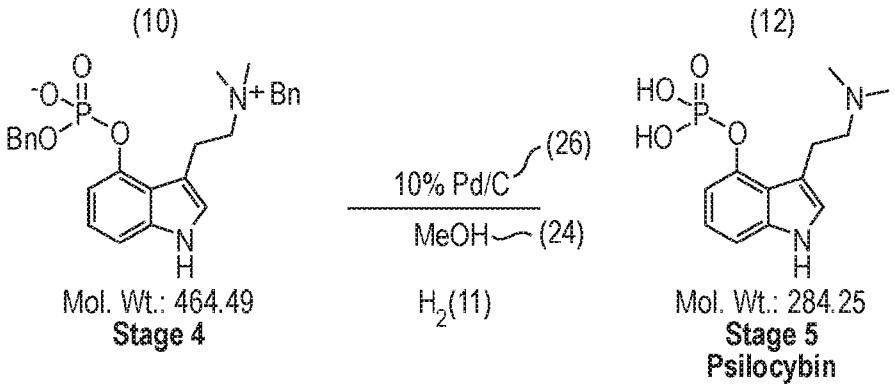

(10)
BnO
O
P
O
N+ Bn
N
H
Mol. Wt.: 464.49
Stage 4
10% Pd/C
(26)
MeOH
(24)
H2(11)
(12)
HO
HO
Mol. Wt.: 284.25
Stage 5
Psilocybin
Add H2O (20)
Filter on celite bed (27)

FIG. 6

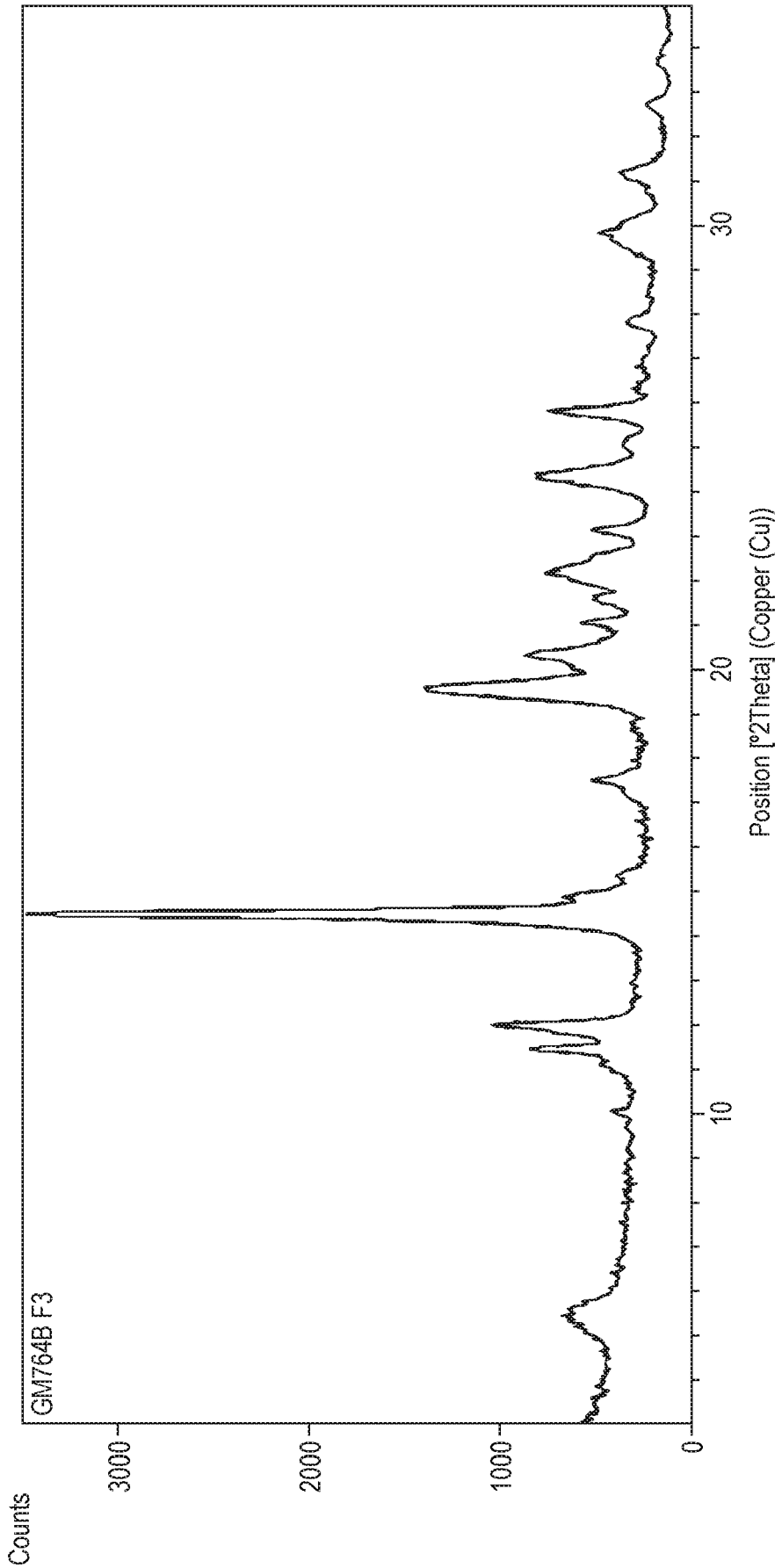


FIG. 7A

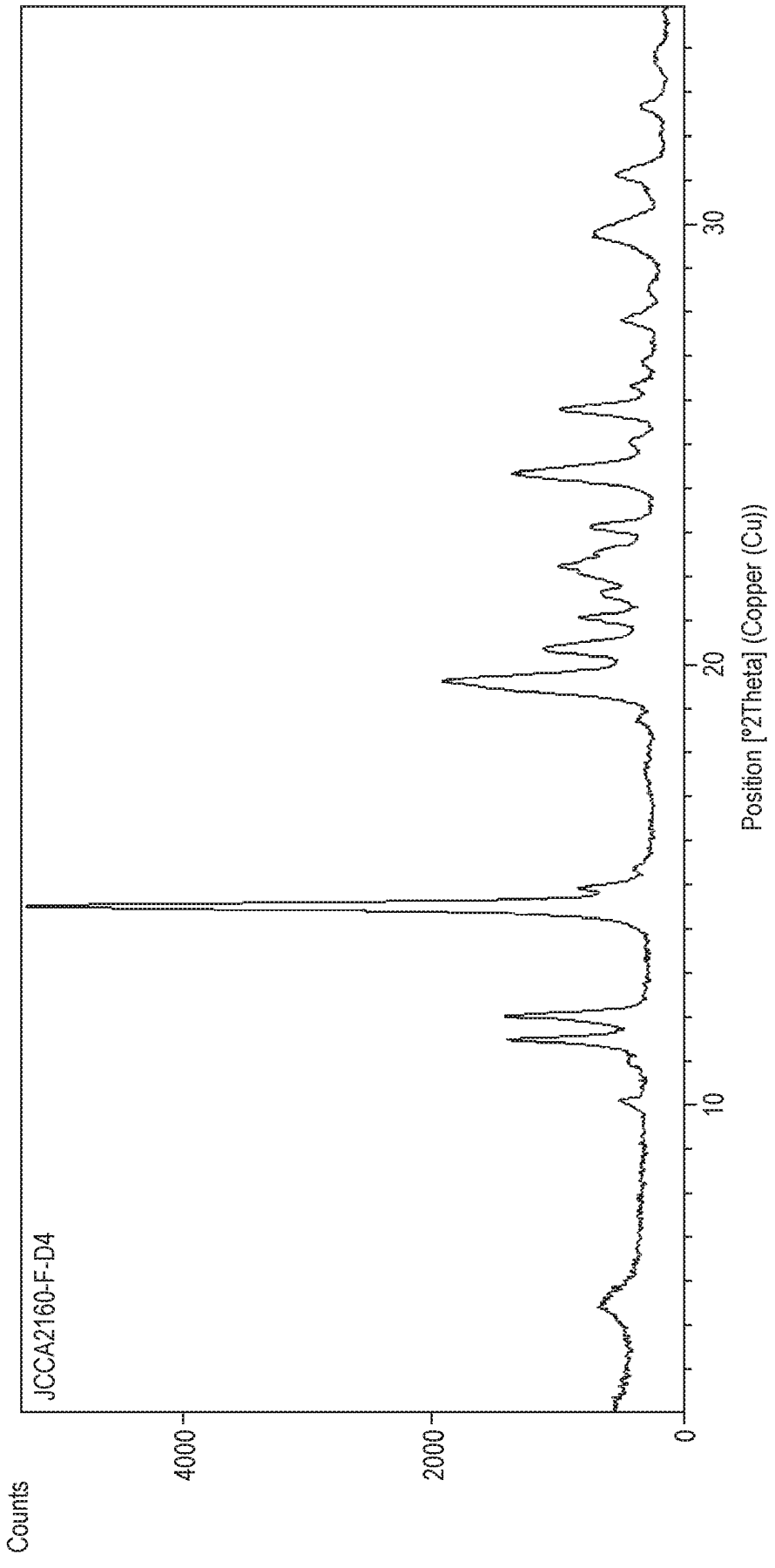

JCCA2160-F-D4
Counts
4000
2000
0
10
20
30
Position [°2Theta] (Copper (Cu))

FIG. 7B

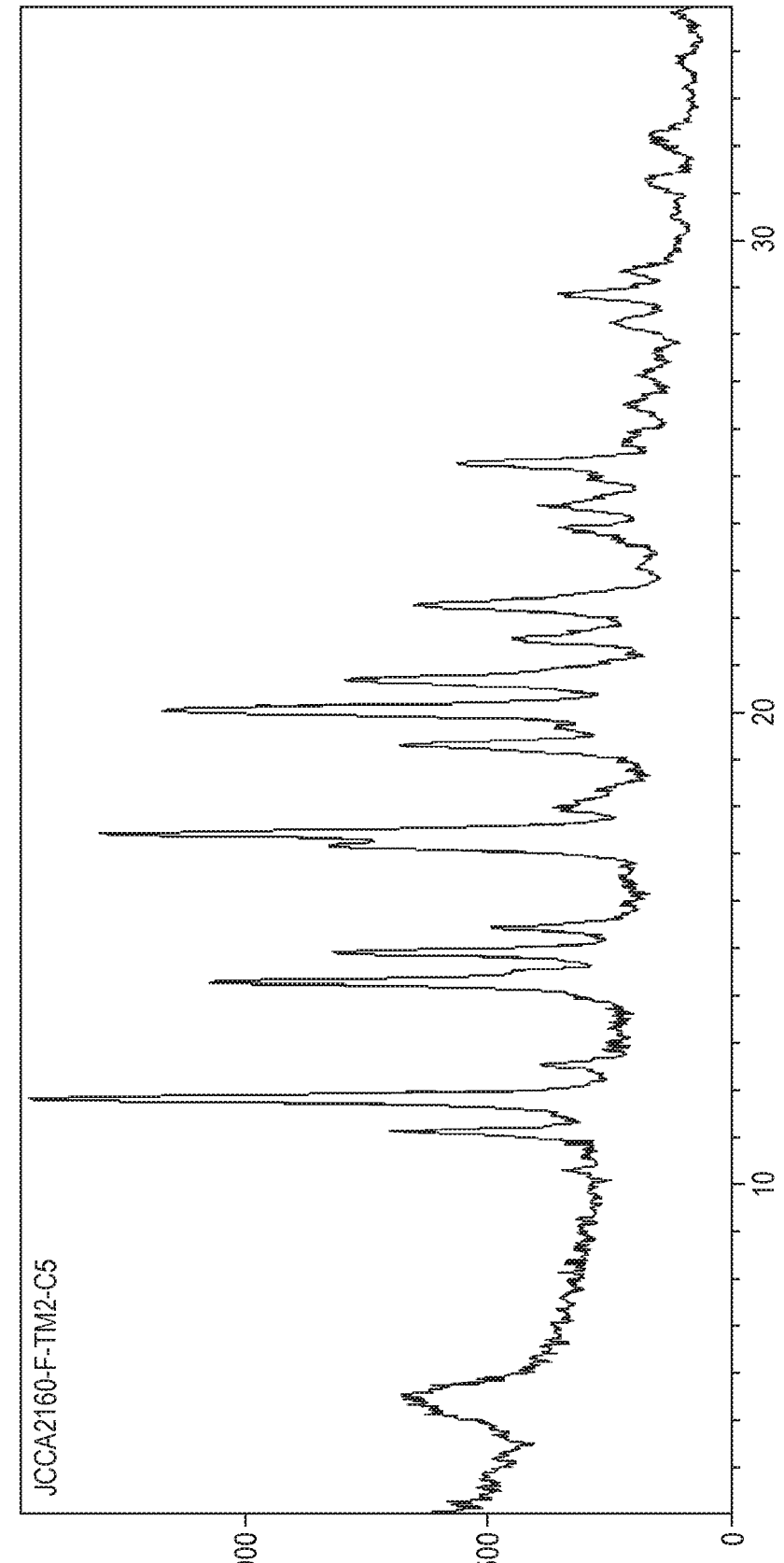


FIG. 7C

08 01 19

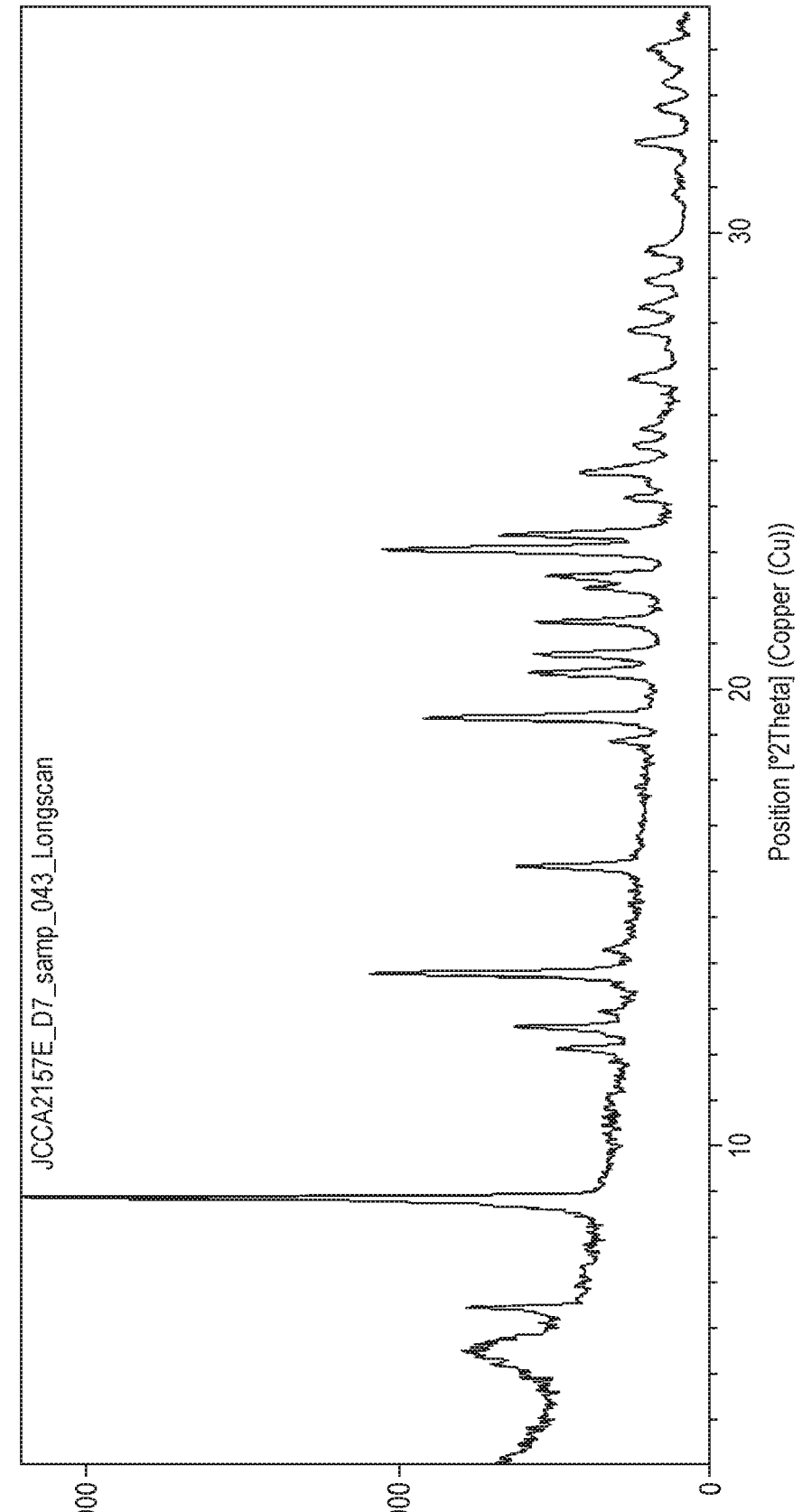


FIG. 7D

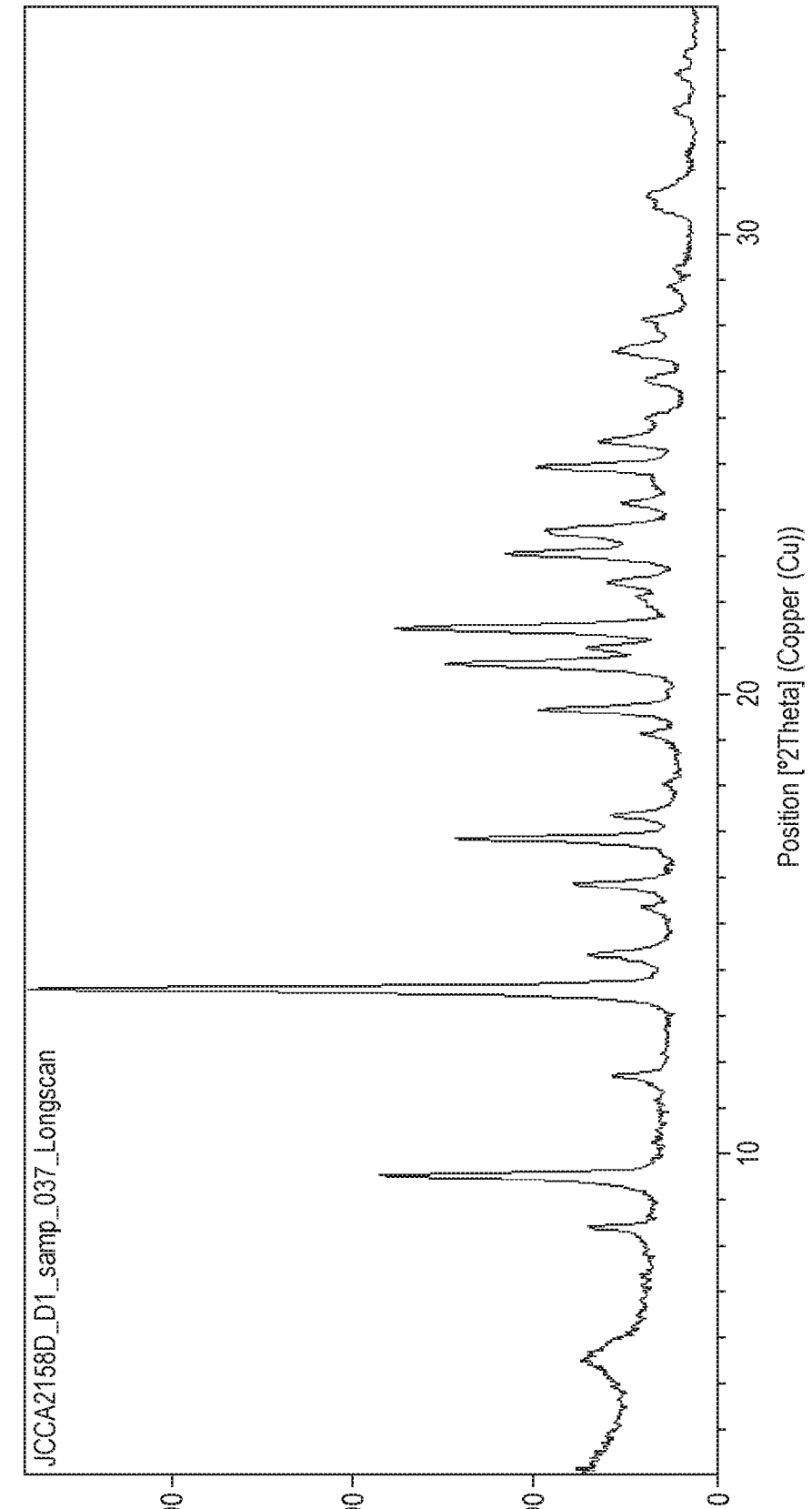
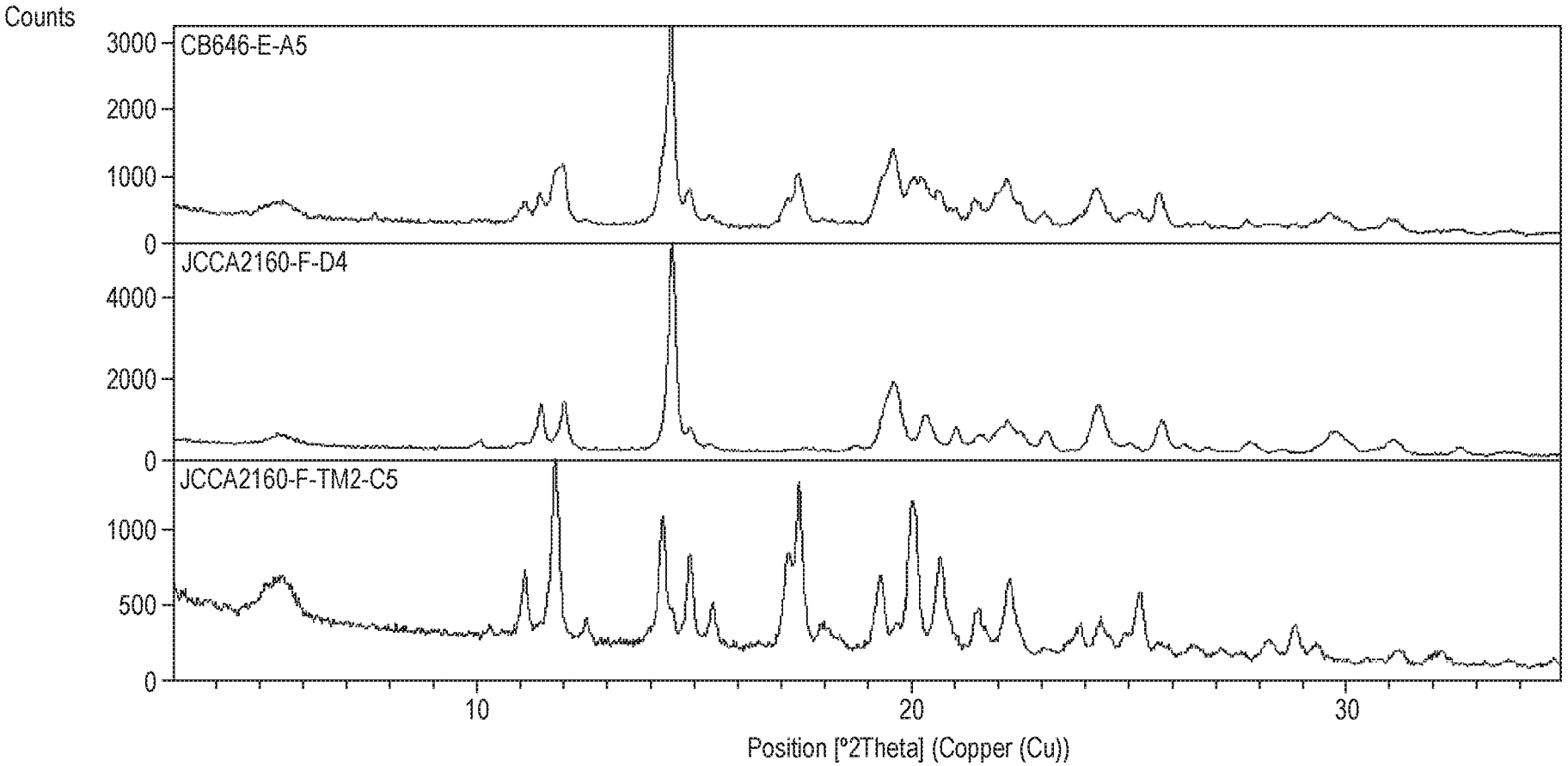


FIG. 7E



CB646 -TOP- MIXED PHASE; JCCA2160-F- MIDDLE PATTERN A'; JCCA2160-F-TM2- BOTTOM PATTERN B

FIG. 7F

08 01 19

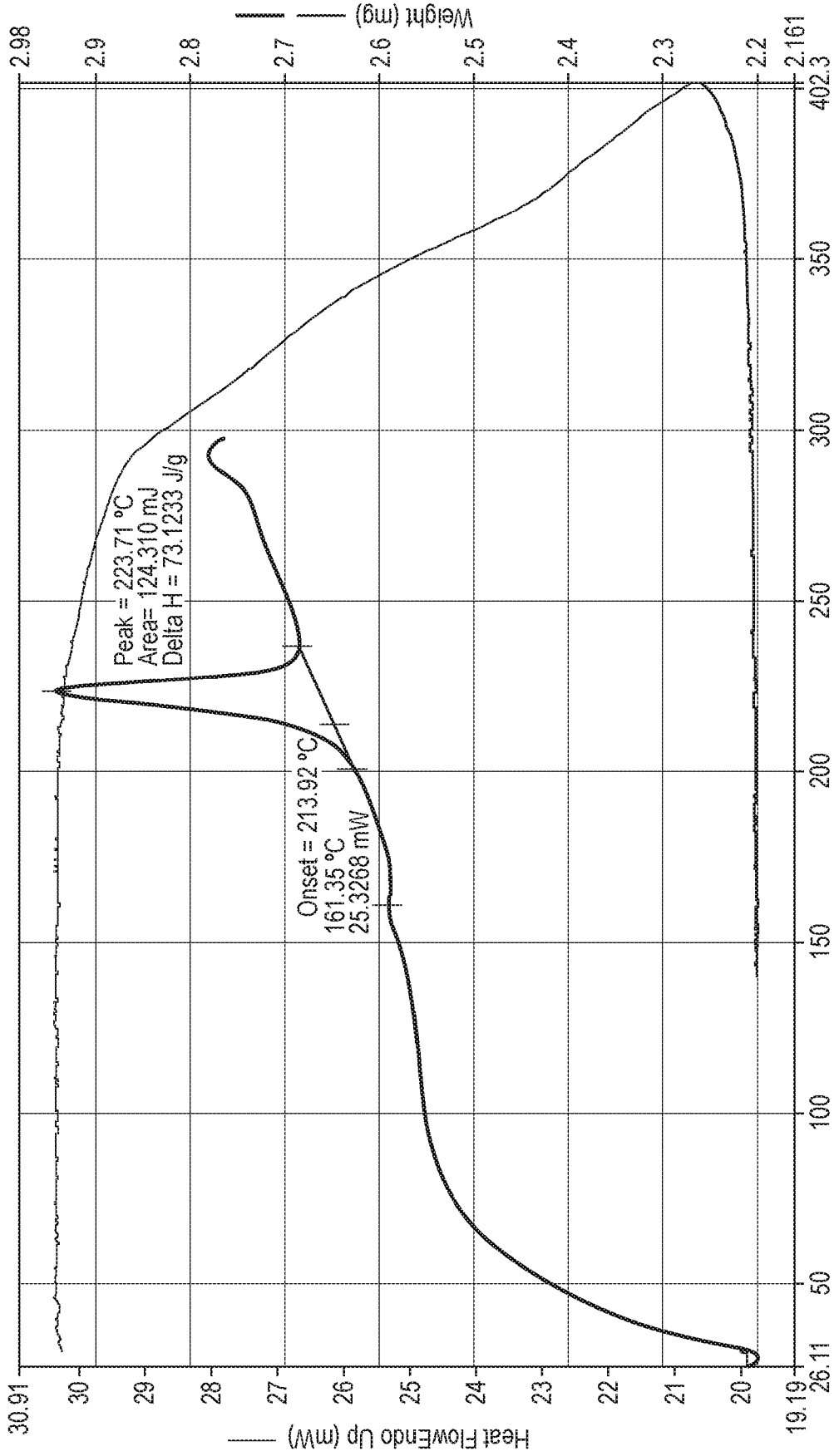


FIG. 8A

08 01 19

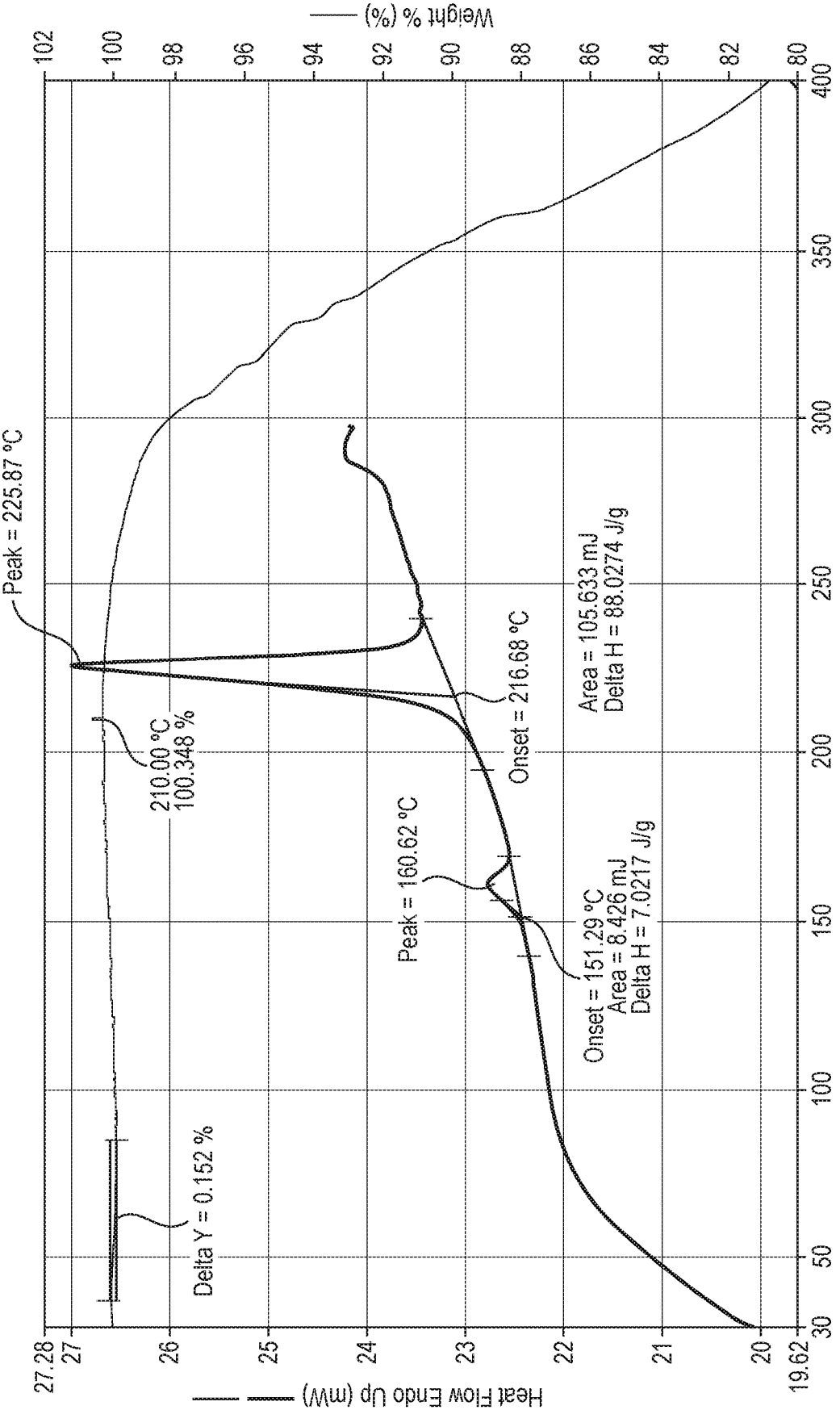


FIG. 8B

08 01 19

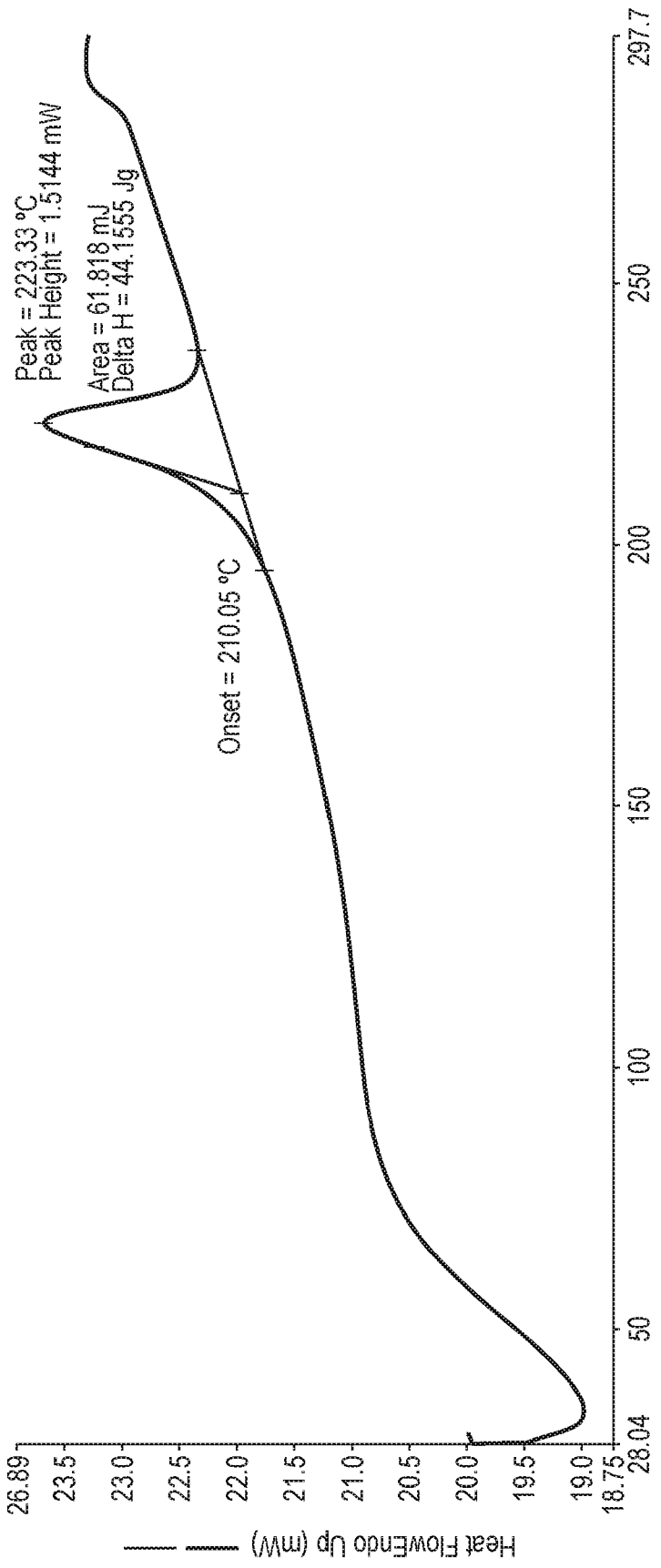


FIG. 8C

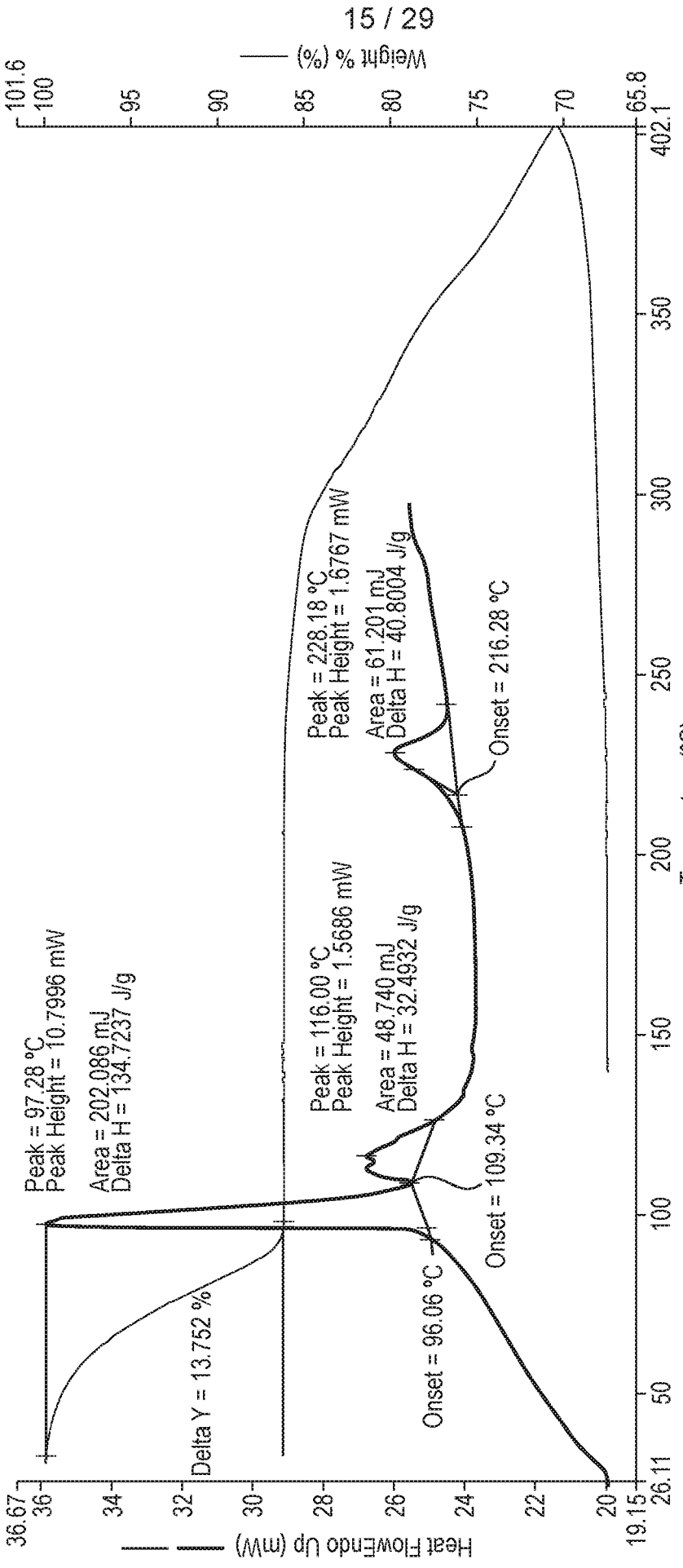

Peak = 97.28 °C
Peak Height = 10.7996 mW
Area = 202.086 mJ
Delta H = 134.7237 J/g
Delta Y = 13.752 %
Onset = 96.06 °C
Peak = 116.00 °C
Peak Height = 1.5686 mW
Area = 48.740 mJ
Delta H = 32.4932 J/g
Onset = 109.34 °C
Peak = 228.18 °C
Peak Height = 1.6767 mW
Area = 61.201 mJ
Delta H = 40.8004 J/g
Onset = 216.28 °C
Heat FlowEndo Up (mW)
Weight % (%)
Temperature (°C)
36.67
19.15
26.11
402.1
65.8
101.6

FIG. 8D

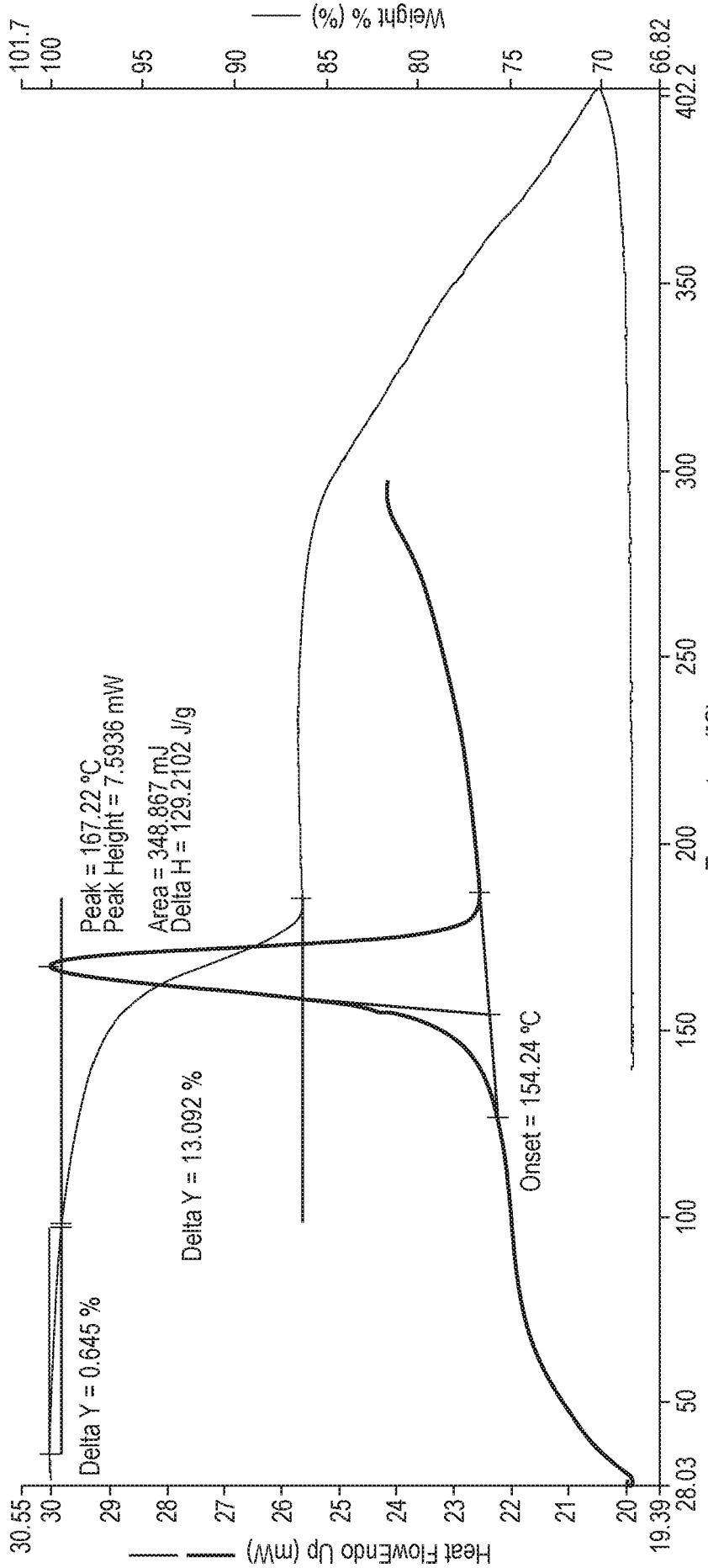

Peak = 167.22 °C
Peak Height = 7.5936 mW
Area = 348.867 mJ
Delta H = 129.2102 J/g
Delta Y = 13.092 %
Delta Y = 0.645 %
Onset = 154.24 °C
Heat FlowEndo Up (mW)
Weight % (%)
Temperature (°C)
30.55
30
29
28
27
26
25
24
23
22
21
20
19.39
28.03
50
100
150
200
250
300
350
402.2
101.7
100
95
90
85
80
75
70
66.82

FIG. 8E

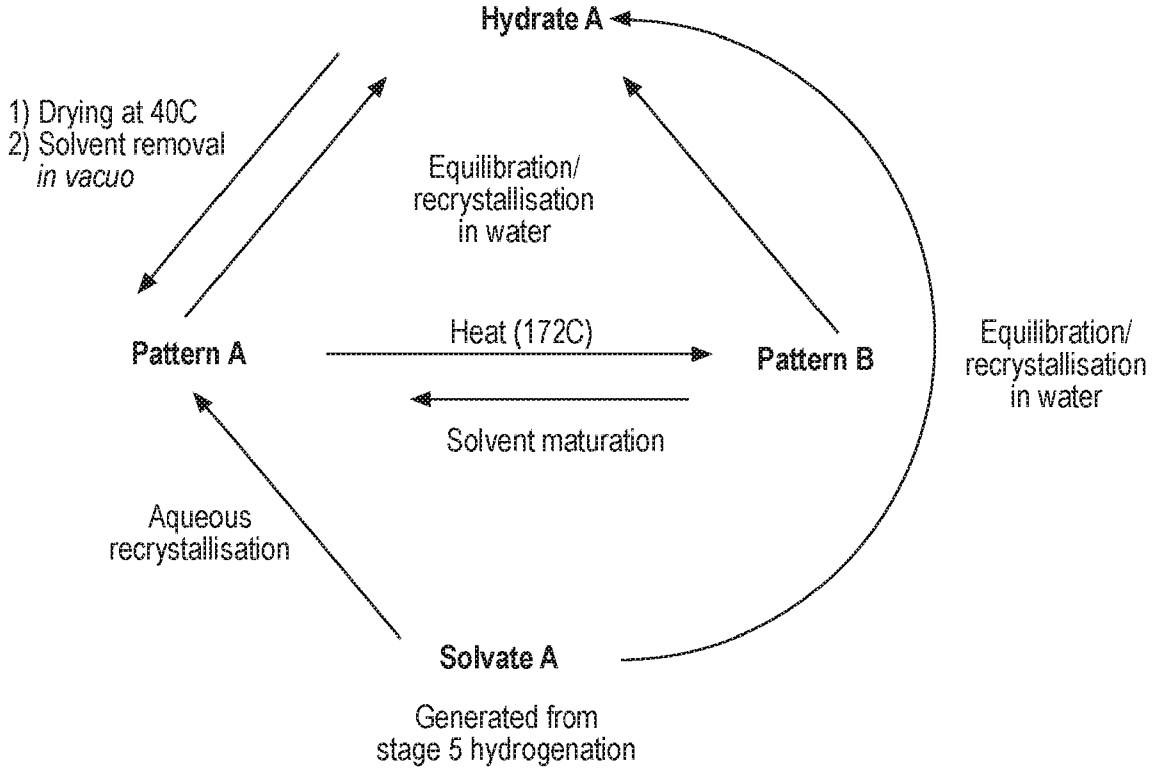

Hydrate A
1) Drying at 40C
2) Solvent removal
in vacuo
Equilibration/
recrystallisation
in water
Pattern A
Heat (172C)
Pattern B
Equilibration/
recrystallisation
in water
Solvent maturation
Aqueous
recrystallisation
Solvate A
Generated from
stage 5 hydrogenation

FIG. 9

08 01 19

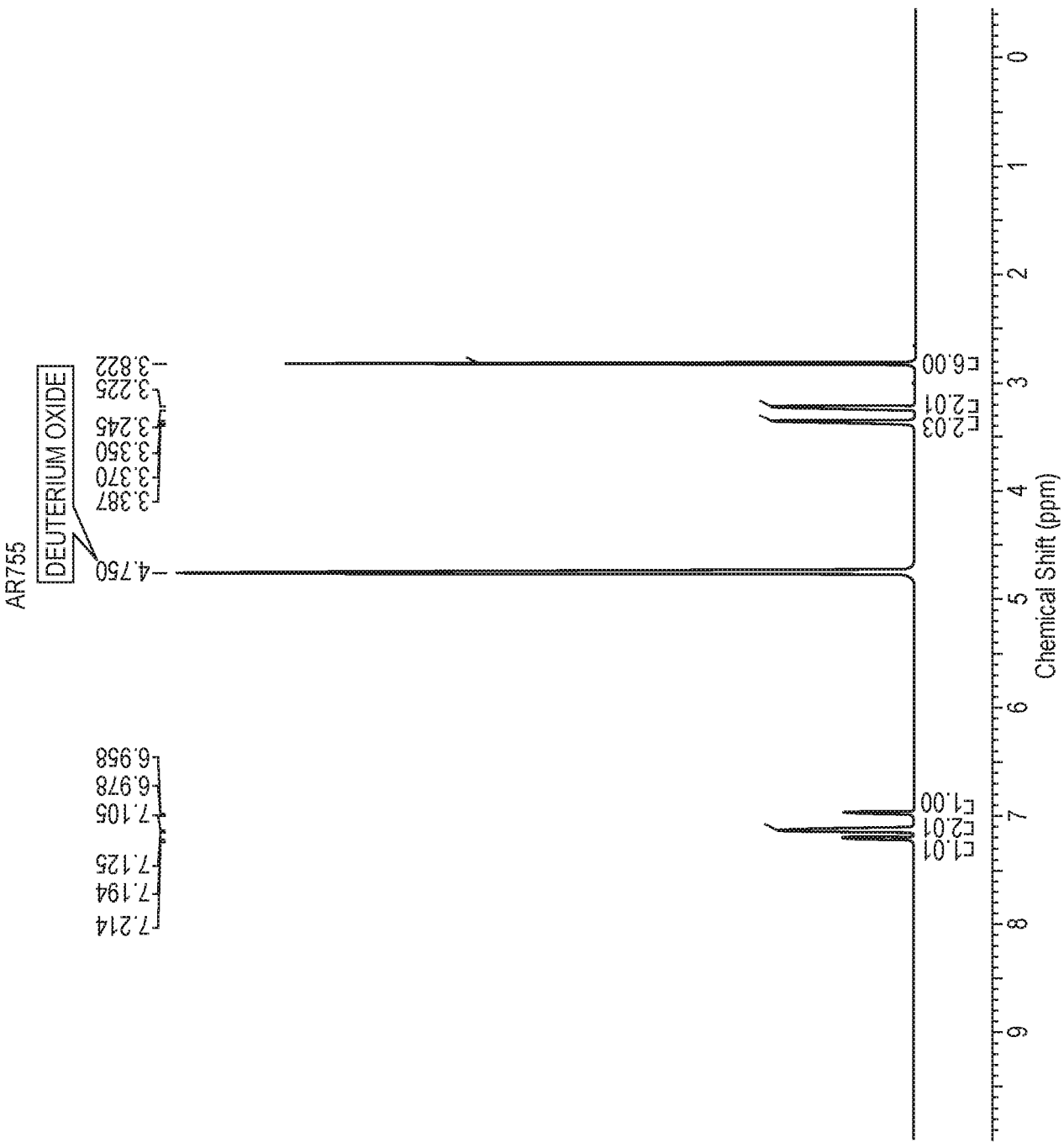


FIG. 10

08 01 19

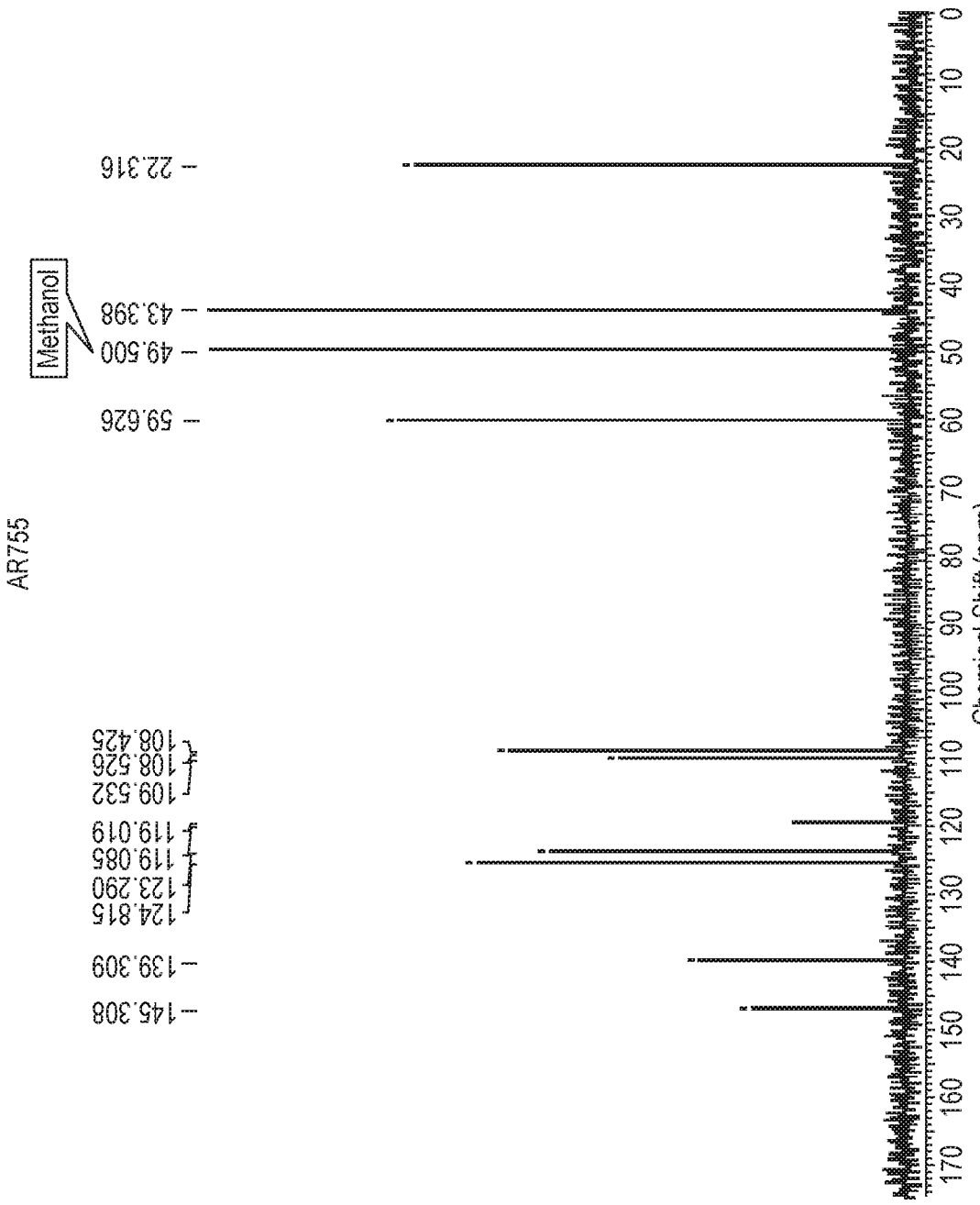
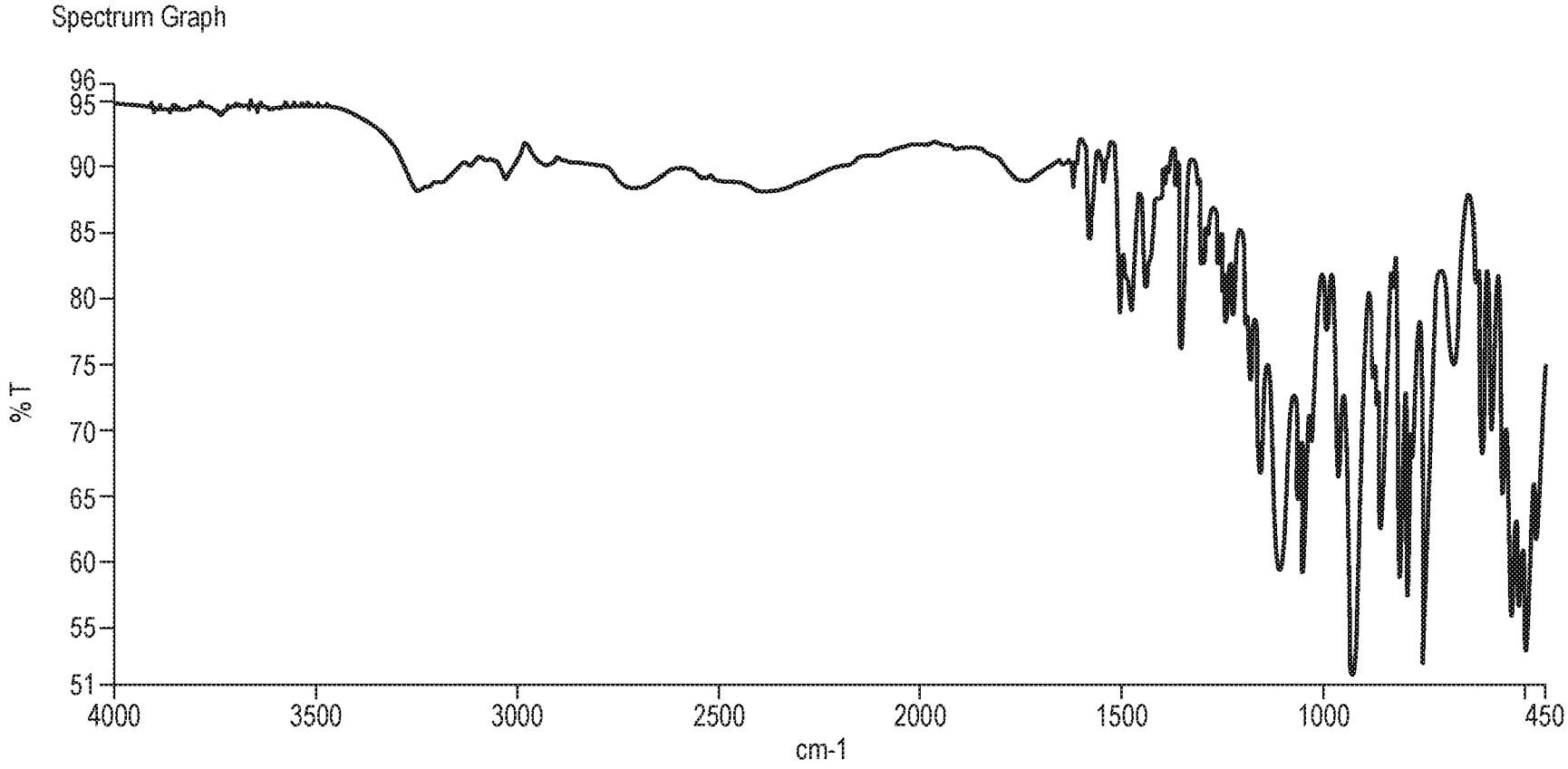


FIG. 11



Name	Description
AR755	Psilocybin by Ryan Date Friday, February 17 2017

FIG. 12

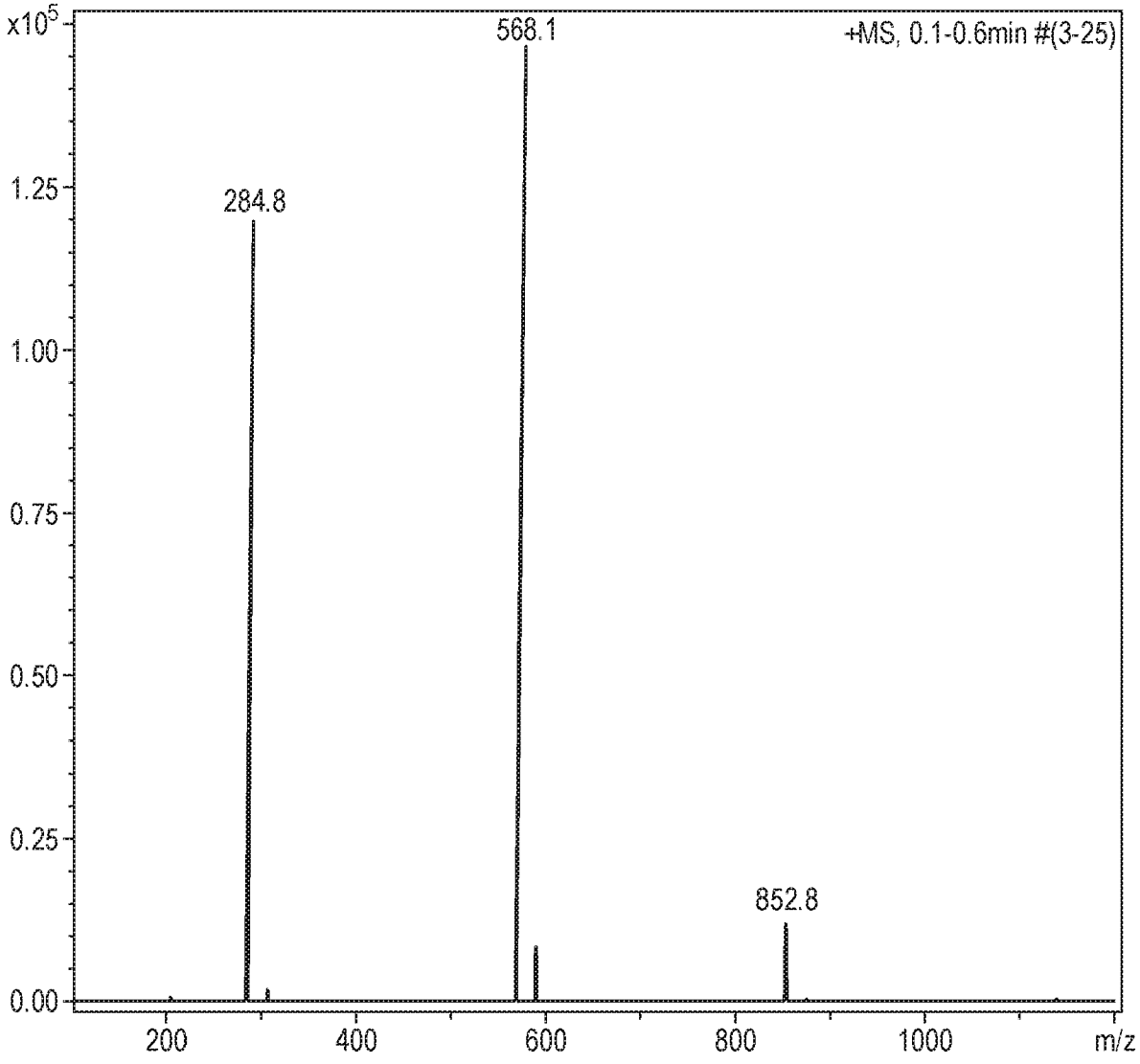
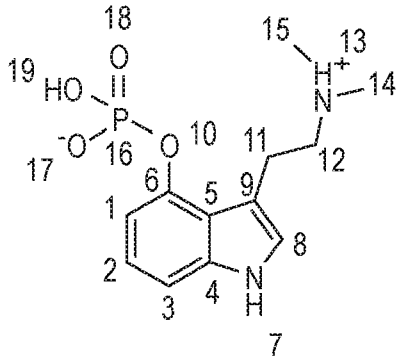


FIG. 13



Psilocybin

$C_{12}H_{17}N_2O_4P$

Exact Mass: 284.09

Mol. Wt.: 284.25

FIG. 14

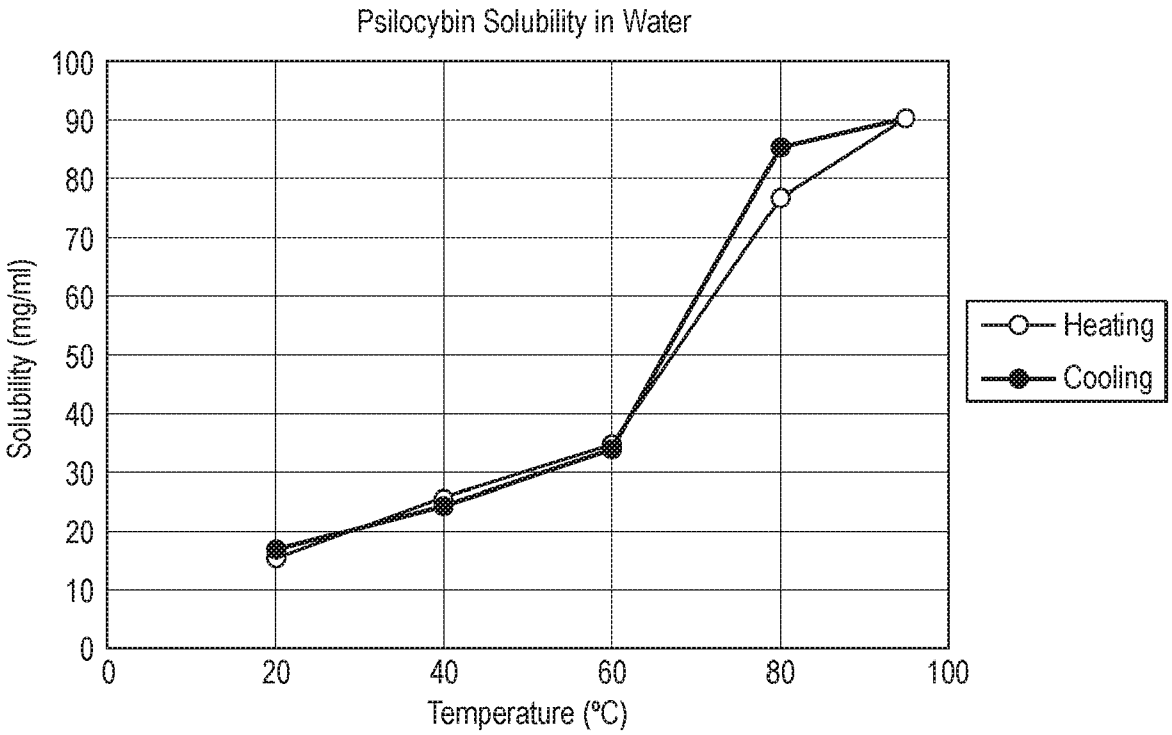


FIG. 15

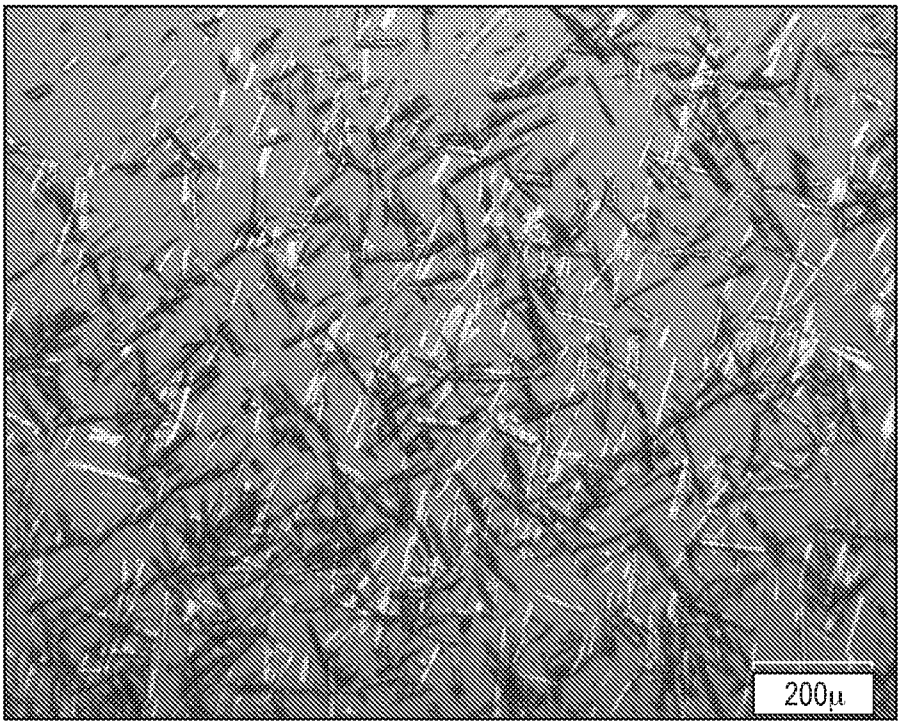


FIG. 16A

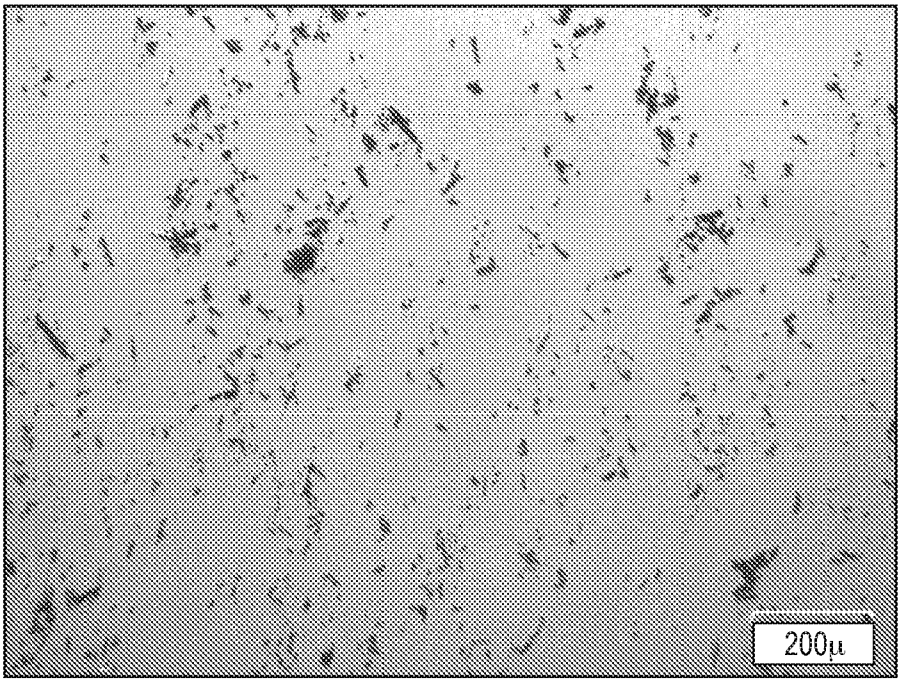


FIG. 16B

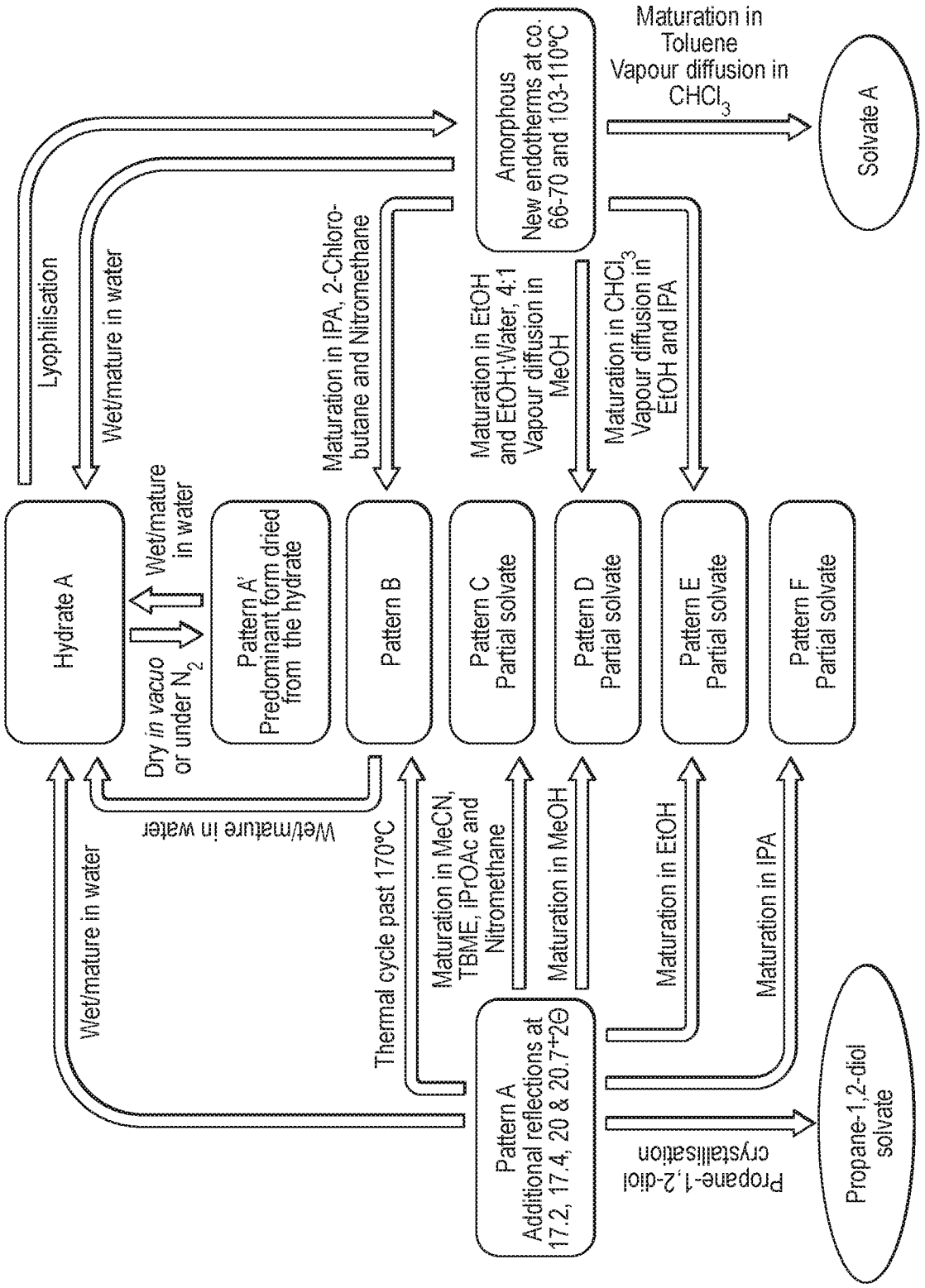

Hydrate A
Pattern A'
Predominant form dried from the hydrate
Pattern B
Pattern C
Partial solvate
Pattern D
Partial solvate
Pattern E
Partial solvate
Pattern F
Partial solvate
Amorphous
New endotherms at ca. 66-70 and 103-110°C
Solvate A
Pattern A
Additional reflections at 17.2, 17.4, 20 & 20.7+2Θ
Propane-1,2-diol solvate
Lyophilisation
Wet/mature in water
Dry in vacuo or under N2
Wet/mature in water
Maturation in IPA, 2-Chloro-butane and Nitromethane
Maturation in EtOH and EtOH:Water, 4:1 Vapour diffusion in MeOH
Maturation in CHCl3 Vapour diffusion in EtOH and IPA
Maturation in Toluene Vapour diffusion in CHCl3
Wet/mature in water
Wet/mature in water
Thermal cycle past 170°C
Maturation in MeCN, TBME, iPrOAc and Nitromethane
Maturation in MeOH
Maturation in EtOH
Maturation in IPA
Propane-1,2-diol crystallisation

FIG. 17

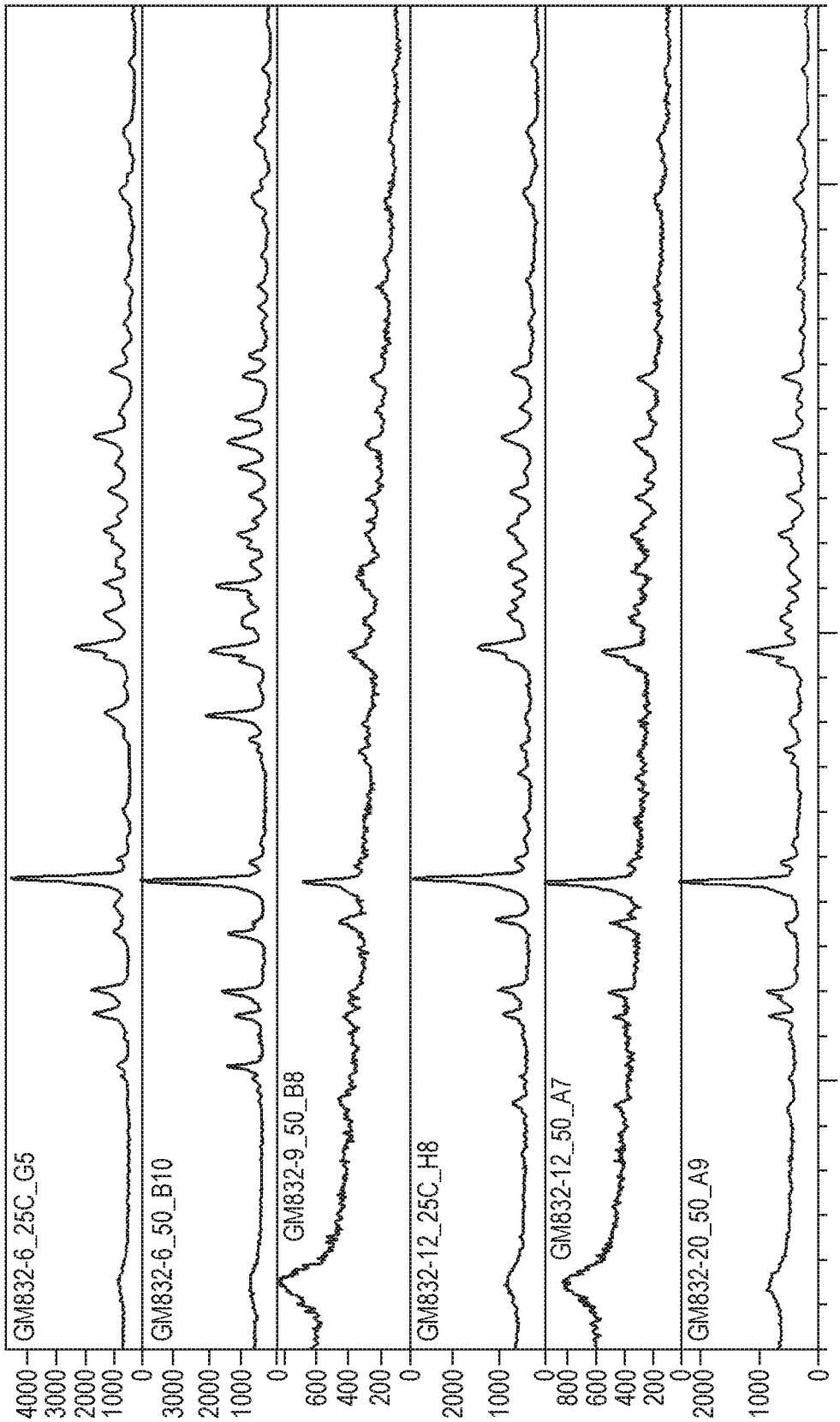

GM832-6_25C_G5
GM832-6_50_B10
GM832-9_50_B8
GM832-12_25C_H8
GM832-12_50_A7
GM832-20_50_A9
4000
3000
2000
1000
0
3000
2000
1000
0
600
400
200
0
2000
1000
0
800
600
400
200
0
2000
1000
0
10
20
30
Position [°2Theta] (Copper (Cu))

FIG. 18

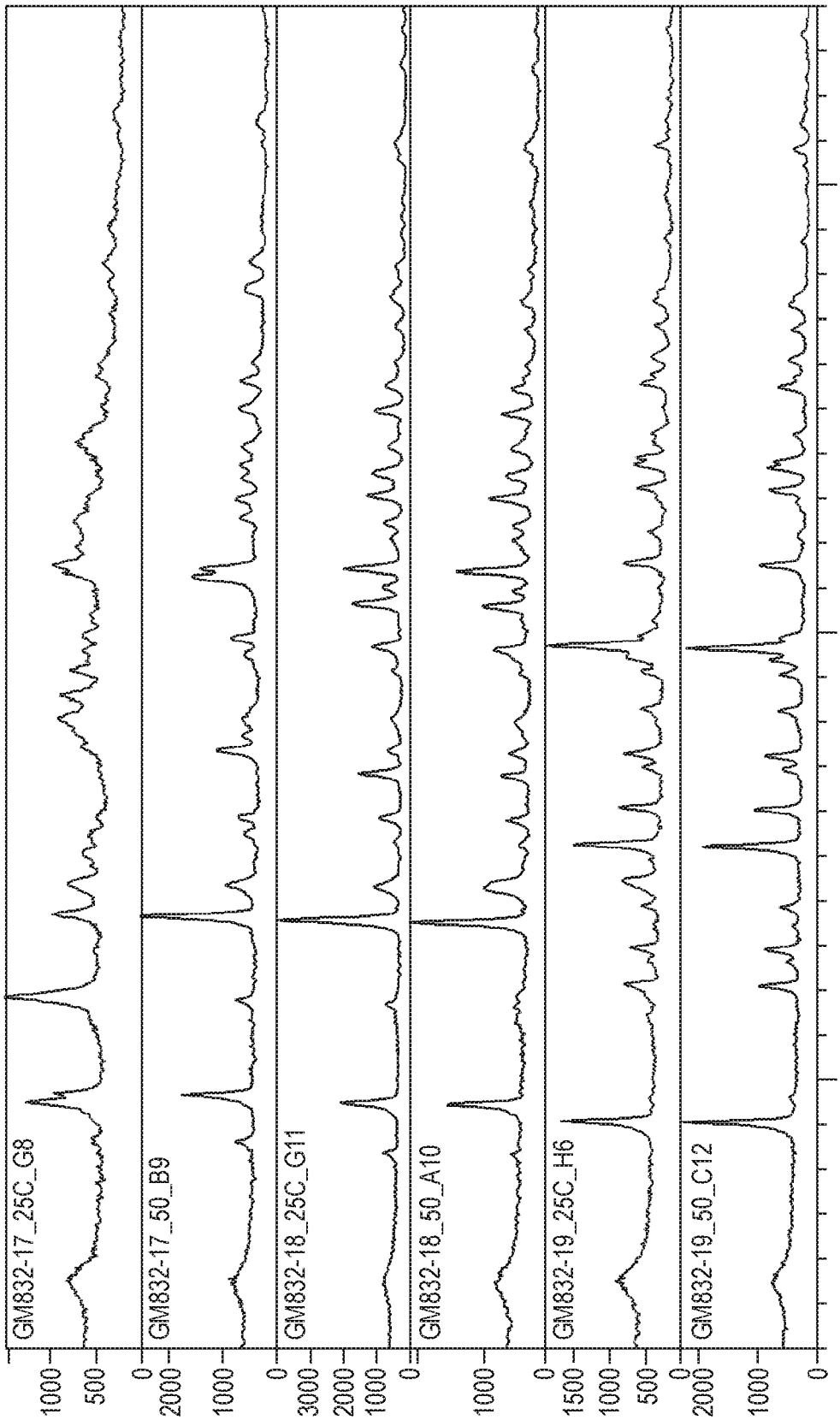

GM832-17_25C_G8
GM832-17_50_B9
GM832-18_25C_G11
GM832-18_50_A10
GM832-19_25C_H6
GM832-19_50_C12
Position [°2Theta] (Copper (Cu))

FIG. 19

08 01 19

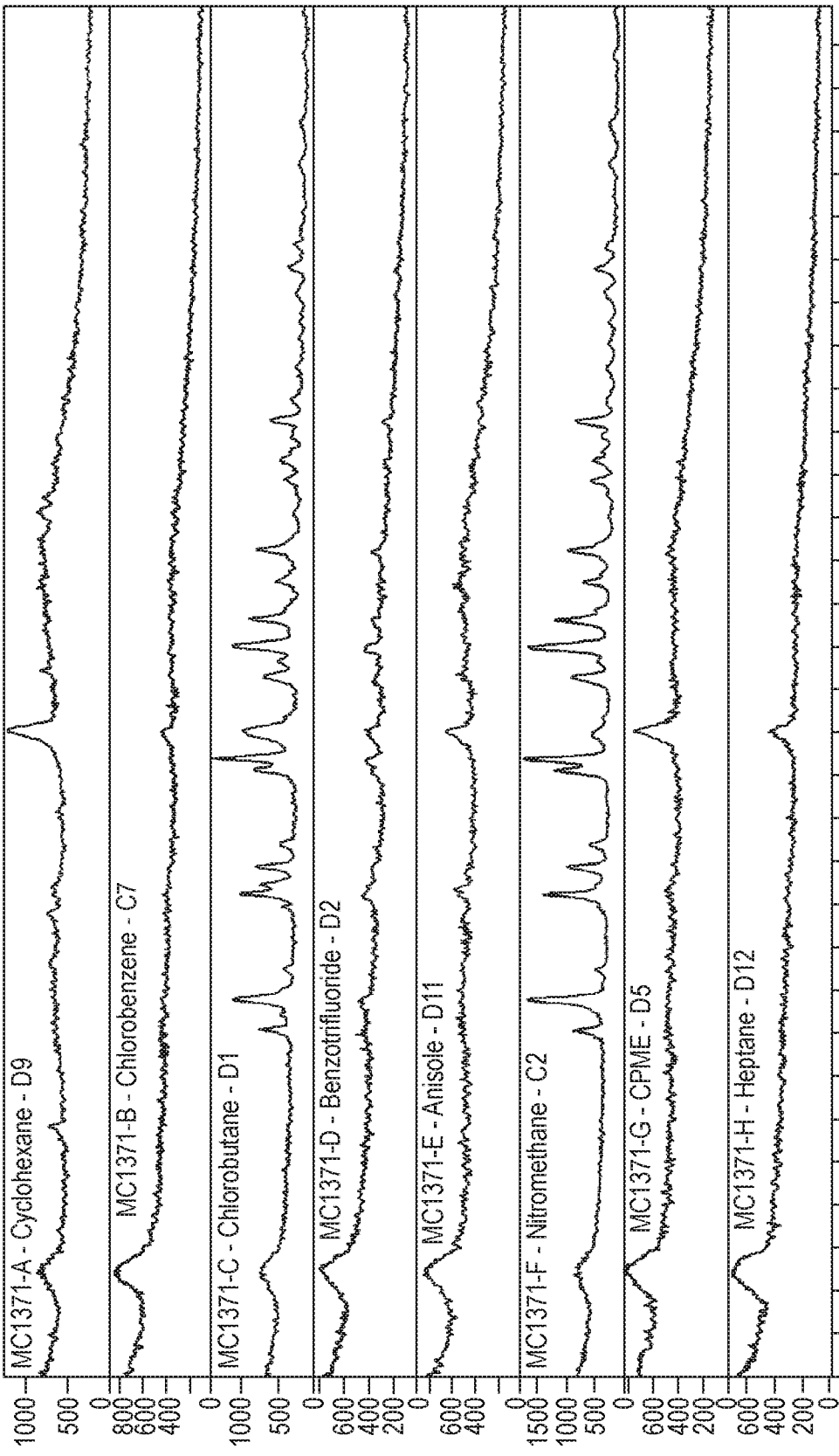


FIG. 20

08 01 19

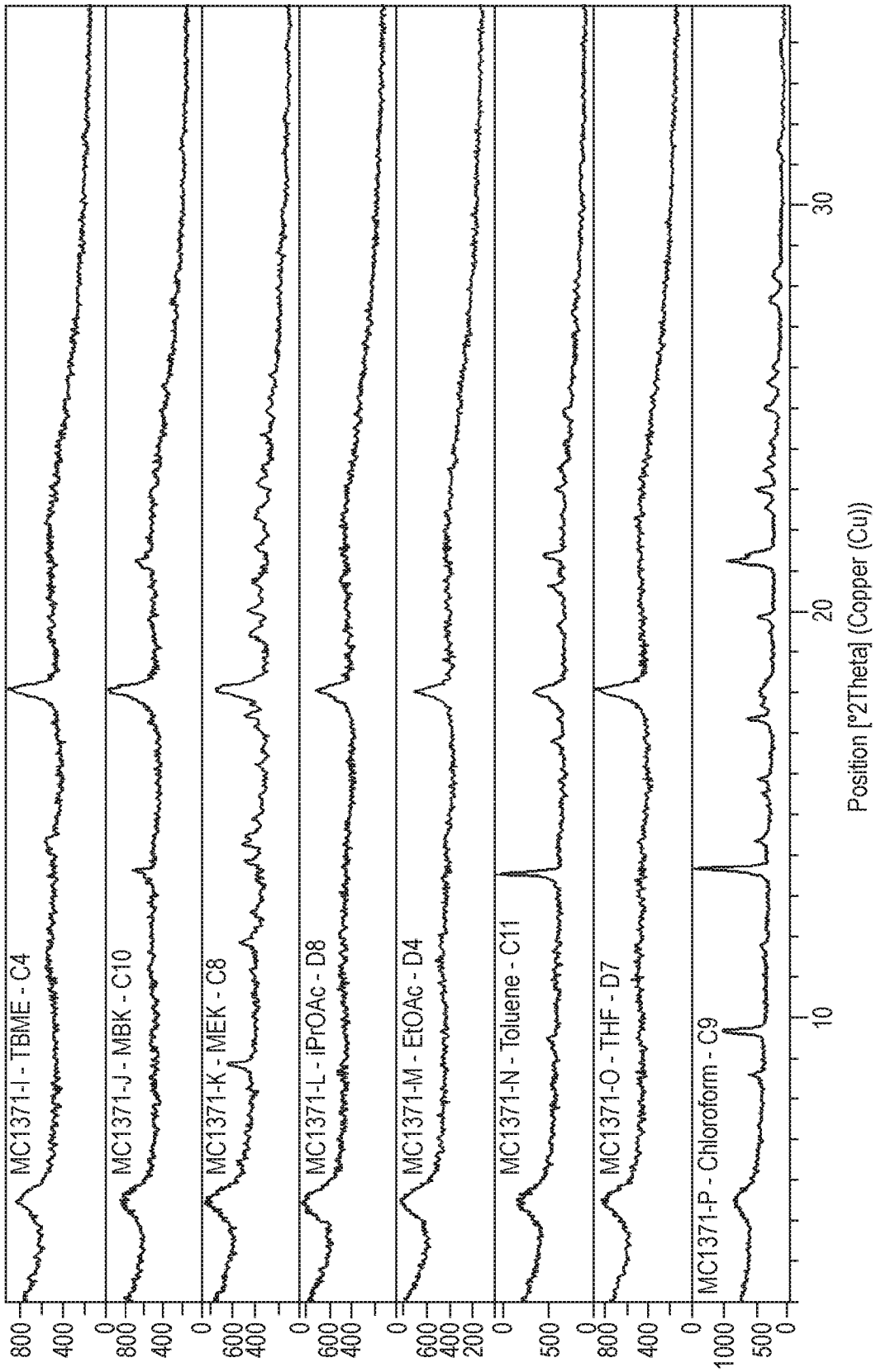


FIG. 21

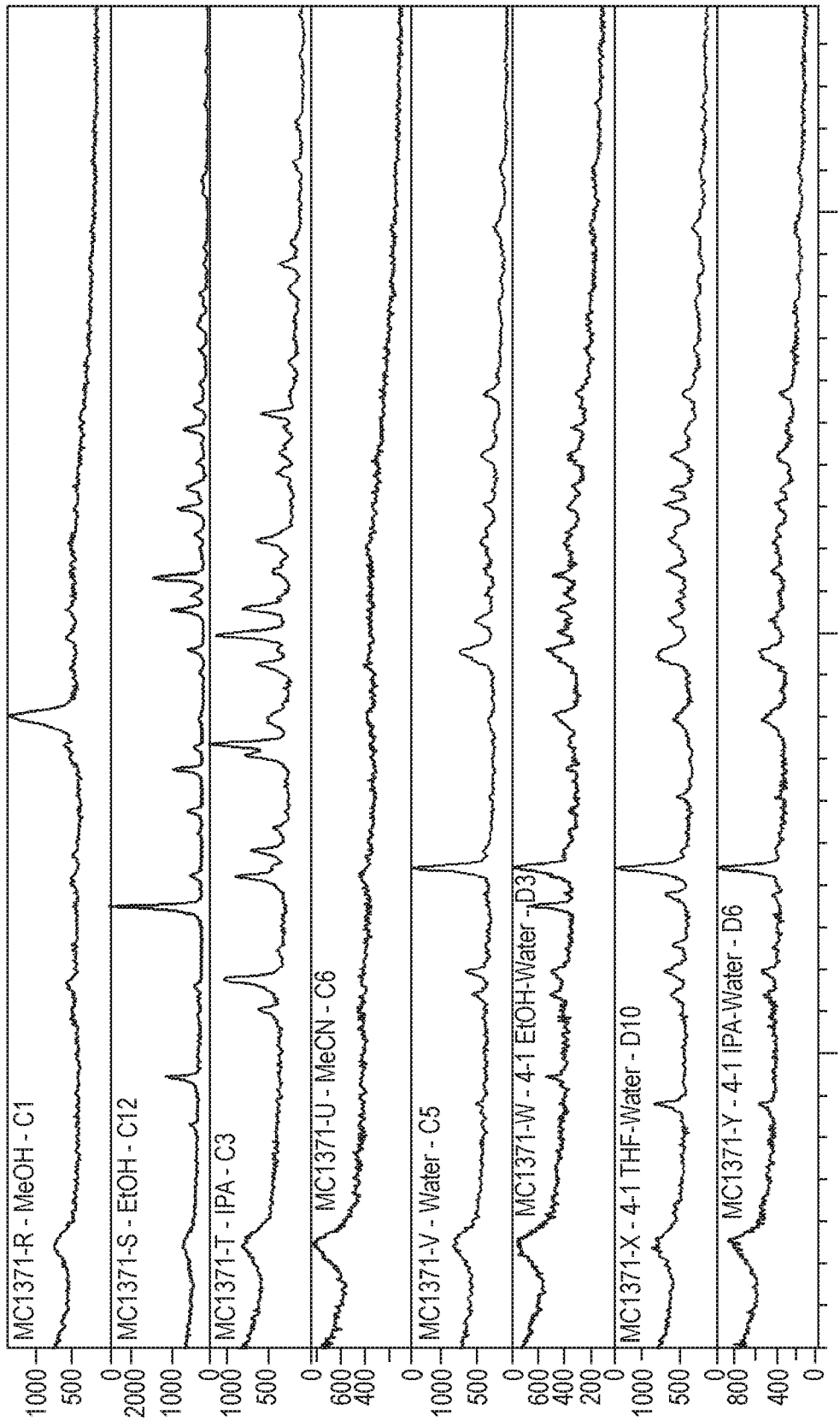

MC1371-R - MeOH - C1
MC1371-S - EtOH - C12
MC1371-T - IPA - C3
MC1371-U - MeCN - C6
MC1371-V - Water - C5
MC1371-W - 4-1 EtOH-Water - D3
MC1371-X - 4-1 THF-Water - D10
MC1371-Y - 4-1 IPA-Water - D6
Position [°2Theta] (Copper (Cu))

FIG. 22

CRYSTALINE PSILOCYBIN POLYMORPH A, A FORMULATION AND USES THEREOF TOGETHER WITH A METHOD OF PREPARATION

[0001] This invention relates to crystalline psilocybin polymorph A, a formulation and uses thereof, together with a method of preparation.

[0002] By production at large scale is meant producing batches of psilocybin with a weight of greater than 10g, more preferably greater than 100g, more preferably still greater than 250g and up to, and above, Kg levels.

[0003] It also relates to formulations and their use in medicine, particularly, but not exclusively for the treatment of treatment resistant depression, as defined in Diagnostic and Statistical Manual, 5th Edition, either alone or in combination with psychological support which may be provided digitally.

BACKGROUND

[0004] Psilocybin was first synthesised in 1958 by Sandoz, see GB912714 and US3075992, and was widely available as a research chemical until the mid -1960's.

[0005] A plant based psychedelic it has been used as an aide to psychotherapy for the treatment of mood disorders and alcoholic disorders and recently 3 clinical trials have reported its use for depressive symptoms.

[0006] Griffiths et al 2016; J Psychopharmacol 30 (12) :1181-1197;

[0007] Ross et al 2016; J Psychopharmacol 30 (12) :1165-1180; and

[0008] Carhart-Harris et al 2016, Lancet Psychiatry 3(7): 619-627;

[0009] Methods of manufacture of psilocybin are limited and include:

[0010] J Nat Prod 2003, 66, pages 885-887;

[0011] Helv Chim Acta 1959, 42, 2073-2103;

[0012] Experientia 1958, 15, 397-399;

[0013] Synthesis 1999, 935-938

[0014] Experientia, 1958, Vol 14, pages 107-109;

[0015] GB912714;

[0016] US3075992; and

[0017] Synthesis, No 6, 1999, pages 935-938.

[0018] Other art identified includes:

[0019] "Folen" J. Forensic Science, April 1975, Vol 20, No.2, Folen, "X-Ray Powder diffraction Data for Some Drugs, Excipients and Adulterants in Illicit Samples", pages 348 to 372;

[0020] "Caira" Topics in Current Chemistry, January 1998, Vol 198, Caira et al, "Crystalline Polymorphism of Organic Compounds", pages 163 – 208;

[0021] "Kuhnert" Archiv der Pharmazie, 1976, Vol 309, Issue 8, Kuhnert-Brandstätter et al, "Polymorphe Modifikationen und Solvate von Psilocin und Psilocybin", pages 625-631; and

[0022] "Weber" J. Chem. Soc. Perkin Transactions 2, 1974, Issue 8, Weber et al, "Crystal structures of the Teonanácatl hallucinogens. Part I. Psilocybin C12H17N2O4P" page 942.

[0023] Based on this literature Applicant believed that the process disclosed in J Nat Prod 2003, 66, pages 885-887 (hereafter JNP) was the most suitable method for development into a commercial scaled process.

[0024] The process disclosed therein produced quantities in the order of 10g and comprised 6 steps numbered (i) to (vi).

[0025] By analogy with the Applicants process, steps ii and iii are hereafter discussed as a single step, ***(Step 2)*** and the JNP process is reproduced as Fig 1 herein.

[0026] ***Step 1 (i)*** comprised reacting 4-hydroxyindole ("3") with acetic anhydride (Ac_2O) in pyridine and anhydrous dichloromethane (CH_2Cl_2) at 0°C. Water was added, the mixture evaporated, and the resulting concentrate was dissolved in ethyl acetate, washed with water, and saturated sodium chloride, and the organic phase dried over sodium sulphate and evaporated to obtain 4-acetylindole ("4"), which was collected by filtration and washed with water and ethyl acetate.

[0027] ***Step 2 (ii and iii),*** a two-step acylation (ii) – amidation step (iii), comprised forming 3-Dimethylaminooxalyl-4-acetylindole ("6") by: (ii) reacting 4-acetylindole ("4") with oxalyl chloride ($(COCl)_2$) in anhydrous diethylether, stirring, adding n-hexane and holding at -20°C to produce an intermediate 3-(2-chloro-2-oxoacetyl)-1H-indol-4-yl acetate ("5") which was separated by filtration. The intermediate was dissolved in anhydrous tetrahydrofuran (THF) and reacted with dimethylamine ($(CH_3)_2NH$) in tetrahydrofuran and pyridine. Anhydrous ether was added because of solidification, and the reaction product separated by filtration and washed with n hexane, ethyl acetate, and water to obtain 3-Dimethylaminooxalyl-4-acetylindole ("6").

[0028] ***Step 3 (iv)*** comprised the formation of psilocin ("1") by reacting the 3-Dimethylaminooxalyl-4-acetylindole ("6") with lithium aluminium hydride ($LiAlH_4$) in anhydrous

THF under an argon atmosphere. After refluxing and cooling, anhydrous sodium sulphate was added, followed by a solution of sodium sulphate, and further anhydrous sodium sulphate. The reaction mixture was diluted with ethyl acetate, quickly concentrated *in vacuo*, and the resulting psilocin crystals briefly washed with methanol.

[0029] ***Step 4 (v)*** comprised the formation of benzyl [2-(4-oxyindol-3-yl) ethyl] dimethylammonio-4-O-benzyl phosphate ("8") by reacting psilocin, dissolved in anhydrous THF, with n-butyl lithium (n-BuLi) in n-hexane at -78ºC and tetrabenzylpyrophosphate $[(BnO)_2PO]_2O$, and the reaction allowed to warm to 0ºC, and the production of intermediate dibenzyl 3-[2-(dimethylamino)ethyl]-1H-indol-4-yl phosphate ("7") monitored. On checking for its presence, aminopropyl silica gel was added, the mixture diluted with ethyl acetate and filtered through a Celite pad by suction, the filtrate concentrated *in vacuo*, re-dissolved in CH_2Cl_2, and the precipitate collected by filtration.

[0030] ***Step 5 (vi)*** comprised the formation of psilocybin ("2") by reaction of ("8"), in methanol (MeOH), with hydrogen (H_2) using a palladium-activated carbon catalyst (Pd/C). Water was added, because of product deposition, and ("8"), its mono de-benzylated derivative were monitored along with the appearance of psilocybin, the reaction solution was filtered through a Celite pad. The product was collected by filtration and washed with ethanol to provide a white needle crystalline form with a melting point 190ºC -198ºC.

[0031] In contrast to most processes, such as JNP, which use non-aqueous solvents, such as methanol or ethanol, Experientia 1958, 15, 397-399 used a single recrystalisation from water to obtain psilocybin from a mushroom extraction. The teaching was to use boiling water to dissolve the starting material, obtained at small scale by chromatography, and the resulting high vacuum dried material was stated to melt indistinctly between 185 and 195 ºC, and showed a weight loss of 25.4%, suggesting it clearly differs in purity and form to that obtained by Applicant. Similar rationale apply to Experientia 1958, Vol 14, pages 107-109.

[0032] During the development of a synthesis to produce Psilocybin the applicant conducted a number of hydrogenation reactions on a 5 g scale which resulted in different crystalline forms of Psilocybin being obtained. The initial hydrogenation reaction yielded Hydrate A (JCCA2157E) which exhibited a XRPD diffractogram as shown in Fig. 7d and DSC and TGA thermograms as shown in Fig. 8d. The DSC exhibits an endotherm at ~97°C which is coincidental with a weight reduction in the TGA indicative of dehydration, and an endothermic event with an onset temperature of ~216°C which was presumed to be the melt. Another hydrogenation reaction yielded an ethanol solvate (JCCA2158D) which when analysed by XRPD (Fig. 7e), DSC (Fig. 8e), TGA (Fig. 8e) and by ^1H NMR indicated 11% entrapped ethanol. The DSC thermogram shows an endotherm having an onset of ~154°C

that appeared to be a melt concurrent with the ~13% weight loss in the TGA. In another experiment performed during development, the applicant performed a crystallisation of psilocybin; rather than remain in solution in hot water allowing for a polish filtration step, precipitation occurred at high temperature (>90°C). The solids formed did not re-dissolve upon further heating or addition of extra water. Upon cooling and isolation of the solid (CB646E) XRPD was performed. The XRPD diffractogram (Fig. 7f) suggested a mixed phase of Polymorph A' (JCCA2160-F-D4) and Polymorph B (JCCA2160-F-TM2-C5). These findings highlight the importance of developing a process which can consistently produce the desired crystalline form so the Applicants set about experiments to determine what these forms were in order they could produce a chemically pure psilocybin, in a controlled form suitable for use in medicine.

[0033] For clinical trials any New Active Substance (NAS) should be capable of large scale production (typically 100g plus, more typically greater than 250g, more preferably still greater than 500g, to Kg plus batches), depending on the amount of active to be dosed to a human subject. It should also be chemically pure, well defined, and stable on storage.

[0034] Furthermore, any method of manufacture must be readily reproducible, and provide batch to batch consistency.

[0035] It is a first object of this invention to provide psilocybin, of consistent polymorphic form, for administration to human subjects.

[0036] It is another object of this invention to provide chemically pure psilocybin, of consistent polymorphic form, for administration to human subjects.

[0037] It is yet a further object to provide chemically pure psilocybin, in large scale batch quantities since for commercial use, the pure psilocybin must be produced at scale.

[0038] It is yet a further object of the invention to provide a method of crystallising psilocybin in a desired polymorphic form.

[0039] It is yet a further object of the present invention to provide a scalable method for manufacturing psilocybin, from psilocin or 4 hydroxy-indole.

[0040] In developing suitable methodology Applicant experienced numerous problems and difficulties which they had to be overcome, and it is a separate, independent, object to overcome those problems identified at each step, and use the inventions either alone or in combination.

[0041] It is yet a further object of the invention to formulate the psilocybin of the invention in a form suitable for administration to human subjects and use it in medicine, particularly in the treatment of central nervous system disorders (CNS), and more particularly, but not

exclusively, in the treatment of depression, particularly, drug resistant depression either alone or in combination with a digital health product or digital solution.

BRIEF SUMMARY OF THE DISCLOSURE

[0042] In accordance with a first aspect of the present inventions there is provided crystalline psilocybin in the form Polymorph A characterised by one or more of:

a. peaks in an XRPD diffractogram at 11.5, 12.0,14.5 and 17.5 °2θ±0.1°2θ, wherein the peak at 17.5 °2θ ±0.1°2θ has a relative intensity compared to the peak at 14.5 °2θ±0.1°2θ of at least 5%, and which is substantially absent of a peak in an XRPD diffractogram at 10.1 °2θ±0.1°2θ; and
b. an endothermic event in a DSC thermogram having a first onset temperature of between 145°C and 155°C and a second onset temperature of between 210 and 220°C; and
having a chemical purity of greater than 97% by HPLC, and no single impurity of greater than 1% including phosphoric acid as measured by 31P NMR, and psilocin as measured by HPLC.

Polymorph A and A'

[0043] Crystalline psilocybin Polymorph A may be characterised by one or more of:

a. peaks in an XRPD diffractogram at 11.5, 12.0,14.5, and 17.5, °2θ±0.1°2θ;
b. peaks in an XRPD diffractogram at 11.5, 12.0, 14.5 and 17.5, °2θ±0.1°2θ, further characterised by at least one further peak at 19.7, 20.4, 22.2, 24.3 or 25.7 °2θ±0.1°2θ;
c. an XRPD diffractogram as substantially illustrated in Figure 7a; or
d. an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C substantially as illustrated in Figure 8a.

[0044] The peak at 17.5 °2θ±0.1°2θ has a relative intensity compared to the peak at 14.5 °2θ±0.1°2θ of at least 5%, preferably at least 6%, more preferably still at least 7%, through 8%, and 9% to at least 10%.

[0045] In one embodiment, psilocybin Polymorph A exhibits an XRPD diffractogram characterised by the diffractogram summarised in Table 1. In one embodiment, described herein, the crystalline psilocybin Polymorph A comprises at least 3 peaks of (±0.1°2θ) of Table 1. In a certain embodiment, described herein, the crystalline psilocybin Polymorph A comprises at least 4 peaks of (±0.1°2θ) of Table 1. In a certain embodiment, described herein the crystalline psilocybin Polymorph A comprises at least 5 peaks of (±0.1°2θ) of Table 1. In a certain embodiment, described herein the crystalline psilocybin Polymorph A

comprises at least 6 peaks of (±0.1°2θ) of Table 1. In a certain embodiment, described herein the crystalline psilocybin Polymorph A comprises at least 8 peaks of (±0.1°2θ) of Table 1. In a certain embodiment, described herein the crystalline psilocybin Polymorph A comprises at least 10 peaks of (±0.1°2θ) of Table 1. In a certain embodiment, described herein the crystalline psilocybin Polymorph A comprising at least 15 peaks of (±0.1°2θ) of Table 1. A peak at about 17.5, °2θ±0.1°2θ distinguishes psilocybin Polymorph A from Polymorph A', in which the peak is absent or substantially absent (i.e. has a relative intensity compared to the peak at 14.5 °2θ±0.1°2θ of less than 2%, more preferably less than 1%).

[0046] Table 1 – XRPD peak positions for Polymorph A

Position [°2Th.]	Relative Intensity [%]
5.6	8.42
11.5	13.05
12.0	26.45
14.5	100
17.5	10.71
19.7	37.29
20.4	20.06
22.2	17.83
23.2	6.99
24.3	17.93
25.7	16.4
26.8	3.15
27.8	4.54
29.7	9.53
31.2	6.51
32.6	2.45
33.7	1.75

[0047] In one embodiment, crystalline psilocybin Polymorph A is characterised by XRPD diffractogram peaks at 11.5, 12.0, 14.5, and 17.5°2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph A is further characterised by at least one additional peak appearing at 19.7, 20.4, 22.2, 24.3 or 25.7°2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph A is further characterised by at least two additional peaks appearing at 19.7, 20.4, 22.2, 24.3 or 25.7 °2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph A is further characterised by at least two additional peaks appearing at 19.7, 20.4, 22.2, 24.3 or 25.7 °2θ±0.1°2θ. In yet a further embodiment, crystalline psilocybin Polymorph A exhibits an XRPD diffractogram substantially the same as the XRPD diffractogram shown in Fig. 7a.

[0048] In one embodiment, crystalline psilocybin Polymorph A is characterised by XRPD diffractogram peaks at 14.5 and 17.5°2θ±0.1°2θ with the peak at 17.5°2θ having an intensity which is at least 5% of the intensity of the peak at 14.5°2θ, more preferably still at least 6%, through at least 7%, at least 8%, at least 9%, to at least 10%.

[0049] In one embodiment, crystalline psilocybin Polymorph A is absent or substantially absent of an XRPD diffractogram peaks at 10.1. By substantially absent is meant than any XRPD diffractogram peaks at 10.1 is less than 2% of the intensity of the peak at 14.5°2θ, such as less than 1%, or is not detectable in the XRPD diffractogram,

[0050] In one embodiment, crystalline psilocybin Polymorph A is characterised by an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C. In another embodiment, crystalline psilocybin Polymorph A is further characterised by an endothermic event in the DSC thermogram having an onset temperature of between 145 and 165°C, such as between 145 and 160°C, or such as between 145 and 155°C. In another embodiment, crystalline psilocybin Polymorph A is characterised by an endothermic event having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C, and an endothermic event having an onset temperature of between 145 and 165°C, such as between 145 and 160°C, or such as between 145 and 155°C, in a DSC thermogram. In yet another embodiment, crystalline psilocybin Polymorph A exhibits a DSC thermogram substantially the same as the DSC thermogram in Fig. 8a.

[0051] In another embodiment, crystalline psilocybin Polymorph A is characterised by having a water content of <0.5% w/w, such as <0.4% w/w, such as <0.3% w/w, such as <0.2% w/w, or such as <0.1% w/w. The skilled person would know of methods to determine the water content of a compound, for example Karl Fischer Titration. In one embodiment, crystalline psilocybin Polymorph A is characterised by having <0.5% w/w loss, such as <0.4% w/w, such as <0.3% w/w, such as <0.2% w/w, such as <0.1% w/w, in the TGA thermogram between ambient temperature, such as about 25°C, and 200°C. In one embodiment, crystalline psilocybin Polymorph A loses less than 2% by weight in a loss on drying test, such as less than 1% by weight, such as less than 0.5% by weight. The loss on drying test is performed at 70°C.

[0052] In one embodiment, crystalline psilocybin Polymorph A is a highly pure crystalline form of Polymorph A, for example, psilocybin comprises at least 90% by weight, such as 95%, such as 99%, such as 99.5% of Polymorph A.

[0053] In one embodiment, crystalline psilocybin Polymorph A is a white to off white solid.

[0054] In another embodiment, crystalline psilocybin Polymorph A is chemically pure, for example the psilocybin has a chemical purity of greater than 97%, such as greater than 98%, or such as greater than 99% by HPLC. In one embodiment, crystalline psilocybin Polymorph A has no single impurity of greater than 1%, more preferably less than 0.5%, including phosphoric acid as measured by ^{31}P NMR, and psilocin as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph A has a chemical purity of greater than 97 area%, more preferably still greater than 98 area%, and most preferably greater than 99 area% by HPLC. In one embodiment, crystalline psilocybin Polymorph A has no single impurity greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph A does not contain psilocin at a level greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph A does not contain phosphoric acid at a level greater than 1 weight%, more preferably less than 0.5 weight% as measured by ^{31}P NMR. In one embodiment, crystalline psilocybin Polymorph A has a chemical assay of at least 95 weight%, such as at least 96 weight%, or such as at least 98 weight%.

[0055] In contrast crystalline psilocybin Polymorph A' may be characterised by one or more of:

a. peaks in an XRPD diffractogram at 11.5, 12.0 and 14.5 °2θ±0.1°2θ, but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ;
b. peaks in an XRPD diffractogram at 11.5, 12.0 and 14.5 °2θ±0.1°2θ, but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ, further characterised by at least one further peak at 19.7, 20.4, 22.2, 24.3 or 25.7 °2θ±0.1°2θ;
c. an XRPD diffractogram as substantially illustrated in Figure 7b; or
d. an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C substantially as illustrated in Figure 8b.

[0056] By substantially absent of a peak at 17.5 °2θ±0.1°2θ is meant, if present, the peak has a relative intensity, compared to a peak at 14.5 °2θ±0.1°2θ, of less than 5%, more preferably less than 4%, through less than 3%, to 2%, 1% or less.

[0057] In one embodiment, psilocybin Polymorph A' exhibits an XRPD diffractogram characterised by the diffractogram summarised in Table 2. In one embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 3 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprising at least 4 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph

A' comprises at least 5 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 6 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 8 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 10 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 15 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 20 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ. In a certain embodiment, described herein the crystalline psilocybin Polymorph A' comprises at least 25 peaks of (±0.1°2θ) of Table 2 but absent or substantially absent of a peak at 17.5 °2θ±0.1°2θ.

[0058] Table 2 – XRPD peak positions for Polymorph A'

Position [°2Th.]	Relative Intensity [%]
5.5	4.89
10.1	4.09
11.5	22.05
12.0	22.77
14.5	100
14.9	11.29
17.5	1.08
18.7	2.44
19.4	23.02
19.6	33.7
20.3	17.01
21.1	12.08
21.6	8.51
22.2	15.54
22.6	8.78
23.1	10.11
24.3	21.83
25.1	4.36
25.8	15.4
26.3	4.28
26.8	2.86
27.8	5.96
28.6	1.91

29.7	10.56
31.1	7.35
32.6	3.72
33.8	1.54

[0059] In one embodiment, crystalline psilocybin Polymorph A' is characterised by XRPD diffractogram peaks at 11.5, 12.0, and 14.5°2θ±0.1°2θ but substantially absent of a peak at 17.5 °2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph A' is further characterised by at least one additional peak appearing at 19.7, 20.4, 22.2, 24.3, or 25.7 °2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph A' is further characterised by at least two additional peaks appearing at 19.7, 20.4, 22.2, 24.3, or 25.7 °2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph A' is further characterised, and distinguished from Polymorph A by the presence of a peak appearing at 10.1 °2θ±0.1°2θ. In yet a further embodiment, crystalline psilocybin Polymorph A' exhibits an XRPD diffractogram substantially the same as the XRPD diffractogram shown in Fig. 7b.

[0060] In one embodiment, crystalline psilocybin Polymorph A' is characterised by XRPD diffractogram peaks at 14.5 and 17.5°2θ±0.1°2θ wherein the intensity of the peak at 17.5°2θ is less than 5% of the intensity of the peak at 14.5°2θ, such as less than 4%, such as less than 3%, such as at less than 2%, such as less than 1%, or such as about 1%.

[0061] In one embodiment, crystalline psilocybin Polymorph A' is characterised by XRPD diffractogram peaks at 10.1 and 14.5°2θ±0.1°2θ wherein the intensity of the peak at 10.1°2θ is at least 1% of the intensity of the peak at 14.5°2θ, such as at least than 2%, such as at least than 3%, or such as about 4%.

[0062] In one embodiment, crystalline psilocybin Polymorph A' is characterised by an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C. In another embodiment, crystalline psilocybin Polymorph A' is further characterised by an endothermic event in the DSC thermogram having an onset temperature of between 145 and 165°C, such as between 145 and 160°C, or such as between 145 and 155°C. In another embodiment, crystalline psilocybin Polymorph A' is characterised by an endothermic event having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C, and an endothermic event having an onset temperature of between 145 and 165°C, such as between 145 and 160°C, or such as between 145 and 155°C, in a DSC thermogram. In yet another embodiment, crystalline psilocybin Polymorph A' exhibits a DSC thermogram substantially the same as the DSC thermogram in Fig. 8b.

[0063] In another embodiment, crystalline psilocybin Polymorph A' is characterised by having a water content of <0.5% w/w, such as <0.4% w/w, such as <0.3% w/w, such as <0.2% w/w, or such as <0.1% w/w. The skilled person would know of methods to determine the water content of a compound, for example Karl Fischer Titration. In one embodiment, crystalline psilocybin Polymorph A' is characterised by having <0.5% w/w loss, such as <0.4% w/w, such as <0.3% w/w, such as <0.2% w/w, such as <0.1% w/w, in the TGA thermogram between ambient temperature, such as 25°C, and 200°C. In one embodiment, crystalline psilocybin Polymorph A' loses less than 2% by weight in a loss on drying test, such as less than 1% by weight, such as less than 0.5% by weight. The loss on drying test is performed at 70°C.

[0064] In one embodiment, crystalline psilocybin Polymorph A' is a highly pure crystalline form of Polymorph A', for example, psilocybin comprises at least 90% by weight, such as 95%, such as 99%, such as 99.5% of Polymorph A'.

[0065] In one embodiment, crystalline psilocybin Polymorph A's is a white to off white solid.

[0066] In another embodiment, crystalline psilocybin Polymorph A' is chemically pure, for example the psilocybin has a chemical purity of greater than 97%, more preferably still greater than 98%, and most preferably greater than 99% by HPLC. In one embodiment, crystalline psilocybin Polymorph A' has no single impurity of greater than 1%, more preferably less than 0.5%, including phosphoric acid as measured by ^{31}P NMR, and psilocin as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph A' has a chemical purity of greater than 97 area%, more preferably still greater than 98 area%, and most preferably greater than 99 area% by HPLC. In one embodiment, crystalline psilocybin Polymorph A' has no single impurity greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph A' does not contain psilocin at a level greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph A' does not contain phosphoric acid at a level greater than 1 weight%, more preferably less than 0.5 weight% as measured by ^{31}P NMR. In one embodiment, crystalline psilocybin Polymorph A' has a chemical assay of at least 95 weight%, such as at least 96 weight%, or such as at least 98 weight%.

[0067] XRPD diffractograms and XRPD peak positions are acquired using Cu Kα radiation.

[0068] DSC and TGA thermograms are acquired using a heating rate of 20°C/min.

[0069]

[0070] Preferably the crystalline psilocybin Polymorph A (12A) exhibits an XRPD diffractogram as illustrated in Fig 7a and a DSC thermograph as illustrated in Fig 8a.

[0071] In contrast the crystalline psilocybin Polymorph A' (12A') exhibits an XRPD diffractogram as substantially illustrated in Fig 7b and a DSC thermograph as substantially illustrated in Fig 8b.

[0072] Preferably the high purity crystalline psilocybin Polymorph A (12A) is characterised by a XRPD diffractogram as substantially illustrated in Fig 7a and a DSC thermograph as substantially illustrated in Fig 8a.

[0073] In contrast the high purity crystalline psilocybin Polymorph A' (12A') is characterised by a XRPD diffractogram as illustrated in Fig 7b and a DSC thermograph as illustrated in Fig 8b.

[0074] Polymorph A (including its isostructural variant Polymorph A') (Figs 7a and 7b) differ from Polymorph B (Fig 7c), the Hydrate A (Fig 7d) and the ethanol solvate (Fig 7e: Solvate A), and the relationship between the different forms is illustrated in Fig 9.

[0075] The crystalline psilocybin Polymorph A according to the invention, is a white to off white solid, and/or has a chemical purity of greater than 97%, more preferably still greater than 98%, and most preferably greater than 99% by HPLC, and has no single impurity of greater than 1%, more preferably less than 0.5%, including phosphoric acid as measured by ^{31}P NMR, and psilocin as measured by HPLC. In one embodiment, there is provided high purity crystalline psilocybin, Polymorph A. In one embodiment, crystalline psilocybin, Polymorph A, has a chemical purity of greater than 97 area%, more preferably still greater than 98 area%, and most preferably greater than 99 area% by HPLC. In one embodiment, crystalline psilocybin, Polymorph A, has no single impurity greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin, Polymorph A, does not contain psilocin at a level greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin, Polymorph A, does not contain phosphoric acid at a level greater than 1 weight%, more preferably less than 0.5 weight% as measured by ^{31}P NMR. In one embodiment, crystalline psilocybin, Polymorph A, has a chemical assay of at least 95 weight%, such as at least 96 weight%, or such as at least 98 weight%.

[0076] The heating of polymorph A results in an endothermic event having an onset temperature of circa 150°C corresponding to solid-solid transition of Polymorph A to Polymorph B. Continued heating of the resulting solid, i.e., Polymorph B, results in a second endothermic event corresponding to a melting point having an onset temperature of between 205 and 220ºC (see Figs 8a and 8b).

[0077] IAn alternative polymorphic crystalline form of psilocybin, Hydrate A, may be characterised by one or more of:

a. peaks in an XRPD diffractogram at 8.9, 12.6 and 13.8°2θ±0.1°2θ;
b. peaks in an XRPD diffractogram at 8.9, 12.6 and 13.8°2θ±0.1°2θ, further characterised by at least one further peak at 6.5, 12.2, 19.4, 20.4 or 20.8°2θ±0.1°2θ;
c. an XRPD diffractogram as substantially illustrated in Figure 7d; or
d. an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C substantially as illustrated in Figure 8d.

[0078] In one embodiment, psilocybin Hydrate A exhibits an XRPD diffractogram characterised by the diffractogram summarised in Table 3. In one embodiment, described herein the crystalline psilocybin Hydrate A comprises at least 3 peaks of (±0.1°2θ) of Table 3. In a certain embodiment, described herein the crystalline psilocybin Hydrate A comprises at least 4 peaks of (±0.1°2θ) of Table 3. In a certain embodiment, described herein the crystalline psilocybin Hydrate A comprises at least 5 peaks of (±0.1°2θ) of Table 3. In a certain embodiment, described herein the crystalline psilocybin Hydrate A comprises at least 8 peaks of (±0.1°2θ) of Table 3. In a certain embodiment, described herein the crystalline psilocybin Hydrate A comprises at least 10 peaks of (±0.1°2θ) of Table 3.

Table 3 XRPD peak positions for Hydrate A

Position [°2Th.]	Relative Intensity [%]
5.6	14.4
6.5	18.84
8.9	100
12.2	11.51
12.6	18.65
13.8	44.22
16.2	21.22
18.9	6.62
19.4	38.68
20.4	21.32
20.8	19.73
21.5	20.75
22.3	12.8
22.5	19.38
23.1	47.53
23.5	25.79
24.3	5.62
24.8	14.62
25.4	5.27
26.9	6.53
27.9	7.82

28.4	5.78
29.0	5.09
29.7	4.83
32.1	8.27
32.8	4.81
33.4	3.74
34.2	5.96

[0079] In one embodiment, crystalline psilocybin Hydrate A is characterised by XRPD diffractogram peaks at 8.9, 12.6 and 13.8°2θ±0.1°2θ. In another embodiment, crystalline psilocybin Hydrate A is further characterised by at least one peak appearing at 6.5, 12.2, 19.4, 20.4 or 20.8°2θ±0.1°2θ. In another embodiment, crystalline psilocybin Hydrate A is further characterised by at least two peaks appearing at 6.5, 12.2, 19.4, 20.4 or 20.8°2θ±0.1°2θ. In yet a further embodiment, crystalline psilocybin Hydrate A exhibits an XRPD diffractogram substantially the same as the XRPD diffractogram shown in Fig. 7d.

[0080] In one embodiment, crystalline psilocybin Hydrate A is characterised by an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C. In another embodiment, crystalline psilocybin Hydrate A is further characterised by an endothermic event in the DSC thermogram having an onset temperature of between 85 and 105°C, or such as between 90 and 100°C. In another embodiment, crystalline psilocybin Hydrate A is characterised by an endothermic event having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C, and an endothermic event having an onset temperature of between 85 and 105°C, or such as between 90 and 100°C, in a DSC thermogram. In yet another embodiment, crystalline psilocybin Hydrate A exhibits a DSC thermogram substantially the same as the DSC thermogram in Fig. 8d.

[0081] In another embodiment, crystalline psilocybin Hydrate A is characterised by having a water content of between 10 and 18%, such as between 12 and 16%, or such as about 13%. The skilled person would know of methods to determine the water content of a compound, for example Karl Fischer Titration. In one embodiment, crystalline psilocybin Hydrate A is characterised by having a weight loss in the TGA thermogram of between 10 and 18%, such as between 12 and 16%, or such as about 13%, between ambient temperature, such as about 25°C, and 120°C.

[0082] In one embodiment, crystalline psilocybin Hydrate A is a highly pure crystalline form of Hydrate A, for example, psilocybin comprises at least 90% by weight, such as 95%, such as 99%, such as 99.5% of Hydrate A.

[0083] A yet another alternative polymorphic crystalline form of psilocybin is Polymorph B, which may be characterised by one or more of:

a. peaks in an XRPD diffractogram at 11.1, 11.8 and 14.3°2θ±0.1°2θ;
b. peaks in an XRPD diffractogram at 11.1, 11.8 and 14.3°2θ±0.1°2θ, further characterised by at least one further peak at 14.9, 15.4, 19.3, 20.0 or 20.6°2θ±0.1°2θ;
c. an XRPD diffractogram as substantially illustrated in Figure 7c; or
d. an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C substantially as illustrated in Figure 8c.

[0084] In one embodiment, psilocybin Polymorph B exhibits an XRPD diffractogram characterised by the diffractogram summarised in Table 4. In one embodiment, described herein the crystalline psilocybin Polymorph B comprises at least 3 peaks of (±0.1°2θ) of Table 4. In a certain embodiment, described herein the crystalline psilocybin Polymorph B comprises at least 4 peaks of (±0.1°2θ) of Table 4. In a certain embodiment, described herein the crystalline psilocybin Polymorph B comprises at least 5 peaks of (±0.1°2θ) of Table 4. In a certain embodiment, described herein the crystalline psilocybin Polymorph B comprising at least 8 peaks of (±0.1°2θ) of Table 4. In a certain embodiment, described herein the crystalline psilocybin Polymorph B comprises at least 10 peaks of (±0.1°2θ) of Table 4.

Table 4 XRPD peak positions for Polymorph B

Position [°2Th.]	Relative Intensity [%]
5.5	21.33
11.1	36.91
11.8	100.00
12.5	12.73
14.3	70.23
14.9	50.01
15.4	23.67
17.1	51.58
17.4	91.25
18.0	12.61
19.3	39.33
20.0	76.61
20.6	50.26
21.5	20.77
22.3	40.19
23.9	13.32
24.3	16.03
25.3	32.94

28.3	7.60
28.9	17.89
29.3	8.96
31.3	6.57
32.2	6.90
33.8	2.37

[0085] In one embodiment, crystalline psilocybin Polymorph B is characterised by XRPD diffractogram peaks at 11.1, 11.8 and 14.3°2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph B is further characterised by at least one peak appearing at 14.9, 15.4, 19.3, 20.0 or 20.6°2θ±0.1°2θ. In another embodiment, crystalline psilocybin Polymorph B is further characterised by at least two peaks appearing at 14.9, 15.4, 19.3, 20.0 or 20.6°2θ±0.1°2θ. In yet a further embodiment, crystalline psilocybin Polymorph B exhibits an XRPD diffractogram substantially the same as the XRPD diffractogram shown in Fig. 7c.

[0086] In one embodiment, crystalline psilocybin Polymorph B is characterised by an endothermic event in a DSC thermogram having an onset temperature of between 205 and 220°C, such as between 210 and 220°C, such as between 210 and 218°C, or such as between 210 and 216°C. In yet another embodiment, crystalline psilocybin Polymorph B exhibits a DSC thermogram substantially the same as the DSC thermogram in Fig. 8c.

[0087] In another embodiment, crystalline psilocybin Polymorph B is characterised by having a water content of <0.5% w/w, such as <0.4% w/w, such as <0.3% w/w, such as <0.2% w/w, or such as <0.1% w/w. The skilled person would know of methods to determine the water content of a compound, for example Karl Fischer Titration. In one embodiment, crystalline psilocybin Polymorph B is characterised by having <0.5% w/w loss, such as <0.4% w/w, such as <0.3% w/w, such as <0.2% w/w, such as <0.1% w/w, in the TGA thermogram between ambient temperature, such as about 25°C, and 200°C. In one embodiment, crystalline psilocybin Polymorph B loses less than 2% by weight in a loss on drying test, such as less than 1% by weight, such as less than 0.5% by weight. The loss on drying test is performed at 70°C.

[0088] In one embodiment, crystalline psilocybin Polymorph B is a highly pure crystalline form of Polymorph B, for example, psilocybin comprises at least 90% by weight, such as 95%, such as 99%, such as 99.5% of Polymorph B.

[0089] In another embodiment, crystalline psilocybin Polymorph B is chemically pure, for example the psilocybin has a chemical purity of greater than 97%, such as greater than 98%, or such as greater than 99% by HPLC. In one embodiment, crystalline psilocybin Polymorph B has no single impurity of greater than 1%, more preferably less than 0.5%, including phosphoric acid as measured by ^{31}P NMR, and psilocin as measured by HPLC. In one

embodiment, crystalline psilocybin Polymorph B has a chemical purity of greater than 97 area%, more preferably still greater than 98 area%, and most preferably greater than 99 area% by HPLC. In one embodiment, crystalline psilocybin Polymorph B has no single impurity greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph B does not contain psilocin at a level greater than 1 area%, more preferably less than 0.5 area% as measured by HPLC. In one embodiment, crystalline psilocybin Polymorph B does not contain phosphoric acid at a level greater than 1 weight%, more preferably less than 0.5 weight% as measured by ^{31}P NMR. In one embodiment, crystalline psilocybin Polymorph B has a chemical assay of at least 95 weight%, such as at least 96 weight%, or such as at least 98 weight%.

[0090] The psilocybin Polymorph A of the invention has the general properties illustrated in Table 5 below:

Table 5

Appearance:	White to off white solid
Major endothermic event in DSC (onset temperature) (corresponding to a melt):	210-215°C
Hygroscopicity:	Psilocybin Polymorph A forms Hydrate A at high humidity and when added to water but the water of hydration is lost rapidly on drying. The anhydrous form is therefore being developed.
Crystalline form:	Anhydrous Polymorph A
pKa (calculated):	1.74, 6.71, 9.75
Solubility	*approx.* 15 mg/ml in Water

[0091] The psilocybin Polymorph A conforms to the spectra as set out in Table 6 below and illustrated in the spectra of Figs 10-13.

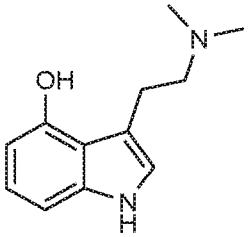
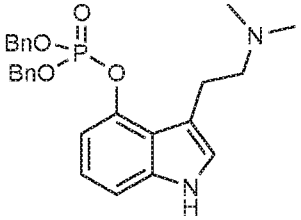
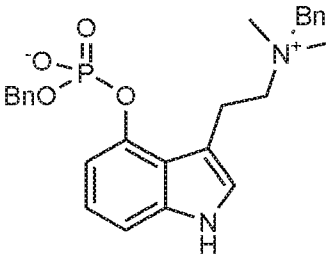
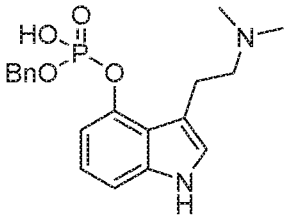
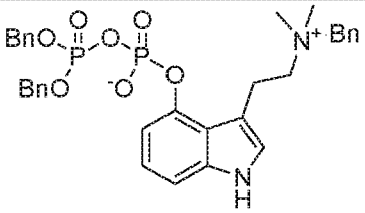
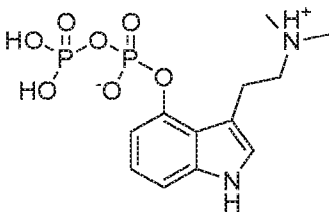
Table 6

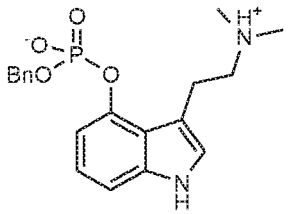
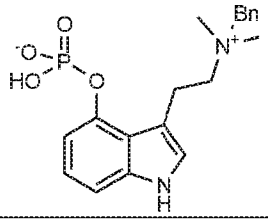
Technique	**Conclusions**
Proton (^{1}H) and Carbon (^{13}C) NMR	Assignment of the proton (Fig 10) and carbon spectra (Fig 11) are concordant with Psilocybin.
FT-Infrared Spectroscopy (FT-IR)	Assignment of the FT-IR spectrum (Fig 12) is concordant with Psilocybin.
Mass Spectroscopy (MS)	Assignment of the mass spectrum (Fig 13) is concordant with Psilocybin.

[0092] The high purity is attained by careful control of reaction conditions to ensure that potential organic impurities are significantly reduced.

[0093] Known and potential impurities in Psilocybin are shown in Table 7 below:

Table 7

Impurity	Relative Retention Time (RRT)	Structure	Origin
Psilocin	1.65		Starting material (stage 3). Also generated by hydrolysis of Psilocybin. Only significant impurity observed in psilocybin batches.
Stage 4A			Initial product formed in the stage 4 reaction. Converts to stage 4 on stirring in THF. Converts to Psilocybin in stage 5.
Stage 4	2.74		Intermediate
N-Denzylated stage 4			Identified by MS in Stage 4. Converts to Psilocybin in stage 5.
Stage 4 Anhydride Impurity			Identified by MS in Stage 4. Converts to Stage 5 Pyrophosphoric acid impurity in stage 5.
Stage 5 Pyrophosphoric acid impurity			Identified by MS Formed from the stage 4 anhydride. Removed in the stage 6 re-crystallisation by a combination of hydrolysis to Psilocybin and increased solubility due to the extra phosphate group.

Stage 5 (intermediates)	1.89 and 2.45	 	2 Intermediates are formed during the hydrogenation. These subsequently convert to product (structures based on chemistry). Monitored and controlled in stage 5 reaction.

[0094] Similarly, the careful processing ensures solvent levels are kept to below levels as indicated in Table 8.

Table 8

Solvent	Controlled to	Chemical Stage solvent is used in
Methanol	3000ppm	Stage 5
Ethanol	5000ppm	Stage 5
THF	720ppm	Stage 4
Toluene	890ppm	Generated as a by-product in stage 5

[0095] Through careful selection of operating methodology the psilocybin Polymorph A drug substance of the invention meets the acceptance criteria set out in Table 9 below:

Table 9

Quality attribute	Acceptance criteria	Test method
1.Appearance	For information only.	Visual
2.Identity by ^{1}H NMR	Compares well with reference. Fig 10	^{1}H NMR
3.Identity by ^{13}C NMR	Compares well with reference. Fig 11	^{13}C NMR
4.Identity by MS	Compares well with reference. Fig 12	MS
5.Identity by FT-IR	Compares well with reference. Fig 13	FT-IR
6.Loss on drying	NMT 2% w/w	European Pharmacopoeia 2.2.32
7.Residue on Ignition	NMT 0.5% w/w	US Pharmacopoeia <281>

8.Chemical purity	NLT 97 area%	HPLC
9.Drug Related Impurities	No single impurity NMT 1.0 area%	HPLC
10. Assay (on a dry basis)	95-103 weight%	HPLC
11. Residual Solvent Content	Methanol NMT 3000ppm Ethanol NMT5000ppm THF NMT 720ppm Toluene NMT 890ppm	HRGC
12. Phosphoric acid content	NMT 1% w/w	^{31}P NMR
13. Elemental analysis by ICP-MS	Cd NMT 1.5ppm Pb NMT 1.5ppm As NMT 4.5ppm Hg NMT 9.0ppm Co NMT 15ppm V NMT 30ppm Ni NMT 60ppm Li NMT 165ppm Pd NMT 30ppm	US Pharmacopoeia <233>
14. Polymorphism	Conforms to reference Fig 7a	XRPD
15. Melting Point	Report result Fig 8a	DSC

Abbreviations used in table: NMT = not more than, NLT = not less than.

[0096] The methodology used to verify the purity is provided in the detailed description.

[0097] In fact, the criteria 6-13 are far exceeded in practice, as noted in Table 10 below:

Table 10

Quality attribute	**Acceptance criteria**	**Typically**	**Test method**
1.Loss on drying		Typically less than 1% w/w	European Pharmacopoeia 2.2.32
2.Residue on Ignition		Typically less than 0.2% w/w	US Pharmacopoeia <281>
3.Chemical purity		Typically NLT 99%	HPLC
4.Drug Related Impurity	No single impurity NMT 1.0%	RRT 1.49: 0.06% RRT 1.69 (Psilocin): 0.39% RRT 1.70: 0.05% Others LT 0.05%: 0.22%	HPLC
5.Assay (on a dry basis)	95 - 103	98.65%	HPLC
6.Residual Solvent Content	Methanol NMT 3000ppm Ethanol NMT 5000ppm THF NMT 720ppm	NMT 5 ppm NMT 10 ppm NMT 5 ppm NMT 5 ppm	HRGC

	Toluene NMT 890ppm		
7. Phosphoric acid content	NMT 1% w/w	0.2% Absence of phosphoric acid (H_3PO_4) which comes at approx. 0ppm	^{31}P NMR
8. Elemental analysis by ICP-MS	Cd NMT 1.5ppm Pb NMT 1.5ppm As NMT 4.5ppm Hg NMT 9.0ppm Co NMT 15ppm V NMT 30ppm Ni NMT 60ppm Li NMT 165ppm Pd NMT 30ppm	LT 0.5 ppm LT 0.5 ppm LT 1 ppm LT 1 ppm LT 5 ppm LT 20 ppm LT 10 ppm LT 20 ppm LT 5ppm	US Pharmacopoeia <233>

Abbreviations used in table: NMT = not more than, LT = less than.

[0098] Thus, crystalline psilocybin Polymorph A, has spectra that conform with Proton (^{1}H) and Carbon (^{13}C) NMR, FT-Infrared Spectroscopy (FT-IR), and Mass Spectroscopy (MS) – Figs 10-13.

[0099] It also conforms to any of the criteria specified in Table 9 or Table 10.

[00100] In accordance with a second aspect of the present invention there is provided a batch of at least 100g of crystalline psilocybin Polymorph A, according to the first aspect of the present invention. In one embodiment, there is provided a batch of crystalline psilocybin, Polymorph A, comprising at least 100g, and most preferably at least 250g.

[00101] In accordance with a third aspect of the present invention there is provided a pharmaceutical formulation comprising crystalline psilocybin Polymorph A, according to the first aspect of the present invention together with one or more excipients. Preferred pharmaceutical excipients for an oral formulation include: diluents, such as, microcrystalline cellulose, starch, mannitol, calcium hydrogen phosphate anhydrous or co -mixtures of silicon dioxide, calcium carbonate, microcrystalline cellulose and talc; disintegrants, such as, sodium starch glycolate or croscarmellose sodium; binders, such as, povidone, copovidone or hydroxyl propyl cellulose; lubricants, such as, magnesium stearate or sodium stearyl fumurate; glidants, such as, colloidal silicon dioxide; and film coats, such as, Opadry II white or PVA based brown Opadry II.

[00102] Studerus et al (2011) J Psychopharmacol 25(11) 1434-1452 classified oral doses of psilocybin as follows: Very low doses at 0.045mg.kg; low doses between 0.115-0.125mg/kg, medium doses between 0.115-0.260mg/kg, and high doses at 0.315mg/kg.

[00103] The psilocybin Polymorpg A would typically be present in a formulated dose in an amount of from 0.01mg/kg to 1mg/kg. A typical human dose (for an adult weighing 60-80kg) would equate to a dose of somewhere between 0.60mg and 80mg. In one embodiment,

between 2 and 50 mg of crystalline psilocybin Polymorph A, is present in a formulated dose, such as between 2 and 40 mg, such as between 2 and 10 mg, such as 5 mg, such as between 5 and 30 mg, such as between 5 and 15 mg, such as 10 mg, such as between 20 and 30 mg, or such as 25 mg. In one embodiment, between 2 and 50 mg of crystalline psilocybin Polymorph A is present in a formulated dose, such as between 2 and 40 mg, such as between 2 and 10 mg, such as 5 mg, such as between 5 and 30 mg, such as between 5 and 15 mg, such as 10 mg, such as between 20 and 30 mg, or such as 25 mg.

[00104] Favoured adult oral doses are likely to be in the range 1mg to 40mg, preferably 2 to 30mg, more preferably 15 to 30mg, for example 5mg,10mg or 25mg. Micro-dosing, typically at about a tenth of these doses, is also possible with micro dose formulations typically lying within the range 0.05mg to 2.5mg.

[00105] A preferred pharmaceutical formulation is an oral dosage form.

[00106] The oral dosage form may be a tablet or a capsule.

[00107] The psilocybin polymorph A will be present together with one or more excipients. Preferred excipients include microcrystalline cellulose and starch.

[00108] In accordance with a fourth aspect of the present invention there is provided the crystalline psilocybin Polymorph A, according to the first aspect of the present invention for use in medicine.

[00109] Preferably the crystalline psilocybin Polymorph A, of the first aspect of the present invention is for use in treating central nervous disorders.

[00110] In one embodiment, there is provided crystalline psilocybin, Polymorph A for use in treating depression. In one embodiment, there is provided crystalline psilocybin, Polymorph A for use in treating drug resistant depression.

[00111] Other conditions that may be treated include: anxiety disorders, including anxiety in advanced stage illness e.g. cancer as well as Generalized Anxiety Disorder, Depression including Major Depressive Disorder, Cluster Headaches, Obsessive Compulsive Disorder, Personality Disorders including Conduct Disorder, Drug Disorders including: alcohol dependence, nicotine dependence, opioid dependence, cocaine dependence and other addictions including Gambling Disorder, Eating Disorder and Body Dysmorphic Disorder. A still further condition is the treatment of pain.

[00112] To produce the psilocybin of the invention the psilocybin was crystallised from water in a controlled manner.

[00113] According to a fifth aspect of the present invention there is provided a method for large scale manufacture of psilocybin in the form Polymorph A (12) having a chemical purity

of greater than 97% by HPLC, and no single impurity of greater than 1% including phosphoric acid as measured by 31P NMR, and psilocin as measured by HPLC characterised in that the method comprises subjecting psilocybin (12) to a water crystallization step, with controlled drying, to produce a crystalline psilocybin in the form Polymorph A as per the first aspect of the present invention.

[00114] . In one embodiment, there is provided a method for large scale manufacture of psilocybin characterised in that the method comprises subjecting psilocybin to a water crystallization step, with controlled drying, to produced crystalline psilocybin Polymorph A with an XRPD diffractogram as illustrated in Fig 7a and a DSC and TGA thermograph as illustrated in Fig 8a.

[00115] Preferably Polymorph A is an isostructural variant with an XRPD diffractogram as illustrated in Fig 7a and a DSC thermograph as illustrated in Fig 8a.

[00116] More preferably the psilocybin is recrystallized in about 10-20 volumes of water, heated with agitation to a temperature of at least 70°C, polish filtered with a suitable cut off (typically, below 5 µm), seeded at a temperature of about 70°C, and cooled in a controlled manner to about 5°C over a period of more than 2 hours.

[00117] More preferably the method comprises controlled cooling which drops the temperature by about 5 °C -15 °C an hour, more preferably about 10°C an hour.

[00118] Preferably the polish filter step is done through an appropriately sized filter such as a 1.2µm in line filter.

[00119] Preferably the agitation is by stirring at about 400-500 rpm, typically about 450 rpm.

[00120] Preferably the seed is psilocybin Hydrate A. In one embodiment, 0.1% weight or less of seed is added to the process.

[00121] Preferably the crystalline psilocybin Polymorph A is isolated by vacuum filtration.

[00122] In one embodiment, the isolated crystals are dried *in vacuo* at a temperature of at least 30°C, such as between 30 and 50°C, or such as between 40 and 50°C. In one embodiment, the isolated crystals are dried *in vacuo* for at least 10 hours, such as between 12 and 18 hours, or such as about 30 hours. In one embodiment, the isolated crystals are dried *in vacuo* at a temperature of at least 30°C, such as between 30 and 50°C, or such as between 40 and 50°C, for at least 10 hours, such as between 12 and 18 hours, or such as about 30 hours. In one embodiment, the isolated crystals are dried until the isolated crystals lose less than 2% weight in a loss on drying test, such as less than 0.5% weight.

[00123] Preferably the isolated crystals are washed, several times, in water and dried *in vacuo* at about 50°C for at least 12 hours.

[00124] The crystals obtained are typically relatively large (range 50 to 200 microns) and uniform when viewed under the microscope x 10, as illustrated in Fig 16a.

[00125] This differs from crystals obtained without controlled cooling which are much smaller in size (typically 5 to 50microns) when viewed under the microscope x 10, as illustrated in Fig 16b.

[00126] The psilocybin manufactured prior to crystallisation may be produced using any method: synthetic or biological, e.g. by fermentation or obtained by extraction from mushrooms.

[00127] Preferred manufacturing methods use psilocin, or 4 hydroxy-indole, as a starting material.

[00128] A preferred method for large scale manufacture of psilocybin from psilocin comprises the steps of:

i) **Stage 4** – Reacting psilocin with tetrabenzylpyrophosphate to form benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate; and

ii) **Stage 5** – Reacting benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate with hydrogen to form psilocybin.

[00129] A more detailed method for large scale manufacture of psilocybin from 4-hydroxyindole comprises the steps of:

i) **Stage 1** - Reacting 4-hydroxyindole with acetic anhydride to form 1H-indol-4-yl acetate;

ii) **Stage 2** – Reacting 1H-indol-4-yl acetate with oxalyl chloride and dimethylamine to form 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate;

iii) **Stage 3** – Reacting 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate with lithium aluminium hydride to form psilocin;

iv) **Stage 4** – Reacting psilocin with tetrabenzylpyrophosphate to form benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate; and

v) **Stage 5** – Reacting benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate with hydrogen to form psilocybin.

[00130] In order to produce psilocybin Polymorph A the method for large scale manufacture of psilocybin above further comprises:

vi) **Stage 6** – a water crystallization step, with controlled drying.

[00131] In one embodiment, there is provided a method for large scale manufacture of psilocybin as described above further comprising:

vi) **Stage 6** – a water crystallization step, with controlled drying, to produce crystalline psilocybin – Polymorph A with an XRPD diffractogram as substantially illustrated in Fig 7a and a DSC thermograph as substantially illustrated in Fig 8a.

[00132] In developing methodology for the large scale production of psilocin or psilocybin the Applicant overcame one or more significant problems at each of **Stages 1 to 5,** and whilst these problems are considered in the context of the large scale production of psilocin or psilocybin each step, or rather the way each problem was overcome, are considered separate and independent inventions as they have application in the manufacture of other actives be they intermediates to psilocin, psilocybin, or other derivatives, salts, esters or the like which provide a prodrug.

[00133] Preferably the **Stage 4 (i)** reaction comprises the use of sodium hexamethyldisilazide (NaHMDS).

[00134] This has the benefits over the use of Butyl lithium in that: i) it is easier to handle, and ii) it does not introduce lithium into the reaction which causes issues in downstream processing.

[00135] Preferably the reaction uses the solvent THF.

[00136] This has the benefit that resulting product is obtained in significantly higher purity.

[00137] Preferably in (i) the reaction is initiated below -50°C.

[00138] This has the benefit of reducing the levels of impurities (m/z 295.2 observed by LCMS) that will subsequently effect purity downstream.

[00139] More preferably still the **Stage 4 (ii)** step uses THF as the solvent.

[00140] This has the benefit of ensuring thickening is avoided and facilitates a simple stir out process for obtaining the product.

[00141] Preferably the Stage 4 (ii) step comprises a stir out process to obtain benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate.

[00142] A stir out process has the advantage that the process is simplified and yields are improved.

[00143] To ensure the Stage 4 (ii) reaction is run to completion, levels of Intermediate 4A are monitored, and on completion, the benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate is filtered and oven dried.

[00144] This has the advantage that impurities are minimised and a purer product is obtained

[00145] Preferably the **Stage 5** reaction is monitored for levels of intermediates by HPLC, using relative retention times (RRT) and completion is determined by the intermediates being present at less than 0.2%.

[00146] Psilocybin crude (**Stage 5** product, (12)) has main stage 5 impurities whose relative retention times (RRT) in the HPLC method are about 1.89 and 2.45 respectively, and psilocin (RRT 1.66). These impurities are illustrated in Table 7. Typically, psilocybin crude (**Stage 5** product (12)) has 0.24 area% of the RRT 1.89 impurity, 0.03 area% of the RRT 2.45 impurity and 1.86 area% of psilocin. In addition, the pyrophosphoric acid impurity (RRT 0.31) is present in psilocybin crude, for example at a level of about 2-6 area% by HPLC.

[00147] At this level subsequent crystallisation processes can be conducted to provide substantially pure psilocybin, for example psilocybin having a purity of at least 95 area% by HPLC, such as at least 98 area%, or such as at least 99 area%. In one embodiment, the pyrophosphoric acid impurity (RRT 0.31) is present in the substantially pure psilocybin at a level of less than 0.3 area% by HPLC, such as less than 0.2 area%, or such as less than 0.1 area%.

[00148] In addition, during this stage water is added to the reaction to maintain the psilocybin in solution.

[00149] Preferably the catalyst is recovered by filtration.

[00150] Preferably in **Stage 1** the reaction is conducted in DCM and pyridine.

[00151] This has the advantage that flammable solvents are avoided.

[00152] Preferably the reaction mixture is washed with citric acid, to give a pH of about 2-3, to remove excess pyridine, and the acid phase is separated from the DCM phase.

[00153] This has the advantage that the Intermediate 2A can be isolated, allowing purification away from excess oxalyl chloride.

[00154] More preferably the DCM phase is further washed with sodium bicarbonate at about pH 8.

[00155] This has the advantage of purer processing.

[00156] Preferably the 1H-indol-4-yl acetate is precipitated in heptane.

[00157] This aids precipitation and overcomes partial solubility issues.

[00158] Preferably magnesium sulphate is used as a drying agent.

[00159] Preferably the solvents tert butyl methyl ether (TBME) and tetrahydrofuran (THF) are used.

[00160] Preferably the reaction with oxalyl chloride is conducted at about 30°C - 40°C.

[00161] This has the advantage that a high reaction rate is ensured giving improved levels of completion.

[00162] Preferably Intermediate 2A is isolated by filtration.

[00163] This has the advantage that the intermediate is purified away from excess oxalyl chloride.

[00164] Preferably in **Stage 2, step i** the Intermediate 2A is also washed to remove excess oxalyl chloride.

[00165] Preferably the Intermediate 2A is washed with TBME.

[00166] Preferably a heptane addition is made to precipitate out further Intermediate 2A.

[00167] Preferably in **Stage 2, step ii,** dimethyl amine is used in excess.

[00168] This has the advantage that a much improved impurity profile and yield is obtained.

[00169] Preferably the pH is maintained at about or above pH 7.

[00170] Preferably the reaction is carried out in TBME.

[00171] Preferably this stage further comprises a purification step that removes dimethyl amine salts.

[00172] This has the advantage that purity is improved.

[00173] Preferably this stage comprises a slurry and filtration step.

[00174] This has the advantage that handling and purity is improved.

[00175] More preferably it comprises slurrying with water and / or IPA, filtering, and drying the isolated 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate.

[00176] This has the advantage that purity and yields are improved and hydrolysis reduced.

[00177] Preferably in **Stage 3** the reaction is conducted in the solvent THF.

[00178] This has the advantage that a suspension/ emulsion is formed without thickening.

[00179] Preferably the 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate is added to a solution of $LiAlH_4$ in THF.

[00180] Preferably the reaction is quenched with acetone, followed by citric acid ensuring the mixture remains strongly basic (pH11 or above).

[00181] This has the advantage that high yields are obtained.

[00182] Preferably the psilocin is filtered and washed in THF and slurried in PrOAc:TBME, filtered, washed in TBME, and dried.

[00183] This has the advantage that a high purity product is obtained, for example at least 95% pure by HPLC, such as at least 98% pure by HPLC, or such as at least 99% pure by HPLC.

[00184] The favoured production method comprises each of **Stages 1 to 6** but it will be appreciated that each of the features of each stage can stand alone or be used in combination with any other feature from the same or a different step of the reaction.

[00185] Psilocybin of a given form, Polymorph A, and psilocybin of such high purity has not previously been obtained, and to Applicants knowledge their production of Polymorph A particularly (as illustrated in Figs 7a and 8a) is novel. Indeed, the production of large batch quantities of Polymorph A, is new. A consequence of the crystallisation methodology of the invention and, in part, the manufacturing process enable such high chemical purity of crystalline Psilocybin to be obtained.

[00186] Furthermore, given the unstable nature of the compound they have obtained a crystalline form which they have shown to be stable, under accelerated conditions, (described later) for at least 12 months.

[00187] Polymorph A and A' (Fig 7a and 7b) differs from Polymorph B (Fig 7c), a Hydrate A (Fig 7d) and ethanol solvate (Fig 7e) and mixture (Fig 7f (upper)) as will be apparent from their XRPD diffractograms and DSC thermographs – as described hereafter.

[00188] The relationship between the different polymorphs is shown in Fig 9.

[00189] Indeed, the size and shape of the crystals are determined by the crystallisation methodology, and these in turn can affect stability and the ability to formulate the product.

[00190] In a particularly preferred embodiment, the psilocybin is manufactured through a 6-stage process as outlined below:

[00191] In accordance with another aspect of the present invention there is provided a method for manufacture of crystalline psilocybin according to the first aspect of the present invention, characterised in that the method comprises subjecting psilocybin to a water crystallization step, with controlled drying, to produce crystalline psilocybin Polymorph A according to the first aspect of the present invention. In one embodiment, there is provided a method for manufacture of crystalline psilocybin according to the first aspect of the present invention, characterised in that the method comprises a water crystallization step, with controlled drying, to produce crystalline psilocybin – Polymorph A with an XRPD

diffractogram as substantially illustrated in Fig 7a and a DSC thermograph as substantially illustrated in Fig 8a

[00192] More preferably the psilocybin is recrystallized in about 10-20 volumes of water, heated with agitation to a temperature of at least 70°C, polish filtered with a suitable cut off (typically, below 5 µm), seeded at a temperature of about 70°C, and cooled in a controlled manner to about 5°C over a period of more than 2 hours.

[00193] More preferably the method comprises controlled cooling which drops the temperature by about 5 °C -15 °C an hour, more preferably about 10°C an hour.

[00194] Preferably the polish filter step is done through an appropriately sized filter such as a 1.2µm or a 0.45µm in line filter.

[00195] Preferably the agitation is by stirring at about 400-500 rpm, typically about 450 rpm.

[00196] Preferably the seed is psilocybin Hydrate A. In one embodiment, 0.1% weight or less of seed is added to the process.

[00197] Preferably the crystalline psilocybin is isolated by vacuum filtration.

[00198] In one embodiment, the isolated crystals are dried *in vacuo* at a temperature of at least 30°C, such as between 30 and 50°C, or such as between 40 and 50°C. In one embodiment, the isolated crystals are dried *in vacuo* for at least 10 hours, such as between 12 and 18 hours, or such as about 30 hours. In one embodiment, the isolated crystals are dried *in vacuo* at a temperature of at least 30°C, such as between 30 and 50°C, or such as between 40 and 50°C, for at least 10 hours, such as between 12 and 18 hours, or such as about 30 hours. In one embodiment, the isolated crystals are dried until the isolated crystals lose less than 2% weight in a loss on drying test, such as less than 0.5% weight.

[00199] Preferably the isolated crystals are washed, several times, in water and dried *in vacuo* at about 50°C for at least 12 hours.

[00200] The crystals obtained are typically relatively large (range 50 to 200 microns) and uniform when viewed under the microscope x 10, as illustrated in Fig 16a.

[00201] This differs from crystals obtained without controlled cooling which are much smaller in size (typically 5 to 50microns) when viewed under the microscope x 10, as illustrated in Fig 16b.

Stage 1: Synthesis of 1H-indol-4-yl acetate (3)

[00202] The core reaction is the reaction of 4-hydroxyindole (1) with acetic anhydride (2) to form 1H-indol-4-yl acetate (3); (Fig 2)

[00203] Most preferably stage 1 is as follows:

[00204] 4-hydroxyindole (1), DCM (12), and pyridine (13) are added to a vessel and cooled to about 0-5°C. Acetic anhydride (2) is added dropwise, and the mixture warmed to about 20-25°C and stirred until complete by HPLC. The reactants are washed with aqueous citric acid solution (14) and aqueous $NaHCO_3$ (15), dried over $MgSO_4$ (16) filtered and evaporated to approximately half volume. Heptane (17) is added, and distillation continued to remove the majority of the DCM. The mixture is cooled to about 5-25°C, filtered, washed with heptane and dried in a vacuum oven overnight to isolate 1H-indol-4-yl acetate (3) as a solid suitable for use in the following stage.

Stage 2: Synthesis of 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate (6)

[00205] The core reaction is the reaction of 1H-indol-4-yl acetate (3) with Oxalyl chloride (4) and dimethylamine (5) to form 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate (6); (Fig 3)

[00206] Most preferably stage 2 is as follows:

[00207] 1H-indol-4-yl acetate (3) is dissolved in a mixture of THF (19) and TBME (18) at room temperature. Oxalyl chloride (4) was added dropwise allowing the reaction to exotherm at about 35-40°C. The temperature range is maintained throughout the remainder of the addition. The reaction is then stirred at about 40°C until complete by HPLC. The reaction is cooled to room temperature and heptane (17) added resulting in precipitation of further solids. The slurry is stirred, then allowed to settle, followed by removal of the majority of the solvent (18/19) by decanting. The solid was washed in the vessel twice with heptane (17). TBME (18) is added to give a yellow slurry and the mixture cooled to about -20°C. Dimethylamine solution (5) is added maintaining the temperature at -20°C to -10°C. The reaction was then warmed to room temperature and stirred until complete, adding extra dimethylamine if necessary. The reaction was filtered, washed with heptane (17) and dried in a vacuum oven. The crude 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate (6) was further purified by a slurry in water (20), then IPA (21) and then dried in a vacuum oven to yield (6) as a solid suitable for use in the following stage.

Stage 3: Synthesis of 3-(2-(dimethylamino)ethyl)-1H-indol-4-ol (Psilocin) (8)

[00208] The core reaction is the reaction of 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate (6) with lithium aluminium hydride (7) to form psilocin (8); (Fig 4)

[00209] Most preferably stage 3 is as follows:

[00210] The 3[(dimethylcarbamoyl)carbonyl]-1H-indol-4yl-acetate (6) was slurried in THF (19) and cooled to about 0°C. A THF solution of $LiAlH_4$ (7) was added dropwise maintaining the temperature at about 0-20°C. The reaction was then refluxed until complete by HPLC. The reaction was cooled to 0°C and the excess $LiAlH_4$ quenched by addition of acetone (22) followed by aqueous citric acid solution (14). The batch was filtered to remove Lithium and Aluminium salts. The filtrate was dried over $MgSO_4$ (16), filtered and concentrated and loaded onto a silica pad (23). The pad was eluted with THF (19) and the product containing fractions evaporated. The resulting solid was slurried in iPrOAc:TBME (24/18) mixture, filtered and washed with TBME. The solid was dried in the oven to yield high purity psilocin (8) as an off white solid.

Stage 4: Synthesis of benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate (10)

[00211] The core reaction is the reaction of psilocin (8) with tetrabenzylpyrophosphate (9) to form benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate (10), (Fig 5)

[00212] Most preferably stage 4 is as follows:

[00213] Charge psilocin (8) to a vessel followed by THF (19). The reaction was cooled to -50°C to -70°C and NaHMDS (25) was added dropwise at about -45°C to -70°C. The temperature was adjusted to about -45°C to -60°C and tetrabenzylpyrophosphate in THF was added. The batch was allowed to warm to 0°C after which the solid by products were removed by filtration and the filtrate concentrated *in vacuo*. The concentrated mixture was then heated to about 40°C and stirred until the intermediate had converted to the stage 4 product (10) – controlled by monitoring and the use of HPLC. The batch was cooled to about 0-5°C and the resulting solid isolated by filtration and dried *in vacuo* to provide benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate (10) as a solid.

Stage 5: Synthesis of Intermediate Grade 3-[2-(dimethylazaniumyl)ethyl]-1H-indol-4-yl hydrogen phosphate (Psilocybin Crude) (12)

[00214] The core reaction comprises reacting benzyl 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate (10) with hydrogen (11) to form psilocybin (12), (Fig 6).

[00215] Most preferably stage 5 is as follows:

[00216] To a vessel was charged Pd/C (26), methanol (24) and 3-[2-(benzyldimethylazaniumyl)ethyl]-1H-indol-4-yl phosphate (10) and the resulting mixture

sparged with hydrogen (11) until complete by HPLC. Purified water (20) is added during this process to retain the product in solution. The mixture was heated to about 35°C - 45°C and then filtered through a bed of Celite (27) washing with methanol (24) and purified water (20). The filtrate was evaporated *in vacuo*, azeotroping with ethanol (28) to obtain intermediate grade psilocybin (12).

Stage 6: Synthesis of 3-[2-(dimethylazaniumyl)ethyl]-1H-indol-4-yl hydrogen phosphate (Psilocybin)

[00217] The core purifying/ polymorph determining step is a water crystallization step, followed by a controlled cooling and drying step, to produce high purity crystalline psilocybin, (Polymorph A or Polymorph A'.

[00218] Most preferably stage 6 is as follows:

[00219] The intermediate grade Psilocybin (12) (stage 5) was charged to a vessel with purified water (20) and the mixture heated until the psilocybin (12) dissolved. The resulting bulk solution was then polish filtered into a pre-warmed vessel. The temperature was adjusted to, preferably, about 68°C - 70°C, and a Psilocybin hydrate seed (i.e., Hydrate A) was added to the reaction. The batch was then cooled in a controlled manner to about 0-10°C and stirred, and the solids were collected by filtration and washed with purified water. The isolated solids were then dried *in vacuo* to yield high purity crystalline Psilocybin, Polymorph A or A' as an off white solid.

BRIEF DESCRIPTION OF THE DRAWINGS

[00220] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Fig 1 is a schematic of the reaction taught in JNP;

Fig 2 is a schematic of the **Stage 1** reaction of one aspect of the present invention;

Fig 3 is a schematic of the **Stage 2** reaction of one aspect of the present invention;

Fig 4 is a schematic of the **Stage 3** reaction of one aspect of the present invention;

Fig 5 is a schematic of the **Stage 4** reaction of one aspect of the present invention;

Fig 6 is a schematic of the **Stage 5** reaction of one aspect of the present invention;

Fig 7a is a XRPD diffractogram of Polymorph A (GM764B);

Fig 7b is a XRPD diffractogram of Polymorph A' (JCCA2160F);

Fig 7c is a XRPD diffractogram of Polymorph B; (JCCA2160-F-TM2);

Fig 7d is a XRPD diffractogram of a Hydrate A (JCCA2157E);

Fig 7e is a XRPD diffractogram of an ethanol solvate (JCCA2158D);

Fig 7f is a XRPD diffractogram of product obtained during development of the process (CB646-E) (top) – compared to the diffractograms Polymorph A' (JCCA2160F) (middle) and Polymorph B (JCCA2160-TM2) (bottom);

Fig 8a is a DSC and TGA thermograph of Polymorph A (GM764B);

Fig 8b is a DSC and TGA thermograph of Polymorph A' (JCCA2160F);

Fig 8c is a DSC thermograph of Polymorph B (GM748A);

Fig 8d is a DSC and TGA thermograph of Hydrate A (JCCA2157E)

Fig 8e is a DSC and TGA thermograph of ethanol solvate (JCCA2158D)

Fig 9 is a form phase diagram showing the inter-relationship of form in water based systems;

Fig 10 is a 1H NMR spectrum of Psilocybin; (Read alongside assignment Example 7);

Fig 11 is a ^{13}C NMR spectrum of Psilocybin; (Read alongside assignment Example 7);

Fig 12 is a FT-IR Spectrum of Psilocybin;

Fig 13 is a Mass Spectrum of Psilocybin;

Fig 14 is a numbered structural formula of Psilocybin;

Fig 15 is a temperature solubility curve for Psilocybin in water;

Fig 16a is a micrograph showing crystals obtained by controlled cooling; and

Fig 16b is a micrograph showing crystals obtained by uncontrolled cooling drying.

Fig. 17 is a form phase diagram showing the inter-relationship of forms in different solvent systems.

Fig. 18 is XRPD diffractogram - Pattern C for solids isolated at 25 and 50°C.

Fig. 19 is XRPD diffractograms - Patterns D, E and F for solids isolated at 25 and 50°C.

Fig. 20 is a comparison of the XRPD diffractograms acquired for the solids isolated from the equilibration of amorphous Psilocybin in solvents A to H

Fig. 21 is a comparison of the XRPD diffractograms acquired for the solids isolated from the equilibration of amorphous Psilocybin in solvents I to P

Fig. 22 is a comparison of the XRPD diffractograms acquired for the solids isolated from the equilibration of amorphous Psilocybin in solvents R to Y

DETAILED DESCRIPTION

[00221] In contrast to the prior art, the present invention sought to produce psilocybin at a commercial large scale, in amounts or batches of at least 100g, and more preferably at least 250g, levels 1 log or 2 logs higher than the levels described in JNP, which describes a "large" scale method to producing gram quantities on a 10g scale.

To demonstrate the many significant development steps from JNP, the description below sets out details of experiments and investigations undertaken at each of the process stages, which illustrate the selections made to overcome the numerous technical problems faced, in producing psilocybin (7) to GMP at a large scale (including the various intermediates (2-6)) starting from 4-hydroxyindole (1).

[00222] Reference to a particular numerical value includes at least that particular value, unless the context clearly dictates otherwise. When a range of values is expressed, another embodiment includes from the one particular value and/or to the other particular value. Further, reference to values stated in ranges include each and every value within that range. All ranges are inclusive and combinable.

[00223] When values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment.

[00224] As used herein, the singular forms "a," "an," and "the" include the plural.

[00225] The term "about" when used in reference to numerical ranges, cut-offs, or specific values is used to indicate that the recited values may vary by up to as much as 10% from the listed value. As many of the numerical values used herein are experimentally determined, it should be understood by those skilled in the art that such determinations can, and often times will, vary among different experiments. The values used herein should not be considered unduly limiting by virtue of this inherent variation. Thus, the term "about" is used to encompass variations of ± 10% or less, variations of ± 5% or less, variations of ± 1% or less, variations of ± 0.5% or less, or variations of ± 0.1% or less from the specified value.

[00226] As used herein, "treating" and like terms refer to reducing the severity and/or frequency of symptoms, eliminating symptoms and/or the underlying cause of said symptoms, reducing the frequency or likelihood of symptoms and/or their underlying cause,

delaying, preventing and/or slowing the progression of diseases and/or disorders, such as cancers or benign proliferative disorders, and improving or remediating damage caused, directly or indirectly, by the diseases and/or disorders such as cancers or benign proliferative disorders.

[00227] The following abbreviations have been used herein:

DSC - Differential Scanning Calorimetry

RT - room temperature

TBME - methyl tert-butyl ether

TGA - Thermogravimetric Analysis

THF - tetrahydrofuran

wrt - with respect to

XRPD - X-Ray Powder Diffraction

EXAMPLE 1

STAGE 6 CRYSTALISATION PROCESS AND RESULTING POLYMORPHS

Experimental to produce Form A':

[00228] 1.0g of crude Psilocybin was charged to a 25 mL flask. Water (12.8 mL/16 volumes based on activity of input material) was added. The mixture was agitated and heated to 80°C. A dark brown solution with visible undissolved solids was obtained. The mixture was polish filtered through a warmed 0.45µm filter into a hot 25mL flask. The undissolved solids were removed to give a dark brown solution. The solution was re-equilibrated at 75°C and then cooled slowly (10°C/hour) to ambient temperature. The resulting pale brown solution was equilibrated at ambient temperature for 16 hours. The suspension was cooled to 5°C prior to isolation of the solid by vacuum filtration. The filter cake was washed with water (0.8mL/1 volume) and dried *in vacuo* at 50°C for 16 hours. Yield of 75%, chemical purity 99%, NMR assay >98%.

[00229] The procedure above was repeated with 14 volumes (11.2mL) of water. Yield of 69%, chemical purity 99%, NMR assay >98%.

[00230] In both cases, dissolution of crude Psilocybin was achieved at ca. 75°C. On gradual cooling, precipitation was observed at ca. 60°C.

[00231] In both cases, psilocybin Polymorph A' was produced, confirmed by XRPD (diffractogram consistent with Fig. 7b) and DSC (thermogram consistent with Fig. 8b).

Experimental to produce Form A:

[00232] 94g of crude Psilocybin obtained from the Stage 5 process (about 93% pure by HPLC with about 4% pyrophosphate impurity) was subject to an aqueous re-crystallisation as set out below:

[00233] The protocol used sufficient water (12 volumes), a rapid agitation rate (450 rpm) and a controlled cooling profile (10°C /hr).

[00234] Psilocybin (94.0g) (CB650E) was charged to a 2L flask. Water (902ml, 12 volumes based upon activity of input material) was added. The mixture was agitated and heated to about 78°C. A dark brown solution with visible undissolved solids was obtained. The mixture was polish filtered through a 1.2µm in-line filter into a hot 5L flask fitted with an overhead stirrer (450 rpm). The undissolved solids were removed to give a clarified dark brown solution. The solution was re-equilibrated at about 75°C for 15 minutes and then cooled slowly (10°C/hour) to ambient temperature. The solution was seeded with Psilocybin Hydrate A (GM758A-XRPD diffractogram consistent with Fig. 7d) following maturation in water) at 68°C - 70°C. The resulting pale brown suspension was equilibrated at ambient temperature for about 16 hours. The suspension was cooled to 5°C for one hour prior to isolation of the solid by vacuum filtration. The filter cake was washed with water (282mL, 3 volumes) and dried *in vacuo* at about 50°C for 30 hours.

[00235] The process was completed successfully with a yield of 75% achieved. Chemical purity of the solid was confirmed as 99.3%. Analysis of the solid by XRPD post drying for 30 hours showed Polymorph A (Fig 7a). A characteristic perturbation was observed at *ca* ~17 °2θ, such as 17.5°2θ, and was pronounced in the bulk material.

Solid state characterisation of Polymorph A and Polymorph A'

[00236] The DSC and TGA thermograms (Fig 8a) obtained for Polymorph A were comparable to the DSC and TGA thermograms obtained for Polymorph A' (Fig 8b). The TGA thermograms (Fig 8a) obtained for Polymorph A and Polymorph A' show no weight loss prior to decomposition. This suggested that the difference between the XRPDs obtained for Polymorph A (Fig 7a; perturbation present at ca. ~17 °2θ) and for Polymorph A' which was obtained at small scale (Fig 7b; perturbation not present) was not due to excess hydration.

[00237] Microscopy of the solid (Fig 16a) shows rod shaped crystals with good uniformity with a size range of between 50 and 200 micron.

[00238] The XRPD diffractogram obtained for Polymorph A' does not demonstrate a perturbation at *ca.* ~17°2θ to the same extent as Polymorph A. The perturbation in the XRPD diffractogram at ca. ~17 °2θ is more pronounced for psilocybin produced at large scale (compared to that obtained at small scale) and was unexpected. Applicant has

demonstrated that the Hydrate A is the only polymorphic form that exists across a range of temperatures with no diffraction peak in the 17 °2 theta region (see Fig 7d). This strongly suggests a collapse of Hydrate A upon dehydration to yield Polymorph A or A' that varies with scale and that Polymorph A is the true form with Polymorph A' formed at a small scale being atypical.

[00239] To test the robustness of this theory and to demonstrate a return to Polymorph A, a small portion of the bulk was re-dried following another soak in water (to reproduce Hydrate A). A small sample (250mg – psilocybin Polymorph A) was equilibrated in water (10 vols) for one hour. The suspension was filtered and analysis of the damp solid confirmed that Hydrate A had been generated (Fig 7d), no perturbation at 17° 2 theta. The material was dried *in vacuo* for 16 hours and the solid reassessed by XRPD. Polymorph A' material was confirmed by XRPD (Figure 7b) with the reduction in the XRPD perturbation noted. Additional drying of the original bulk solid and ageing at ambient temperature did not change the XRPD diffractogram of the solid. The two solid versions obtained, the XRPD diffractograms for Polymorph A and Polymorph A' are virtually identical other than the ~17.5° 2 theta peak. The thermal properties are also identical. The distinction between the XRPD diffractograms for Polymorph A versus Polymorph A' is subtle and both Polymorphs kinetically convert to the hydrated state very rapidly.

[00240] Additional experiments were performed to ascertain if the differences in the XRPD diffractograms for Polymorph A and Polymorph A' were due to the larger scale crystallisation process delivering solids of a larger particle size that subsequently did not dry as effectively and caused the change, or whether the habit and size difference of the crystalline solid was the cause. Psilocybin Polymorph A (polymorphic form confirmed prior to experiment) was ground via a mortar and pestle and assessed by XRPD. No change in polymorph was observed. Another portion (51 mg) was charged with water (<1 mL) and assessed damp to confirm that the hydrate was formed. Both lots were dried *in vacuo* at 50°C for ca. 18 hours and re-assessed by XRPD. The ground sample remained as Polymorph A. The hydrated sample after dehydration was shown to be Polymorph A' (i.e., no reflection at ~17.5°2θ). This suggested that size/habit alone were not the sole reason for the original reflection peaks.

[00241] TGA assessment revealed that the input lot demonstrated a small mass loss (0.139% weight) by ca. 70°C. The particle size reduced and subsequently dried solid demonstrated a greater mass loss of 0.343wt% by *ca.* 75°C whereas the hydrated and dried solid demonstrated the smallest mass loss of 0.069wt% by *ca.* 80°C. The particle size reduced and subsequently dried solid was held at 80°C for 10 minutes (past the point of mass loss by TGA) but assessment by XRPD revealed no change from the input, meaning

that low levels of hydration and partial swelling of the crystalline lattice were not the cause of the variation

[00242] It is possible to generate Polymorph A' via the hydration of Polymorph A and subsequent drying of the isolated solid on a small scale.

[00243] Psilocybin Polymorph A and Polymorph A', *ca.* 60mg each, were charged with water, 0.2ml, to deliver Hydrate A from both lots. Half of each Hydrate A was dried *in vacuo* at 25°C for *ca.* 17¼ hours and the remainder of each Hydrate A was dried at ambient temperature under a stream of N_2 for *ca.* 17¼ hours. The solids were isolated following drying and assessed by XRPD. XRPD assessment of the solids isolated from the Polymorph A input confirmed that Hydrate A was successfully generated and that the solids dried to give Polymorph A' from both drying methods. XRPD assessment of the solids isolated from the Polymorph A' input confirmed that Hydrate A was successfully generated and that the solids dried to remain as Polymorph A' from both drying methods.

[00244] On the small scale investigated, Polymorph A and Polymorph A' will dry to give Polymorph A' via conversion to Hydrate A.

[00245] Psilocybin Polymoprh A (100mg) was particle size reduced via mortar and pestle grinding. The ground lot was subject to two different drying regimes in order to assess whether reducing the particle size affected the dehydration of the sample. The first sample was held at 80°C for 10 minutes and the second sample held at 110°C for 10 minutes. Both solids were assessed by XRPD which revealed that Polymorph A was retained. It was considered whether the ground lot in the prior isothermal stresses were not held at 110°C for long enough to impact the form and so a portion of the ground lot was dried *in vacuo* at 110°C for *ca.* 24 hours. Assessment by XRPD revealed a subtle change in form with the Polymorph A reflections at *ca.* 17 still present but at a slightly reduced intensity.

[00246] It was concluded that Polymorph A would not readily convert to Polymorph A' via particle size reduction and/or drying at high temperature.

Methodology

[00247] Stability assessments of Psilocybin, not containing the pyrophosphate impurity, indicated that at temperatures in excess of 80°C, the level of the Stage 3 intermediate impurity (psilocin) generated by hydrolysis of Psilocybin is of concern. For example, when a 83mg/mL Psilocybin aqueous solution is heated to 90°C and analysed by HPLC at 1, 2 and 4 hours, the level of the Stage 3 impurity were determined as 0.28, 1.82 and 7.79 area% respectively. In comparison, when 50 mg of psilocybin is dissolved in water (1.2 - 1.8 ml; volume sufficient to maintain a solution) and heated to 70, 75 and 80°C for 4 hours, the level

of the Stage 3 impurity was determined, by HPLC, as 0.53, 0.74 and 2.30 area% respectively. The recrystallization heats the crude Psilocybin to between 75°C and 80°C in order to achieve dissolution, and polish filtration. The immediate cooling of the solution limits the level of Psilocybin hydrolysis by reducing the residency time of the material to excessive temperature.

[00248] Further trial re-crystallisation's of Psilocybin were conducted introducing the following variations:

[00249] Varying the volumes of water used;

[00250] Varying agitation;

[00251] Having a controlled cooling profile;

[00252] Having a rapid (uncontrolled) cooling profile.

[00253] Using smaller volumes of water (as little as 12 volumes) did not hinder the re-crystallisation process and dissolution of Psilocybin was achieved at a temperature to enable a polish filtration step. Different cooling rates were shown to result in different crystal size distributions; a slow controlled cool at *ca* 10°C per hour produced a relatively larger and more even average crystal size (Fig 16a) whereas a rapid cooling profile delivered smaller crystals (Fig 16b). A controlled cooling profile is preferred and this was reflected in an improved purity for the controlled cool.

[00254] Using the process resulted in Psilocybin of 99.3% chemical purity, with a 75% yield. Thermal characteristics of the solid corresponded with those desired. Differences in the XRPD diffractogram of the dry solid have suggested that the drying profile may be important in determining how the Hydrate A collapses to give the preferred solid form. Polymorph A has been demonstrated to be stable under accelerated stability testing conditions for 12 months.

[00255] Experimental

[00256] Stage 5 was charged to vessel under N_2 followed by water (approx. 12-15vol based on active Stage 5). The mixture was heated to about 80°C to achieve dissolution and polish filtered through a 1.2µm in-line filter into a clean new flask heated to 80°C. The agitation rate was set to a high level (450rpm) and the solution equilibrated at 70-75°C. The solution was cooled to ambient temperature, at approx. 10°C/hour, seeding with Psilocybin Hydrate A (0.001 x stage 5 charge) at 68-70°C. The suspension was held at ambient temperature overnight then cooled to approx. 5°C and held for 1 hour. The suspension was filtered, washing with water (2-3 volumes based upon active charge of Stage 5). The pure Psilocybin was dried in vacuo at 50°C. Crystalline material psilocybin (Polymorph A or Polymorph A'

dependent on scale) was obtained, for example using 94 g input of psilocybin yielded Polymorph A and using 1 g input of psilocybin yielded Polymorph A'. Typically, batch sizes of greater than 5 g deliver Polymorph A, while batch sizes less than 5 g deliver Polymorph A'.

[00257] The differences from JNP and the benefits can be summarised as follows:

i) This additional crystallisation step gives rise to a defined crystalline form – Polymorph A (or A').

ii) Heating to about 80°C, for a short period, has the advantage that solubility is maximised (and hydrolysis avoided), which ensures good yields.

iii) At about 70-80°C polish filtration can be used to remove insoluble impurities. This is best achieved using an in-line filter – typically about 1.2µm. This ensures good chemical purity.

iv) Using a high agitation rate (typically about 450 rpm) ensures speedy dissolution allowing the time at which the solution is kept at 80°C to be minimised, thus avoiding increased levels of the Stage 3 intermediate impurity formed by hydrolysis of Psilocybin.

v) The provision of controlled cooling, typically cooling at about 10°C per hour, delivers a more uniform crystal size and maintains form as crystalline Hydrate A.

vi) Seeding the solution at about 70°C with Psilocybin Hydrate A facilitates crystallisation as the Hydrate A.

vii) The crystals are washed in water and dried at about 50°C to maximise purity and deliver Polymorph A or A' depending on scale.

EXAMPLES 2 TO 6

STAGES 1 TO 5 PRODUCTION OF PSILOCYBIN

[00258] The following Examples illustrate significant developments from the process described in JNP, and illustrated in Fig 1, as described herein before.

EXAMPLE 2

STAGE 1 (Fig 2)

[00259] The stage 1 conditions in JNP used 1.1 eq Ac_2O, and 1.2 eq Pyridine in the solvent DCM. The reaction was found to be complete (99% product, 0% SM) after stirring overnight. The reaction mixture was washed with water and concentrated *in vacuo* giving a brown oil. In

the literature, the oil was taken up in EtOAc and concentrated by evaporation, giving precipitation of solids at low volume.

Investigation

[00260] However, in the Applicants hands precipitation of solids from EtOAc was not observed. Precipitation of solids was encouraged by trituration with heptane, however this would not form a scalable process. The solids were collected giving high purity stage 1 product (75% yield, >95% pure by NMR).

[00261] While the reaction worked well, the isolation procedure required further development in order that an easy to handle solid could be obtained. It was hoped that isolation of the solids by filtration would then also offer a means of purification.

[00262] The reaction was first trialled in EtOAc to see if precipitation of solids could be encouraged allowing isolation directly from the reaction mixture. However, the reaction profile in EtOAc was found to be less favourable than in DCM and therefore the reaction was abandoned.

[00263] Applicant washed out the pyridine from the DCM reaction mixture, as it was believed this may be preventing re-crystallisation of the product. The reaction was repeated (completion of 0.4% SM, 98.7% product by HPLC) and the reaction mixture washed with 20% citric acid to achieve pH 2/3, removing pyridine and then saturated $NaHCO_3$ (aq) to avoid low pH in the evaporation steps. The organics were dried and a solvent swap to heptane was carried out giving precipitation of stage 1. The solids were collected by filtration yielding pure stage 1 after drying *in vacuo* (87% yield, >95% purity by NMR).

[00264] Stability trials were carried out that confirmed the reaction mixture was stable overnight when stirred with 20% citric acid, and also saturated $NaHCO_3$. The product was found to be stable when oven dried at 40°C and 60°C.

Scale up

[00265] The stage 1 reaction was successfully scaled up processing >100g of 4-hydroxyindole. The reaction progressed as expected and was worked up to give stage 1 product (93% yield, ~98% NMR purity).

GMP raw material synthesis

[00266] A large scale stage 1 reaction was carried out to supply GMP starting material (processing >500g of 4-hydroxyindole). The reaction proceeded as expected giving consumption of SM by HPLC (99.2% product, <0.1% SM). The reaction was worked up using the established procedure to give stage 1 product after drying (94% yield, 99.1% by HPLC, 99% NMR assay).

[00267] It was also noted in development that the stage 1 procedure was effective in removing minor impurities present in some batches of 4-hydroxyindole. The low level impurities present in 4-hydroxyindole were completely removed after the stage 1 reaction providing clean material in high yield (89%) and purity (99% by HPLC, 99% by NMR assay).

[00268] Experimental

[00269] 4-hydroxyindole (1eq. limiting reagent) was charged to a vessel under N_2 followed by DCM (dichloromethane; 6 vol based on 4-hydroxyindole charge). The reaction was cooled to 0-5°C and pyridine added (1.2 eq) dropwise at 0-5°C. Acetic anhydride (1.1 eq) was added dropwise at 0-5°C and the reaction warmed to 20-25°C for 1 – 1.5 hrs and stirred at 20-25°C for a further 3 hours. The reaction was sampled and analysed for completion. The reaction was then washed three times with 20% aqueous citric acid solution (3 x 3 vol based on 4-hydroxyindole charge) and once with sat. $NaHCO_3$ (3 vol based on 4-hydroxyindole charge). The DCM solution was dried over $MgSO_4$ and filtered and the DCM layer concentrated to half volume by distillation. Heptane (6 vol based on 4-hydroxyindole charge) was added and further DCM was removed by distillation until full precipitation of the Stage 1 had occurred. The reaction was cooled to 15-25°C and the solids collected by filtration, washing with heptane (1 vol based on 4-hydroxyindole charge) dried under vacuum overnight at 60°C.

[00270] The differences from JNP and the benefits can be summarised as follows:

i) Applicant washed out the pyridine using citric acid at a pH of about 2 - 3. This facilitated improved isolation and crystallisation. In practice the DCM phase is separated and the aqueous citric acid phase discarded.

ii) An additional wash in sodium bicarbonate resulted in further improvement.

iii) A solvent swap to heptane improved solid precipitation maximising yield and resulting in reproducible high purity Stage 1

EXAMPLE 3

STAGE 2 (Fig 3)

Step i - Acid chloride formation

[00271] Formation of reactive Intermediate 2A by reaction of the stage 1 product with oxalyl chloride (1.5 eq) was initially trialled in a mixture of TBME and THF (6 vol/1 vol) to determine if it was a viable alternative to volatile and highly flammable Et_2O as used in the literature.

The reaction gave completion after ~18 hours with a similar solubility profile to Et_2O (stage 1 in solution, precipitation of stage 2A).

[00272] As the acid chloride intermediate is prone to hydrolysis, leading to variable analytical results a more robust sample make up and analysis was developed in which the reaction was quenched into THF/NMe_2 (to give stage 2) and then analysed by HPLC.

[00273] The ratio of TBME and THF was optimised to give the highest purity and yield of intermediate with a preferred ratio of TBME:THF of 6:1 chosen for scale up. Other ratios of TBME:THF may be used.

[00274] A scale up reaction was carried out using the preferred solvent mixture (1 vol THF, 6 vol TBME) but with the oxalyl chloride addition carried out at 30-35°C. The resulting solution was then heated at 40°C for 2.5 hrs giving a completion with ~1% stage 1 product remaining. Carrying out the addition hot to maintain a solution ensures a high reaction rate and gave an improved level of completion with a much shorter reaction time (2.5 hrs vs overnight). The product was still seen to precipitate at temperature after ~15 min and no detrimental effect on the reaction profile was observed by HPLC.

[00275] As the stability of Intermediate 2A was not known, telescoping of material through to stage 2 was attempted rather than isolating the intermediate and risking degradation (hydrolysis). The reaction profile was complex with multiple components present at a low level. TBME was added and the precipitate collected. However this was also found to be a complex mixture by HPLC/NMR.

[00276] Due to the poor reaction profile it was deemed necessary to isolate Intermediate 2A to allow for purification away from excess oxalyl chloride. The reaction was repeated and the yellow precipitate collected by filtration and washed with TBME to remove the excess oxalyl chloride (80% yield). NMR analysis confirmed the product to be of sufficient purity (~95% by NMR). However despite storing under nitrogen, some decomposition was noted over the following days giving partial hydrolysis including de-protection of the acetate group.

[00277] In order to try and reduce the potential for hydrolysis of the intermediate acid chloride during isolation, further investigations into a telescoped procedure were carried out. It was found that by allowing the reaction mixture to settle the TBME liquors could easily be decanted and then the residual solids washed with further portions of TBME in a similar manner. This allowed purification of Intermediate 2A away from excess oxalyl chloride whilst minimising exposure to moisture.

[00278] It was felt that some reaction yield may be lost due to partial solubility of the intermediate in the THF/TBME mixture. This was confirmed by adding heptane to the decantation liquors which gave precipitation of further solids. To limit this solubility, a heptane

(8 vol) addition was made prior to decantation. Rather than washing the solids with TBME, heptane was also used for the washes (3 x 6 vol) which maximised the yield while maintaining the high purity of the intermediate. This methodology was successfully scaled up and is the preferred process.

Step ii – Reaction with dimethylamine

[00279] The literature (Synthesis, 1999, 6, 935-938; D.E. Nichols) suggested $HNMe_2$ gas was effective for this transformation. However to simplify large scale processing this was substituted for either solid $HNMe_2$.HCl, with an additional excess of base, or a solution of $HNMe_2$ in THF. JNP uses $HNMe_2$ in the presence of excess base (pyridine).

[00280] Initially isolated Intermediate 2A was used to optimise the reaction with dimethylamine *via* a series of trial reactions (see Table 11).

[00281] Table 11 - Stage 2 reaction optimisation trials

#	Dimethylamine Source	Base	Solvent	HPLC completion	Isolated solids
1	2M $HNMe_2$/THF 1.2 eq	Pyridine 1.3 eq	THF/TBME	80% product,	64% yield, ~70% pure by NMR,
2	$HNMe_2$.HCl 1.5eq	K_2CO_3	THF/H_2O	70% product	40% yield ~98% by NMR.
3	2M $HNMe_2$/THF 1.33 eq	Pyridine 3.6 eq	Et_2O	81% product	63% yield, ~90% by NMR.
4	2M $HNMe_2$/THF 2.9 eq	N/A	Et_2O	93% product	72% yield, ~98% by NMR.

[00282] The literature supplied conditions with pyridine (#1) were trialled along with a similar reaction in Et_2O (#3), a biphasic reaction using Me_2NH.HCl and aqueous K_2CO_3 (#2) and a reaction with excess 2M Me_2NH in THF (#4). The major component by HPLC was the desired product in all cases with the conditions using aqueous base and excess Me_2NH being generally much cleaner than those with pyridine. While significant hydrolysis product was seen in all cases this was thought to be the result of unreacted Intermediate 2A which was quenched during sample makeup for HPLC analysis. The reactions were worked up by addition of water and then the organic solvent was removed *in vacuo* giving precipitation of solids.

[00283] The reaction with excess amine showed a much improved impurity profile which translated into a higher yield (72% vs 63%) and purity (98% vs 90%). This approach limited the water content of the reaction and therefore minimised the opportunity for hydrolysis to occur. Purification was also expected to be more facile due to the absence of pyridine in the

isolated solids. For these reasons the conditions with excess $HNMe_2$ as base were chosen for scale up.

[00284] The reaction in Et_2O gave a clean (#4) profile. However, to facilitate large scale processing it proved advantageous to switch to a less volatile solvent, such as TBME. This would facilitate telescoping the acid chloride into this reaction. For these reasons it was chosen to carry out the reaction in TBME using excess 2M Me_2NH in THF.

[00285] It was believed that the addition of water would aid the workup by solubilising the $HNMe_2$.HCl salts that were present and resulted in a very thick mixture and slow filtrations. This was trialled. However, when water was added to the reactions in TBME and THF a poor recovery was obtained with analysis of the liquors showing additional impurities and extensive acetate de-protection (phenol product). Further development of the purification was therefore required.

Purification development

[00286] It was desirable to develop a purification strategy that would remove the hydrolysis product and other impurities observed. It was also desirable to include water in the crystallisation to reduce the salt content of the isolated material (assumed $HNMe_2$.HCl). To this end a series of 15 solvents and solvent mixtures were screened (100 mg scale, 10 vol solvent, heat cycling to 60°C).

[00287] Table 12 - Stage 2 purification trials

Solvent	Observations	Recovery	HPLC Purity (hydrolysis imp of Intermediate 2A)
TBME	Slurry	47 mg*	96.4% (2.8%)
DCM	Slurry	60 mg	99.5% (0.5%)
Toluene	Slurry	30 mg*	95.8% (3.4%)
EtOAc	Slurry	66 mg*	97.8% (1.6%)
iPrOAc	Slurry	40 mg*	97.3% (2.0%)
IPA	Slurry	65 mg	99.4% (0.4%)
EtOH/H_2O, 1:1	Slurry	62 mg	99.4% (0.5%)
MeCN/H_2O, 1:1	Partial solution at RT Solution at 60°C	30 mg	99.0% (0.7%)
Acetone/H_2O, 1:1	Slurry at RT Solution at 60°C	51 mg	99.4% (0.5%)
THF/H_2O, 1:1	Partial solution at RT Solution at 60°C	No precipitate	n/a
Heptane	Slurry	34 mg*	95.0% (3.6%)
MIBK	Slurry	65 mg	97.9% (1.4%)
MEK	Slurry	60 mg	99.6% (0.3%)
Cyclohexane	Slurry	41 mg*	94.4% (4%)
Xylenes	Slurry	56 mg*	96.1% (3%)

[00288] * Recovery not representative due to thick suspensions and solids adhering to the glass vial.

[00289] From the solvents screened acetone/water gave a re-crystallisation with little observed solubility at room temperature. Since this was an aqueous system it had the advantage of helping to purge $Me_2NH.HCl$ from the solids.

[00290] The acetone/water re-crystallisation was scaled up. A solution was obtained at temperature (5 vol acetone, 1 vol water) prior to addition of further water (4 vol) and the mixture cooled to RT giving crystallisation (62% recovery, >99% HPLC purity). This process was subsequently scaled up further with addition of more water to aid the recovery (in total 5 vol acetone, 10 vol water, 78% recovery).

[00291] The process was scaled up further (30 g) and the crude solids taken through the re-crystallisation procedure. While product purity was high, there was a drop in yield (56% yield, 99% by NMR assay, 99.4% by HPLC).

[00292] In order to improve the recovery, the amount of water added was further increased from 10 vol to 15 vol. This maintained product purity at greater than 99% and gave a higher recovery on a small scale (90% recovery, 56-70% previously observed). However, scale up of this amended procedure again gave a low recovery (58% yield). Therefore, due to the issues encountered when scaling up the re-crystallisation, an alternative means of purification was sought based on the original slurry screen that was carried out (Table 12 above).

[00293] Redevelopment of the purification strategy took place using material isolated from a large scale, stage 2, reaction. The reaction progressed as expected to give crude product after oven drying (70% by NMR assay, 79% active yield). To remove the significant salt component (presumed to be $HNMe_2.HCl$) a portion was water slurried at RT. After drying this gave 75% recovery (95% by NMR assay) showing this to be an effective means of reducing the salt content. HPLC purity remained unchanged at ~93%. A method was then sought to increase the chemical purity of the solids.

[00294] From the initial screen both EtOH:H_2O, IPA and acetone:H_2O appeared to give high purity product with a good recovery and so these solvent systems were chosen for further investigation. Input purity was 92.7% with the main impurities at levels of 1.4%, 1.0% and 0.8%.

[00295] Table 13 - Further purification development (slurry at 40°C 1 hr, filtration at RT, 1 vol wash)

Solvent system	Recovery	HPLC purity (main impurity levels)

		Product	Imp 1	Imp 2	Imp 3
Acetone/Water 6/16 vol	67%	98.8%	0.7%	0.2%	0.1%
1:1 EtOH:H_2O 10 vol	79%	98.0%	0.9%	0.5%	0.2%
IPA 10 vol	90%	97.5%	0.8%	0.8%	0.3%
IPA 5 vol	92%	97.2%	1.0%	0.7%	0.3%
4.5:2.5 IPA:H_2O * 7 vol	79%	98.7%	0.6%	0.4%	0.1%
6:1 IPA:H_2O 7 vol	82% **	98.0%	0.9%	0.6%	0.2%

[00296] * The reaction was initially run in 1:1 IPA:H_2O at 5 vol. However it became too thick to stir and so a further 2 vol of IPA was added.

[00297] ** The mixture was thick and the solids present were very fine making filtration difficult with some solids beating the filter.

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[00298] The results of these trials suggested that good recoveries were possible from these systems, particularly those based on IPA. EtOH:H_2O gave a marginally better impurity profile than IPA alone; however the recovery was not as good (79 vs 90%). The impurity profile with IPA was greatly enhanced by the presence of water (98.7% vs ~97.5%) however this led to a lower recovery (79 vs 90%). This suggested a certain level of water solubility for the compound. A final trial in IPA and EtOH:Water was conducted with reduced water volumes to see if a balance could be found that provided high purity and a recovery of ~90%. While this system improved the yield the filtration was slow and therefore further solvent mixtures were also evaluated.

[00299] Table 14 - Examination of further solvent mixtures (500 mg scale, slurry at 40°C 1 hr, filtration at RT, 1 vol wash)

Solvent system	Recovery	HPLC purity (impurity levels)			
		Product	Imp 1	Imp 2	Imp 3
IPA 10 vol	460 mg (92%)	97.6%	1.1%	0.7%	0.3%
1:3 EtOH:H2O 5 vol	451 mg (90%)	96.2%	1.4%	0.8%	0.5%
1:2 EtOH:H2O 5 vol	440 mg (88%)	97.5%	1.3%	0.7%	0.4%
1:1 EtOH:H2O 5 vol	375 mg (75%)	97.9%	1.0%	0.7%	0.3%
2:1 EtOH:H2O	354 mg (71%)	97.6%	1.0%	0.6%	0.4%

5 vol					
3:1 EtOH:H2O 5 vol	369 mg (74%)	97.9%	0.9%	0.5%	0.3%

[00300] The 5 vol EtOH/Water slurries were very thick and not easily handled. Since the purity of the solids was comparable from all the trials (slight variations are likely due to the quality of the filtration and wash), the 100% IPA conditions were scaled up as they offered a high recovery and the resulting suspension was easily handled.

[00301] An initial scale up of the preferred slurry gave (92% recovery) with HPLC purity of 96.4% (Impurity levels of 1.2%, 0.7%, 0.4%). Liquors analysis showed them to be enriched in all of the main impurities - 72% (8.6%, 3.8%, 3.4%) by HPLC. This was deemed a suitable purification method offering a high recovery and the material was use tested in the following stage to ensure tracking and removal of the impurities was achieved downstream (>99% at stage 3, no single impurity >0.5%).

[00302] The slurry proved scalable when remaining crude stage 2 material (70% assay) was water slurried to remove inorganics, and then slurried in IPA to give material of improved purity (97% by NMR assay, 76% yield for stage 2, 96.8% by HPLC, impurities at 1.1%, 0.8%, 0.4%).

GMP raw material synthesis

[00303] A large scale, stage 2 reaction was carried out to supply the GMP campaign. The reaction progressed as expected to provide crude product that was water slurried and filtered to provide stage 2 that was 93% pure by HPLC. This was further slurried in 8 vol IPA and filtered to give stage 2 product (93.7% by HPLC, 92% assay, 66% active yield). Since the purity obtained was lower than that observed during the development campaign, a use test was conducted which confirmed that high purity stage 3 obtained was suitable for onward processing (GMP raw material).

[00304] A second batch was carried out under identical conditions to give crude product which after a water slurry was 90% pure by HPLC. This material was subsequently water slurried and purified by IPA slurry to give 384 g of stage 2 product (93.0% by HPLC, 91% NMR assay, 60% active yield).

[00305] A third batch was carried out to resupply the GMP synthesis. The crude product was successfully purified by a water then IPA slurry to deliver stage 2 (79% yield) with an increased purity when compared to previous batches (97.3% by HPLC, 96% NMR assay).

[00306] Experimental

[00307] Stage 1 (1eq. limiting reagent) was charged to a vessel under N_2 followed by THF (1 vol wrt stage 1 charge) and TBME (6 vol wrt stage 1 charge). Oxalyl chloride was then added dropwise to the vessel (1.5 eq,) allowing the exotherm to initially raise the temperature to 35-40°C and then applying cooling as required to maintain 35-40°C. Immediately following the addition the reaction was heated to 40°C and stirred for 2-6 hours. The reaction was sampled and analysed for completion, then cooled to RT and heptane (8 vol wrt stage 1 charge) added giving precipitation of further solids. The reaction was stirred for 10 min and then the solids were allowed to settle. The majority of the solvent was decanted from the solid which was then washed twice with heptane (2 x 6 vol wrt stage 1 charge), decanting in a similar manner after each wash. The solids were then sampled and analysed. TBME was charged to the vessel (4 vol wrt stage 1 charge) to give a yellow slurry which was cooled to -20°C using a dry ice/acetone bath. A 2M solution of Me_2NH in THF (2 eq,) was added dropwise to the vessel over ~15 min maintaining the temperature at -20°C to -10°C. The reaction mixture was allowed to warm slowly to RT and stirred overnight. Further Me_2NH can be added at this point if required. The reaction was sampled and analysed for completion. The reaction was filtered, washing with heptane (2 x 2 vol wrt stage 1 charge) and the isolated solids dried at 60°C under vacuum. The crude stage 2 was slurried in water (8 vol wrt stage 1 charge) for 2-18 hours and then filtered, washing with water (2 vol wrt stage 1 charge). The solids were dried at 60°C under vacuum to obtain crude stage 2 with <2% w/w water (determined by Karl Fischer titration (KF)). The crude stage 2 was slurried in IPA (10 vol) for 2-18 hrs and then filtered, washed with IPA (1 vol wrt mass of crude Stage 2) and oven dried under vacuum at 60°C.

[00308] The differences from JNP and the benefits can be summarised as follows:

Step 1

i) Firstly, the use of a THF/TBME solvent system in place of diethyl ether was less volatile and flammable.

ii) Secondly, the addition of oxalyl chloride was conducted at an elevated temperature, heated to 40°C, giving rise to improved solubility and preventing entrapment of stage 1 product in the precipitate. It also provided a high reaction rate, improved levels of completion and shorter reaction times.

iii) Thirdly, the Intermediate 2A was isolated to allow for purification away from excess oxalyl chloride.

iv) Fourthly, heptane was added to help precipitate Intermediate 2A.

Step 2

v) By ensuring the amine was used in excess much improved purity and yields were obtained, due to minimal water being present, and hence reduced hydrolysis.

vi) Finally, the use of water and IPA slurries provided good purity of the Stage 2 product.

EXAMPLE 4

Stage 3

[00309] An initial stage 3 trial reaction was carried out using purified stage 2 material (>99% by HPLC) and the supplied reaction conditions. The stage 2 input material was found to be largely insoluble in THF, and so rather than adding a solution of stage 2 to $LiAlH_4$ the reverse addition was carried out. 4 eq of $LiAlH_4$ was used as a 1M solution in THF with the addition made at 20-25°C, over ~2 hrs. At this point 10% product was observed with several intermediate species present. The reaction was heated at reflux for ~7 hrs to give 93% product conversion (by HPLC). The reaction was worked up to give crude stage 3 product (~90% by HPLC, ~90% by NMR, ~87% corrected yield).

[00310] A trial reaction was carried out in which the $LiAlH_4$ charge employed was successfully reduced (3 eq vs 4 eq). It was hoped this would benefit the work up by reducing the quantity of Li and Al salts generated. After prolonged heating at reflux (10-18 hrs) the reaction intermediate was largely consumed (2-3% remaining) with ~95% product by HPLC.

Workup development

[00311] Although the first trial reaction was successfully worked up using Rochelles salt, the volumes employed were very high (~100 vol) and this procedure would not form a viable process for scale up. A variety of alternative workup procedures were examined in order to try and reduce the volumes required and aid removal of Li/Al salts.

[00312] A reduced volume quench was trialled with EtOAc and then Rochelles salt. Grey solids were present as a thick paste which settled to the bottom of the flask. While filtration failed, the liquors could be decanted and the solids re-slurried in THF/EtOAc to extract the product. An aqueous workup was then carried out and the product isolated by concentration. This yielded product of good purity (90-95% by NMR) in good yield (94% uncorrected for purity). However the process was not readily amenable to scale up.

[00313] A reaction was quenched by addition of EtOAc and then sat. Na_2SO_4 in the presence of anhydrous Na_2SO_4 to act as a binding agent. The reaction gave granular solids which could be readily filtered. An aqueous workup was then carried out and the product isolated by concentration. A good yield was obtained (~94% uncorrected for purity) but the

product contained higher levels of the main impurity by NMR (10% vs 2-4% usually observed).

[00314] A reaction was quenched with 20% AcOH at 0°C leading to the formation of a gel which could not be filtered. The reaction was abandoned.

[00315] A reaction was quenched with EtOAc and then 20% citric acid to give solids which could be separated by filtration. The liquors were concentrated to obtain the product. While this procedure was slightly lower yielding (~77% uncorrected for purity), the product was of very high purity (>95%).

[00316] A further reaction was quenched by the addition of EtOAc and then water (3 mL per g of $LiAlH_4$ in THF). A gel formed which could not be readily filtered and the reaction was abandoned.

[00317] Finally, a reaction was quenched by the Fieser method. An addition of water (1 mL per g of $LiAlH_4$) was made then 15% NaOH (1 mL per g of $LiAlH_4$) and finally water (3 mL per g of $LiAlH_4$). This gave solids which could be filtered from the reaction mixture. The liquors were then partitioned and concentrated *in vacuo* (87% yield, 90-95% by NMR).

[00318] These Experiments are summarised in Table 15 below:

[00319] Table 15 - Summary of alternative workup conditions

#	Workup procedure	Yield	Purity
3.1	EtOAc quench Rochelles salt (reduced vol.)	2.8 g (94% uncorrected)	90-95% by NMR
3.2	EtOAc quench in presence of Na_2SO_4	2.8 g (94% uncorrected)	~80-85% by NMR, 10% imp.
3.3	20% AcOH quench	Emulsion (reaction to waste)	
3.4	EtOAc quench 20% citric acid	i) 529 mg (71% uncorrected) ii) 2.3 g (77% uncorrected)	>95% purity by NMR
3.5	Water quench	Emulsion (reaction to waste)	
3.6	Water/NaOH quench	i) 615 mg (83% uncorrected) ii) 2.6 g (87% uncorrected)	90-95% by NMR

[00320] Both quenches with citric acid and NaOH gave solids that could be readily filtered from the reaction mixture and required minimal solvent volumes. While the conditions with NaOH were higher yielding (~10%), the product obtained from this procedure was less pure and would likely require further purification before use in the next stage. The lower yield with citric acid was likely due to some precipitation of the product citrate salt. This had a purifying effect with clean product obtained directly after concentration. These conditions were chosen for scale up and it was hoped that further optimisation of the citric acid charge would enable clean product to be isolated in high yield from this process.

[00321] The reaction was repeated with a slightly reduced citric acid charge in order to try and maximise the recovery. This reaction yielded product in 57% yield with a further 20% yield obtained by re-slurry of the filter cake in THF (both samples 97.7% by HPLC).

[00322] The reaction was scaled up. However during the EtOAc quench, where the reaction was previously seen to thicken, the reaction gummed in the flask to form a thick mass which restricted mixing. While the addition of citric acid then led to the usual slurry/gel this did not represent a viable process. This reaction was worked up with the filter cake re-slurried in THF to maximise the recovery giving 76% active yield, 95.0% by HPLC.

[00323] The reaction was repeated in order to develop a better quench and avoid the gum formation seen with EtOAc. A portion was quenched by addition of acetone which led to a readily stirred suspension/emulsion with no sign of thickening. The citric acid treatment was then carried out to give a filterable mixture. This quench was successfully carried out on the remainder of the reaction and worked up to provide crude product in good yield (71% assay, 82% corrected yield, 98.0% by HPLC).

[00324] After the quench the reaction mixture was generally found to be pH 8/9. As part of the workup optimisation process different pHs were investigated. A reaction was split for workup with half receiving a slightly reduced citric acid charge (to obtain pH 11/12 after quench) and the other half taken to pH 7 by addition of further citric acid. The pH 11 reaction was worked up to give material of 85% NMR assay (73% yield) with the pH 7 reaction giving 60% NMR assay (62% yield). It was clear from this result that obtaining the correct pH after quench was critical in order to give a >70% yield. By reducing citric acid charge only slightly (still approx. 2 vol of 20% citric acid) an approx. 8% increase in yield was obtained. With this information in hand the pH of future reactions was monitored during the quench in order to ensure the mixture remained strongly basic.

Purification development

[00325] A purification screen was carried out using 100 mg portions of crude Psilocin product which were slurried in 10 vol of solvent with heat cycling to 60°C. The slurries were

cooled to RT over the weekend and then any solids collected by filtration. Stability to acid and base was also tested with the view to carrying out an acid/ base workup. The results of the screen are presented in Table 16 below:

[00326] Table 16 - Psilocin purification screen. (Input purity and 3 main impurity levels: 90.2%, 3.8%, 0.9%, 0%).

Solvent	Observations	Recovery (approx.)	HPLC Purity (and main impurity levels)
MeOH	Solution at RT	n/a	n/a
EtOH	Solution at 60°C Precipitate at RT	35 mg	97.2%, 0.4%, 1.1%, 0.8%
IPA	Slurry at 60°C	51 mg	97.6%, 0.5%, 0.5%, 1.1%
MeCN	Solution at 60°C Precipitate at RT	46 mg	96.8%, 0.6%, 0.4%, 1.6%
EtOAc	Slurry at 60°C	58 mg	97.1%, 0.9%, 0.2%, 1.3%
^{i}PrOAc	Slurry at 60°C	58 mg	98.2%, 0.8%, 0.2%, 0.4%
Toluene	Slurry at 60°C	70 mg	93.3%, 3.6%, 0.2%, 0.8%
Heptane	Slurry at 60°C	77 mg	91.3%, 3.8%, 0.2%, 0.7%
Acetone	Solution at 60°C Precipitate at RT	30 mg	97.7%, 0.4%, 0.3%, 0.9%
MEK	Solution at 60°C Precipitate at RT	24 mg	97.3%, 0.5%, 0.5%, 1.2%
MIBK	Slurry at 60°C	49 mg	97.4%, 0.6%, 0.2%, 1.3%
THF	Solution at RT	n/a	n/a
TBME	Slurry at 60°C	67 mg	95.5%, 2.0%, 0.1%, 1.5%
DCM	Solution at RT	n/a	n/a
1M HCl	Solution at RT	n/a	83.7%, new imp 8%
1M KOH	Slurry at RT (black)	n/a	89.1%, 5.4%, 0.9%, 2.2%

[00327] The first of the three highlighted impurities corresponded with the most stable reaction intermediate that is observed at ~70%, when the $LiAlH_4$ addition is complete (requiring refluxing to convert to product). The third impurity was not present in the input and appeared to be generated during the slurry procedure. Of the solvents that remained as a slurry, ^{i}PrOAc gave the highest purity. Several re-crystallisations were found with MeCN having the potential to remove impurities during the crystallisation and having a recovery which had the potential to improve during development. Some degradation was observed in both acid and base with the KOH sample rapidly turning black.

[00328] Purification of the crude stage 3 material was scaled up using the two most promising solvents (MeCN and ^{i}PrOAc). The solvent volumes were reduced to a minimum in order to improve the recovery. The results of these trials are presented in Table 17 below.

[00329] Table 17 – Further development of MeCN / ^{i}PrOAc purification

Solvent	Observations	Recovery (%)	HPLC Purity (and main impurity levels)
MeCN (5 vol)	Recryst in 5 vol	512 mg (51%)	97.6%, 0.7%, 0.8%, 0%
^{i}PrOAc (3 vol)	Slurry in 3 vol	706 mg (71%)	95.8%, 1.3%, 0.6%, 0%

[00330] A re-crystallisation was obtained from MeCN in 5 vol and a hot slurry was achieved in ^{i}PrOAc at 3 vol (both at 75°C). The recovery from MeCN was again poor despite reduced volumes, however the product was of very high purity (>>95% by NMR). The recovery from ^{i}PrOAc was better with a large increase in product purity when analysed by NMR (~95%).

[00331] Although HPLC and NMR purity of material from the iPrOAc slurry was high, a low assay value (85% by NMR assay) was observed. In order to improve the assay value of the material, as well as remove colour, (all materials obtained so far were strongly purple, green or brown) purification by silica pad was investigated.

[00332] Crude Psilocin (71% assay, 98.0% by HPLC) was passed through 4 eq of silica eluting with THF. An 80% recovery of an off white solid with slightly improved HPLC purity (98.4%) and assay value (~82% assay) was obtained. This proved to be an effective means of increasing the product assay value and was therefore included as part of the reaction workup.

[00333] A series of ^{i}PrOAc / anti-solvent slurries (Table 18) were then performed using the silica treated input (100 mg per slurry) to try and improve the recovery, whist maintaining chemical purity (input purity 98.4%).

[00334] Table 18 - Results of ^{i}PrOAc / anti-solvent additions.

Solvent system	Recovery	HPLC purity
^{i}PrOAc 5 vol	78%	99.7%
1:1 ^{i}PrOAc:Heptane 5 vol	70%	99.6%
1:1 ^{i}PrOAc:TBME 5 vol	83%	99.7%
1:1 ^{i}PrOAc:Toluene 5 vol	84%	99.4%
^{i}BuOAc 5 vol	80%	99.6%

[00335] Since all purity values were comparable, two solvent systems were chosen for scale up based on the highest recoveries obtained. The two favoured slurries (TBME and Toluene as anti-solvent) were scaled up (1.0 g per slurry) to better assess the recovery.

[00336] Table 19 - Scale up of favoured purification methods

Solvent system	Recovery	HPLC purity
1:1 ^{i}PrOAc:TBME 5 vol	79.9%	99.1%
1:1 ^{i}PrOAc:Toluene 5 vol	79.4%	99.6%

[00337] Both of these options provided material of >99% HPLC purity at ~80% recovery and, combined with a silica pad, appear to provide an effective means of purification for the Psilocin product. Further colour was removed into the liquors during the slurry giving Psilocin as a white solid. All impurities were effectively removed to below 0.5%. The ^{i}PrOAc:TBME slurry was chosen for scale up as this used non-toxic ICH class 3 solvents.

Scale up

[00338] The developed stage 3 conditions were scaled up and the reaction progressed to give a completion of 94.4% product with 2.9% of the reaction intermediate present by HPLC after overnight at reflux (typical of the process). After a silica pad Psilocin was obtained in 83% purity by NMR assay, 66% active yield, 97.0% by HPLC. This material was further purified by slurry in iPrOAc/TBME to give material 100% by NMR assay in 62% yield and 99.7% by HPLC.

[00339] Due to the crude yield from the reaction being lower than expected (66% vs ~75%) the filter cake and silica pad were reinvestigated in order to try and recover additional material. However, this was unsuccessful.

[00340] The lower than expected yield may have been due to decomposition of the product during workup, although previous stress tests had indicated the material to be stable under the conditions used. To investigate this further the reaction was repeated. The crude product was isolated before the silica pad and additional stress test samples taken to confirm degradation of the product was not occurring during workup.

[00341] The reaction progressed as expected to give completion (93.7% product, 2.9% intermediate) and was concentrated yielding crude material (77% NMR assay, 66% active yield). The filter cake was re-slurried in THF/MeOH but no significant Psilocin was isolated. In order to try and displace any product that was coordinated to the aluminium salts, further citric acid was added to take the pH to 4 (from pH 8) and the cake re-slurried in THF, but

again no significant Psilocin was isolated. Mass balance was not obtained from the reaction with the 66% active yield closely matching what was previously obtained. This batch was purified by silica pad and slurry in ^{i}PrOAc/TBME to give a 62% yield of high purity material (99.8% by HPLC).

[00342] Despite the solvent volumes employed being relatively high and a silica pad being required for removal of aluminium and lithium species, the process was still well suited to the required scale.

[00343] The stage 3 reaction was further scaled up to process. The reaction proceeded as expected to give completion after 18 hours (~91% product, ~3% reaction intermediate remaining). Workup by silica pad and slurry gave a 57% yield of high purity Psilocin (>99% by HPLC, 99% NMR assay, 0.35% w/w water Karl Fischer).

[00344] Experimental

[00345] Stage 2 (1eq. limiting reagent) was charged to the vessel followed by THF (5 vol wrt stage 2 charge). The mixture was cooled to 0°C and a 1M THF solution of $LiAlH_4$ (3 eq,) added dropwise over 30-45min maintaining the temperature at 0-20°C. Following the addition, the reaction was stirred for 30 min at 10-20°C and then heated to reflux and stirred for ~16 hrs. The reaction was sampled and analysed for completion, cooled to 0°C and quenched by dropwise addition of acetone (9.3 eq.) at 0-30°C followed by a 20% aq citric acid soln (1.9 vol wrt stage 2 charge) at 0-30°C. The pH of the addition was monitored to ensure that it remains at pH>11 and the addition was stopped early if required. The resulting suspension was stirred for 1 hr and filtered, washing with THF (2 vol wrt stage 2 charge) to remove Li and Al salts. The filter cake was slurried in THF (12.5 vol wrt stage 2 charge) for ~1 hr and filtered, washing with THF (5 vol wrt stage 2 charge) to recover product from the Li and Al salts. The combined organics were dried over $MgSO_4$ and filtered. The filtrate was evaporated *in vacuo* until approximately 10 volumes remained (wrt stage 2 charge) and this solution was applied to a silica pad (3 eq wrt stage 2 charge). The silica pad was eluted with THF and the product fractions were combined and evaporated to dryness *in vacuo*. The crude stage 3 (Psilocin) was slurried in 1:1 iPrOAc:TBME (5 vol wrt mass at step 18) for 2-18 hrs, filtered, washing with TBME (2.5 vol wrt mass at step 18) and dried *in vacuo* at 40°C to isolate pure Psilocin.

[00346] The differences from JNP and the benefits can be summarised as follows:

i) Firstly, Applicant, whilst using THF as a solvent, quenched the reaching using acetone. This lead to a suspension/ emulsion without thickening.

ii) Secondly, Applicant quenched with citric acid maintaining a basic pH, typically about 11. The pH control ensured high yields were obtained.

iii) Thirdly, following purification by silica pad, to remove residual Li/Al salts, eluting with THF, a iPrOAc:TBME slurry provides a highly purified product which was then dried.

EXAMPLE 5

STAGE 4

[00347] Initially the literature conditions were used to process a 2.58 g sample giving ~88% conversion to Intermediate 4A when analysed by HPLC. The product was purified by the addition of aminopropyl silica and filtration through Celite. The resulting green oil (5.5 g) was slurried in DCM giving benzyl transfer and precipitation of the zwitterionic stage 4 (4.1 g, 70% yield, ~95% by NMR).

Step i

[00348] Initial development at this stage was focussed on finding an alternative to ^{n}BuLi that was easier to handle and ideally did not introduce further lithium into the synthesis. An initial screen of alternative conditions was carried out including the following bases: Li^{t}BuO, K^{t}BuO, NaH, NaHMDS, and $NaNH_2$. All of the reactions gave product with NaHMDS performing as well as ^{n}BuLi. All of the reactions became very thick with gelling observed and overhead stirring was recommended for efficient stirring.

[00349] The initial screen suggested NaHMDS would be a suitable alternative to ^{n}BuLi (81% conversion to product/Intermediate 4A). These conditions were scaled up to 1.5 g alongside a reference reaction with ^{n}BuLi. Overhead stirring was used in both cases.

[00350] Table 20 - Comparison of ^{n}BuLi and NaHMDS

Timepoint	HPLC - ^{n}BuLi	HPLC - NaHMDS
1 hr, -30°C	6.6% St 3, 78% Int 4A, 1% St 4	<1% St 3, 78% Int 4A, 4% St 4
2 hr, 0°C	6.5% St 3, 77% Int 4A, 1% St 4	<1% St 3, 76% Int 4A, 4% St 4
Crude product	4.89 g <1% St 3, 2% Int 4A, 60% St 4	4.38 g <1% St 3, 5% Int4A, 68% St 4

Abbreviations used in the table: St 3 = Stage 3, Int 4A = Intermediate 4A, St 4 = Stage 4

[00351] The reaction profile obtained in both cases was very similar with the NaHMDS reaction giving consumption of stage 3. Both reactions were filtered on Celite to remove a white precipitate and concentrated. By NMR excess benzyl protons were present in both cases (especially in the example with ^{n}BuLi) and the isolated yield was >100%. The NaHMDS conditions proved successful giving a favourable reaction profile and were chosen for further scale up. However, workup and purification development was required.

Step ii

[00352] HPLC data indicated the material isolated from the trials above using NaHMDS and ^{n}BuLi had rearranged to give zwitterionic stage 4 upon concentration. Purification of this material away from the benzylphosphoric acid by-products and other impurities was attempted by slurry in a number of solvents.

[00353] Table 21 - Trial purification of crude stage 4 product

Solvent	Mass recovered	HPLC Purity
DCM	White solid	84% St 4
EtOH	Gum	-
EtOAc	Gum	-
IPA	White solid	88% St 4
Toluene	Gum	-
TBME	White solid	62% St 4
MIBK	Gum	-
MeCN	Gum	-
Acetone	White solid	86% St 4

Abbreviations used in table: St 4 = Stage 4

[00354] *filtration poor

[00355] White solids were obtained from several solvents however the solids obtained from DCM and TBME turned to a pale purple gum when stored over the weekend. Those obtained from IPA and acetone remained as free flowing white solids on storage suggesting that the stability of these solids was likely to be higher and that they would allow for easier handling.

[00356] The slurries in IPA and acetone were scaled up to 1 g. However, gumming was noticed immediately on addition of the solvent. The gum was slowly dispersed by vigorous stirring and eventually showed signs of crystallisation, with a white slurry forming after an overnight stir. However, this process was not suitable for scale up. Solids were isolated in good yield with IPA providing the highest purity.

[00357] THF was also investigated as this had advantages in that it was also the reaction solvent. However, when this was trialled initial gum formation was again observed (isolated ~80% yield, ~92% by HPLC). In order to try and avoid the gum formation and give a more controlled crystallisation the crude stage 4 was first solubilised in a low volume of DMSO (2 vol). THF was then added to this (10 vol) and the solution stirred over the weekend. This slowly gave precipitation of the product which was collected by filtration and washed with THF to yield stage 4 (86% yield) with 96% HPLC purity (>95% by NMR).

[00358] As the THF crystallisation was successful and it was previously noted that complete conversion to zwitterionic stage 4 occurred during concentration of the reaction liquors (THF/EtOAc) at 40°C, it was hoped that the changes of solvent could be avoided and the product crystallised directly by stirring out the reaction mixture at 40°C.

[00359] Two 4 g NaHMDS reactions were carried out with both reactions reaching completion with ~80% conversion to Intermediate 4A. One reaction was diluted with EtOAc and the other with THF and both were filtered to remove phosphate by-products. In order to further reduce the phosphate impurity levels a brine wash was carried out and the organics dried and concentrated to 10 vol. These solutions were stirred overnight at 40°C to give conversion to, and precipitation of, stage 4 (~1% stage 3, ~0.2% Intermediate 4A, ~82% stage 4). The solids were collected by filtration giving 8.03 g (88% yield) from EtOAc / THF and 5.65 g (62% yield) from THF. The brown/grey solids obtained from EtOAc / THF were of lower purity (~90% by HPLC, 78% assay) when compared to the white solids obtained from THF (97% by HPLC, 88% assay). Analysis of the aqueous layer from the THF reaction showed product to be present and additional losses were incurred to the final THF filtrate.

[00360] Due to the higher purity obtained from THF, this solvent was investigated further in order to optimise the recovery. The brine wash was omitted due to product losses to the aqueous layer and the reaction mixture was further concentrated after the reaction to minimise losses during the final filtration step. This new procedure was trialled on a 75 g scale with portions of the reaction mixture concentrated to 8 vol and 6 vol. Upon filtration no difference in yield was noted between the two portions with an overall yield of 140.4 g (90% by NMR assay, 74% active yield, 90% by HPLC).

Impurity tracking

[00361] Three main impurities were observed in the isolated product, with identities for two of these species proposed based on MS data.

[00362] The debenzylated impurity (typically ~2-5% by HPLC) was shown to give psilocybin during the following hydrogenation and could therefore be tolerated at a higher level. The main observed impurity in the isolated stage 4 (typically ~5-8% by HPLC) was the anhydride impurity. This was tracked through the subsequent hydrogenation and shown to be readily removed by re-crystallisation from water as the highly soluble pyrophosphorate impurity that results from debenzylation. The other main observed impurity (m/z 295.2 observed by LCMS) was found to be controlled to less than 2% by limiting the reaction temperature (below -50°C) and was not observed in Psilocybin after hydrogenation.

[00363] The impurity profile of the 140 g batch produced above showed 90.0% stage 4, 6.4% anhydride impurity, 0.2% N-debenzylated impurity and 1.2% of the m/z 295.2 impurity.

GMP synthesis

[00364] The first large scale stage 3 batch (544 g input) was completed using the established procedure to give 213.5 g (53% yield, 99% by HPLC). A second batch (628.2 g input) was also processed successfully to give 295.2 g (66% yield, 99% by HPLC).

[00365] Some variability in yield at this stage was noted over 3 large scale batches (57%, 53% and 66%). This is probably a consequence of minor differences in the workup and quench procedure.

[00366] Experimental

[00367] Stage 3 was charged to a vessel followed by THF (15 vol wrt stage 3 charge) and cooled to ≤ -50°C using a dry ice/acetone bath. 1M NaHMDS solution in THF (1.13 eq) was charged maintaining a temperature of ≤ -45°C, target <-50°C. The reaction was stirred for 30 minutes at -60 to -50°C. Tetrabenzylpyrophosphate (2.26 eq) was charged to the reaction in a single portion followed by additional THF (20 vol) while maintaining the reaction temperature < -30°C. The reaction was warmed to 0°C over 1.5 – 2 hours and sampled for completion. The reaction was filtered to remove phosphate salts washing with THF (8 vol). The filtrate was concentrated until 6-8 vol remains and stirred overnight at 40°C to convert Intermediate 4A to stage 4 product. The reaction was sampled for completion and then filtered and the solid washed with THF (2vol). The stage 4 product was dried in a vacuum oven at 40°C.

[00368] The differences from JNP and the benefits can be summarised as follows:

Step i

i) Firstly, sodium hexamethyldisilazide was introduced to support deprotonation. This proved an effective alternative to Butyl Lithium, which was easier to handle, and did not introduce further lithium into the reaction.

ii) Secondly, by diluting the reaction with THF, a much higher purity Intermediate 4A was obtained.

iii) Thirdly, by controlling the reaction temperature at below -50°C, undesirable mz 295.2 observed by LCMS was controlled to levels of less than 2%.

Step ii

iv) Fourthly, by monitoring levels of stage 4A impurities, particularly the N-debenzylated Stage 4 (Table 7) and anhydride Stage 4 (Table 7) a pure product can be produced reproducibly.

v) The intermediate stage 4A to stage 4 conversion can be carried out in the reaction solvent, avoiding the need for time consuming solvent swaps.

vi) Finally, the obtained solid is washed with THF and oven dried to obtain stage 4.

EXAMPLE 6

STAGE 5

[00369] Catalyst poisoning was noted during development of this stage and a charcolation step can be included in the process when required to prevent incomplete hydrogenation. However, charcolation is not routinely required.

[00370] After sparging with hydrogen for 3 hours typical reactions showed high levels of completion (>90% product, 3-5% SM remaining). A small amount of water was added to aid solubility and after sparging with hydrogen for a further 1 hour, consumption of stage 4 was achieved.

[00371] A successful reaction was worked up by filtration, followed by evaporation to remove methanol, leaving the product as a thick suspension in water. Ethanol was added and the solid filtered to give Psilocybin in 69% yield. ^{1}H NMR confirmed the identity of the product but indicated a minor related impurity was present. LCMS analysis indicated a purity of 95.2% with the major impurity (4.1%) being identified as the pyrophosphoric acid impurity. (Table 7) deriving from the anhydride impurity at stage 4. It was later shown that this impurity was effectively purged during the final product re-crystallisation (Stage 6).

[00372] A further reaction was then carried out using stage 4 material from the finalised THF workup which was 88.0% pure by HPLC and contained 7.3% N-debenzylated stage 4 (converted to product), with none of the anhydride impurity. Again completion was noted and the reaction worked up as previously to give Psilocybin in 46% yield. The low yield was believed to result from precipitation of the product during the catalyst filtration step. ^{1}H NMR confirmed the identity of the product and HPLC indicated a purity of 98.9%.

[00373] Further development of the reaction conditions was carried out to optimise the water volume employed and minimise product losses during the filtration step. After the reaction, a solution was obtained by addition of 10 volumes of water with heating to 40°C. This allowed for removal of the catalyst by filtration without incurring product losses on the filter.

[00374] Some stage 3 was generated by hydrolysis during the reaction and workup with levels of *approx.* 1 - 2.5% appearing to be typical of the process. A reduction in the stage 3 level was demonstrated during the final product re-crystallisation.

Scale up

[00375] The large scale stage 4 batch (non-GMP) was processed as a single batch (148 g active input). Consumption of stage 4 was achieved with 88% product and 0.9% stage 3 resulting from hydrolysis. The anhydride impurity (6.4%) was completely converted to the corresponding pyrophosphoric acid impurity (5.2%).

[00376] The large scale hydrogenation was filtered and concentrated to yield 109 g of crude product after stripping back from ethanol to reduce the water content (~71% by NMR assay, 86% yield).

Experimental

[00377] 10% Pd/C (~50% water wet, type 87L, 0.1 x stage 4 charge) was charged to a vessel under N_2 followed by Methanol (20 vol wrt stage 4) and Stage 4. The N_2 was replaced with H_2 and the reaction was stirred under H_2 (atmospheric pressure) for 1-2 hours. The reaction was sampled for completion and then water was added (10 vol wrt stage 4) maintaining a temperature of <25°C. The mixture stirred for a further 1-2 hours under H_2 (atmospheric pressure). The reaction was sampled and checked for completion.

[00378] If the reaction was incomplete, H_2 was recharged and the reaction continued for a further 1-12 hr until completion was observed. The reaction was then placed under N_2 and warmed to 40°C and held for 15-45 minutes. The reaction was filtered through celite to remove catalyst, washing with methanol (13.3 vol wrt stage 4 charge) and water (6.7 vol wrt stage 4 charge). The filtrate was concentrated *in vacuo*, azeotroping with ethanol to remove water until a solid was obtained. The differences from JNP and the benefits can be summarised as follows:

[00379] Primarily, the reaction is monitored for levels of intermediates by HPLC, using relative retention times (RRT) and completion controlled with intermediates being present at less than 0.2%. The stage 5 pyrophosphoric acid impurity is also carefully monitored to confirm that it can be controlled in the final re-crystallisation.

[00380] The final Stage 6 process is as described in Example 1.

EXAMPLE 7

TESTING METHODOLOGY AND PROTOCOLS

[00381] To test for purity etc the following methodology/ protocols were employed.

7.1 NMR

[00382] ^{1}H and ^{13}C NMR spectra of Psilocybin in D_2O were obtained using 400MHz spectrometer. Chemical shifts are reported in ppm relative to D_2O in the ^{1}H NMR (δ=4.75ppm) and relative to MeOH (δ=49.5ppm), which was added as a reference, in the ^{13}C NMR the spectrum. Literature values for Psilocybin are reported in JNP. Analysis of Psilocybin by NMR gave data that was consistent with the structure and consistent with that reported in the literature with only minor variations in chemical shifts for protons near the ionisable groups which is expected as the zwitterionic nature of the compound makes the material very sensitive to small changes in pH.

[00383] The ^{1}H NMR and ^{13}C NMR data are outlined below and the spectra are shown in Figs 10 and 11.

[00384] **^{1}H NMR Data (400MHz, D_2O):** 2.79 (s, 3H), 3.18 (t, J=7.4Hz, 2H), 3.31 (t, J=7.4Hz, 2H), 6.97 (d, J=8.0Hz, 1H), 7.08 (s, 1H), 7.10 (t, J=8.0Hz, 1H), 7.19 (d, 8.2Hz, 1H).

[00385] **^{13}C NMR Data (400MHz, D_2O (+ trace MeOH):** 22.3 (1 x CH_2), 43.4 (2 x CH_3), 59.6 (1 x xCH_2), 108.4 (1 x CH), 108.6 (1 x C), 109.5 (1 x CH), 119.1 (d, $^{3}J_{P\text{-}H}$=6.7Hz, 1 x C), 123.3 (1 x CH2), 124.8 (1 x CH), 139.3 (1 x C), 146.3 (d, $^{2}J_{P\text{-}H}$=6.7Hz, 1 x C)

29 07 22

7.2 FT-IR

[00386] Data was collected on a Perkin Elmer Spectrum Two™ Spectrometer with UATR Two accessory. Analysis of Psilocybin (Batch: AR755) by FT-IR spectroscopy gave a spectrum (Fig 12) that is consistent with the proposed structure. The broad peak at 3244cm^{-1} is typical of an amine salt. The remainder of the peaks are in the fingerprint region and therefore can't be assigned individually.

7.3. MASS SPECTROMETRY

[00387] The mass spectrum of Psilocybin (AR755) was obtained on a Bruker Esquire 3000 plus Ion Trap Mass Spectrometer and was concordant with the structure. The mass spectrum (Fig 13) showed a main peak at m/z = 284.8 and 568.1 that corresponded to $(M+H)^+$ and $(2M+H)^+$ of Psilocybin. This implied the molecular ion has m/z 284 corresponding to the molecular formula of Psilocybin ($C_{12}H_{17}N_2O_4P$) (Fig 14).

7.4 RESIDUE ON IGNITION

[00388] The residue on ignition method follows the pharmacopeia method with one adjustment. Inconsistent results were obtained when the crucible was heated to 500°C and it is believed this is due to low volatility of the phosphate residues that are generated. The temperature was therefore increased to 800°C for Psilocybin and consistent and accurate results were obtained.

7.5 HPLC - ASSAY AND PURITY DETERMINATION

[00389] The HPLC method used for assay, chemical purity and quantifying the impurities of Psilocybin is a gradient HPLC-UV method and the conditions are outlined in Table 22. External standards are used for quantification. Approximately 1 mg/mL of Psilocybin was dissolved in Purified Water:MeOH (95:5). Sonicate to fully dissolve.

[00390] Purity by HPLC is calculated in the basis of area% and is correlated against a known retention time standard.

[00391] Assay by HPLC is calculated on an anhydrous basis, based on weight% versus a standard of known purity and composition.

[00392] Table 22: Typical HPLC Conditions for Identification, Purity and Assay

Parameter	**Conditions**
System	Agilent 1100 series liquid chromatograph or equivalent
Column	XBridge C18, 4.6 x 150mm; 3.5 µm (Ex; waters PN:186003034)
Flow Rate	1.0ml.min^{-1}
Injection Volume	5µl
Detection	UV @ 267nm
Column Temperature	30°C
Mobile Phase	A – Purified Water : Methanol : TFA (95:5:0.1) B – Methanol : Purified Water : TFA (95:5:0.1)
Gradient	Time (mins) / %A / %B 0 / 100 / 0 2 / 100 / 0 15 / 0 / 100 20 / 0 / 100 22 / 100 / 0

7.6 RESIDUAL SOLVENT CONTENT BY HRGC

[00393] The HRGC method for quantifying residual solvents is a headspace method and is described in Table 23 below:

[00394] Table 23: Typical Residual Solvent GC Method

Parameter	Conditions
System	Agilent 6890/7890 HRGC or similar
Column	DB-624 60m x 0.32mm, 1.80 µm film thickness (or equivalent)
Oven Program	40°C (hold for 15min) then ramp (20°C.min^{-1}) to 200°C (hold 5min)
Headspace Parameters Oven Temp Loop Temp Transfer Line Temp	 125°C 140°C 150°C
Split Ratio	10:1
Injector temperature	200°C
Detector temperature	250°C, FID
Head pressure	15 psi, constant pressure
Carrier gas	Nitrogen
Column flow	2.0 ml.min^{-1} @ 40°C
Internal Standard	1,2-Difluorobenzene

[00395] Levels of the following solvents and reagents are determined: Methanol, Ethanol, THF and Toluene.

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7.7 MELTING POINT BY DSC

[00396] DSC data was collected on a PerkinElmer Pyris 6000 DSC (or similar). The instrument was verified for energy and temperature calibration using certified indium. The sample was weighed (typically 0.5 to 3.0 mg) into a pin-holed aluminium sample pan. The pan was crimped with an aluminium pan cover. The pan was heated at 20°C/min from 30 to 300°C with a purge of dry nitrogen at 20 mL/min. During the melting point procedure, each batch of Psilocybin Polymorph A or A' exhibited two endothermic events the latter; the first of which was attributed to solid-solid transition of Polymorph A or A' to Polymorph B, and the second of which was attributed to melting of Polymorph B.

7.8 POLYMORPHISM BY XRPD

[00397] The solid state form of Psilocybin is determined by XRPD. XRPD diffractograms were collected on a diffractometer (such as a PANalytical X'Pert PRO or equivalent) using Cu Kα radiation (45kV, 40mA), θ-θ goniometer, focusing mirror, divergence slit (1/2"), soller slits at both incident and divergent beam (4 mm) under ambient conditions. The data collection range was 3-35°2θ with a continuous scan speed of 0.2°s^{-1}. The resulting diffractogram is compared to that of a reference diffractogram of Polymorph A or A' to ensure that it is concordant (Fig. 7a or 7b respectively).

7.9 THERMO-GRAVIMETRIC ANALYSIS (TGA)

[00398] TGA data was collected on a PerkinElmer Pyris 1 TGA (or similar). The instrument was calibrated using a certified weight and certified Alumel and Perkalloy for temperature. A predefined amount of sample (typically ca. 5 mg) was loaded into an aluminium crucible, and was heated at 20°C/min from ambient temperature to 400°C. A nitrogen purge at 20 mL/min was maintained over the sample.

7.10 LOSS ON DRYING

[00399] Determine in duplicate the loss on drying of the sample using a 1g portion, accurately weighed, dried at 70°C, under vacuum to constant weight.

[00400] Calculation:

$$\%\ \text{Loss on Drying} = \frac{(W_{\text{INTIAL}} - W_{\text{FINAL}})}{W_{\text{SAMPLE}}} \times 100$$

Where:

$W_{INITIAL}$ = Initial weight of dish and sample prior to drying (g)

W_{FINAL} = Final weight of dish and dried sample (g)

W_{SAMPLE} = Weight of sample (g)

[00401] EXAMPLE 8

Forced Degradation Studies

[00402] Psilocybin drug substance was stressed under various conditions in solution and in the solid state to provide samples to evaluate analytical method selectivity.

[00403] The forced degradation study was performed on Psilocybin; based on the requirements of ICH Q1A(R2). Testing under stressed conditions has provided information on potential degradation pathways and the intrinsic stability of Psilocybin. The optimised analytical method employed demonstrated specificity to Psilocybin; it was shown to be suitable and changes to identity, purity and potency of the product can be detected using this method. The method used has also been shown to be free from interferences from likely impurities and degradation products in accordance with ICH Q2(R1) (Validation of Analytical Procedures) with reference to specificity. Therefore, the HPLC method is deemed suitable for determining purity of Psilocybin and related impurities.

[00404] The control sample of Psilocybin was stable in solution over the study period (study period was 7 days for non-photostability samples). Psilocybin degraded slowly when heated

in solution producing psilocin as the major impurity. Psilocybin was also stable under acid conditions at room temperature. However, at 60°C a slow and steady degradation was observed producing psilocin as the main impurity. Psilocybin was slightly unstable at room temperature in the presence of base with slow degradation to a range of impurities over the study period. Only very low levels of impurities were formed under the peroxide conditions with the overall purity dropping by ~0.5%. In the solid state, slow chemical degradation was noted (3 days at 150°C) predominantly producing psilocin (stage 3) as an impurity. Psilocybin was stable under photostability conditions both as a solid and when in solution.

Stability Studies

[00405] Stability studies were undertaken with two batches of Psilocybin as shown in Table 24.

[00406] Table 24

Study number/Study start	Drug Substance Lot No.	Packaging	Site of Manufacture	Lot Use	Storage Condition	Intended time points/Study Status
ON-YXSTAB0138	GM764B	Double food grade Polythene bags. Outer Polythene container	Onyx Scientific	Ref. Std.	2-8°C 25°C/60%RH 40°C/75% RH	1,3,6 months ongoing 1,3,6 months ongoing 1,3,6 months ongoing
ON-YXSTAB0139	170231	Double food grade Polythene bags. Outer Polythene container	Onyx Scientific	Clinical	2-8°C 25°C/60%RH 40°C/75% RH	1,3,6,9,12,18,24,36 months ongoing 1,3,6,9,12,18,24,36 months ongoing 1,3,6 months ongoing

[00407] Samples were double bagged in food grade polythene bags and sealed in an outer polythene container and placed on storage at 2-8°C, 25°C/60% RH and 45°C/75% RH, a desiccant bag is included between the inner polythene bags to prevent moisture uptake. Tests for appearance, water content, purity and assay were carried out.

[00408] The protocols for the two studies are shown in Table 25 and Table 26.

The one month and three month stability data for batch GM764B are detailed in Table 27and Table 28 below.The one, three, six, nine and twelve month stability data for GMP batch

170231 are provided in Table 29, Table 30, Table 31, Table 32 and Table 33 respectively below.

[00409] Table 25

Onyx Stability Trial Protocol Sheet

Product:		Psilocybin		Onyx trial number:		ONYXSTAB0138
Batch number:		GM764B		Trial due start date:		10 MAR 2017
Test method:		N/A		Date of manufacture:		06 FEB 2017
Additional information:		1200mg of material required in each container.				
Packaging components:		Double polythene bagged lined contained within 300ml HDPE container (food grade). Insert a desiccant bag between the two polythene bags.				
Test parameters Routine tests		Appearance Assay (Anhydrous basis) by ^{1}H-NMR Water Content by loss on drying Chemical Purity / Impurities by HPLC				
Months	1	3	6		Spares	Total
2°C-8°C	X	X	X		2	5
25°C/60%RH	X	X	X		2	5
40°C/75%RH	X	X	X		0	3
Date due off	10APR17	10JUN17	10SEP17			13

[00410] Table 26

Onyx Stability Trial Protocol Sheet

Product:	Psilocybin	Onyx trial number:	ONYXSTAB0139
Batch number:	170231	Trial due start date:	31 MAR 2017
Test method:	SS/PSILOCYBIN/	Date of manufacture:	27FEB2017
Additional information:	2200mg of material required in each container.		
Packaging components:	Double polythene bagged lined contained within 300ml HDPE container (food grade). Insert a desiccant bag between the two polythene bags.		

Test parameters
Routine tests

Appearance
Assay (on a dry basis) by HPLC
Water Content by loss on drying
Chemical Purity / Impurities by HPLC

Timepoint	1 month	3 months	6 months	9 months	12 months	18 months	24 months	36 months	Spares	Total
2-8°C	X	X	X	X	X	X	X	X	2	10
25°C/60%RH	X	X	X	X	X	X	X	X	2	10
40°C/75%RH	X	X	X						1	4
Date due off	31APR17	30JUN17	30SEP17	31DEC17	31MAR18	30SEP18	31MAR19	31MAR20		24

[00411] Table 27: One Month Stability Data for Batch GM764B

Test	Specification Limit	T=0	T=1 month	T=1 month	T=1 month
Condition	N/A	N/A	2°C-8°C	25°C/60%RH	40°C/75%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Assay by ^{1}H-NMR	For information only.	97% $^{w}/_{w}$	99% $^{w}/_{w}$	98% $^{w}/_{w}$	96% $^{w}/_{w}$
Water content by loss on drying	For information only.	0.86% $^{w}/_{w}$	0.35% $^{w}/_{w}$	0.20% $^{w}/_{w}$	0.14% $^{w}/_{w}$
Chemical Purity By HPLC	For information only.	99.24%	99.24%	99.22%	99.23%
Impurities by HPLC: (Quote all GT 0.05%)	For Information only. RRT 0.86 RRT 1.46 RRT 1.59 (Psilocin) Total Impurities	 0.05% 0.05% 0.37% 0.76%	 0.05% 0.09% 0.35% 0.76%	 0.05% 0.10% 0.34% 0.78%	 0.05% 0.10% 0.34% 0.77%

[00412] Table 28: Three Month Stability Data for Batch GM764B

Test	Specification Limit	T=0	T=3 month	T=3 month	T=3 month
Condition	N/A	N/A	2°C-8°C	25°C-60%RH	40°C-75%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Assay by ^{1}H-NMR	For information only.	97 % $^{w}/_{w}$	97% $^{w}/_{w}$	99% $^{w}/_{w}$	97% $^{w}/_{w}$
Water content by loss on drying	For information only.	0.86% $^{w}/_{w}$	0.26% $^{w}/_{w}$	0.08% $^{w}/_{w}$	0.14% $^{w}/_{w}$
Chemical Purity By HPLC	For information only.	99.24%	99.31%	99.27%	99.26%
Impurities by HPLC: (Quote all GT 0.05%)	For Information only. RRT 0.86 RRT 1.46 RRT 1.59 (Psilocin) Total Impurities	 0.05% 0.05% 0.37% 0.76%	 LT0.05% 0.10% 0.37% 0.69%	 LT0.05% 0.09% 0.36% 0.73%	 LT0.05% 0.10% 0.37% 0.74%

[00413] Table 29: One Month Stability Data for Batch 170231

Test	Specification Limit	T=0	T=1 month	T=1 month	T=1 month
Condition	N/A	N/A	2°C-8°C	25°C/60%RH	40°C/75%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Chemical Purity By HPLC	For information only.	99.28%	99.20%	99.16%	99.17%
Impurities by HPLC: (Quote all GT 0.05%) RRT 1.49 RRT 1.62 (Psilocin) RRT 1.70 Impurity at RRT 1.89 Impurity at RRT 2.45 Impurities LT 0.05% Total Impurities	For information only.	 0.06% 0.39% 0.05% LT 0.05% LT 0.05% 0.22% 0.72%	 0.05% 0.36% LT 0.05% LT 0.05% LT 0.05% 0.39% 0.80%	 0.05% 0.37% LT 0.05% LT 0.05% LT 0.05% 0.42% 0.84%	 0.06% 0.36% LT 0.05% LT 0.05% LT 0.05% 0.41% 0.83%
Assay by HPLC (on a dry basis)	For information only	98.65%$^{w}/_{w}$	98.76%$^{w}/_{w}$	97.98%$^{w}/_{w}$	98.52%$^{w}/_{w}$
Water content by loss on drying	For information only.	0.32%$^{w}/_{w}$	0.27%$^{w}/_{w}$	0.17%$^{w}/_{w}$	0.19%$^{w}/_{w}$

[00414] Table 30: Three Month Stability Data for Batch 170231

Test	Specification Limit	T=0	T=3 months	T=3 month	T=3 month
Condition	N/A	N/A	2°C-8°C	25°C/60%RH	40°C/75%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Chemical Purity By HPLC	For information only.	99.28%	99.30%	99.31%	99.17%
Impurities by HPLC: (Quote all GT 0.05%) RRT0.69 RRT 1.49 RRT 1.62 (Psilocin) RRT 1.70 Impurity at RRT 1.89 Impurity at RRT 2.45 Impurities LT 0.05% Total Impurities	For information only.	 LT0.05% 0.06% 0.39% 0.05% LT 0.05% LT 0.05% 0.22% 0.72%	 0.05% 0.05% 0.37% LT 0.05% LT 0.05% LT 0.05% 0.22% 0.70%	 LT0.05% 0.05% 0.36% LT 0.05% LT 0.05% LT 0.05% 0.27% 0.69%	 LT0.05% 0.06% 0.39% LT 0.05% LT 0.05% LT 0.05% 0.34% 0.79%
Assay by HPLC (on a dry basis)	For information only	98.65%$^{w}/_{w}$	98.45%w/w	99.46%$^{w}/_{w}$	98.64%$^{w}/_{w}$
Water content by loss on drying	For information only.	0.32%$^{w}/_{w}$	0.17% w/w	0.01% $^{w}/_{w}$	0.19%$^{w}/_{w}$

[00415] Table 31: Six Month Stability Data for Batch 170231

Test	Specification Limit	T=0	T=6 months	T=6 months	T=6 months
Condition	N/A	N/A	2°C-8°C	25°C-60%RH	40°C-75%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Chemical Purity By HPLC	For information only.	99.28%	99.20%	99.19%	99.12%
Impurities by HPLC: (Quote all GT 0.05%) RRT0.69 RRT 1.49 RRT 1.62 (Psilocin) RRT 1.70 Impurity at RRT 1.89 Impurity at RRT 2.45 Impurities LT 0.05% Total Impurities	For information only.	 LT 0.05% 0.06% 0.39% 0.05% LT 0.05% LT 0.05% 0.22% 0.72%	 0.06% 0.07% LT 0.05% 0.35% LT 0.05% LT 0.05% LT 0.05% 0.32% 0.80%	 0.06% 0.07% LT 0.05% 0.34% LT 0.05% LT 0.05% LT 0.05% 0.34% 0.81%	 0.06% 0.08% LT 0.05% 0.38% LT 0.05% LT 0.05% LT 0.05% 0.36% 0.88%
Assay by HPLC (on a dry basis)	For information only	98.65%$^{w}/_{w}$	97.97%$^{w}/_{w}$	98.04%$^{w}/_{w}$	100.10%$^{w}/_{w}$
Water content by loss on drying	For information only.	0.32%$^{w}/_{w}$	0.06% $^{w}/_{w}$	0.32% $^{w}/_{w}$	2.26% $^{w}/_{w}$

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[00416] Table 32: Nine Month Stability Data for Batch 170231

Test	Specification Limit	T=0	T=9 months	T=9 months
Condition	N/A	N/A	2°C-8°C	25°C-60%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Chemical Purity By HPLC	For information only.	99.28%	99.16%	99.16%
Impurities by HPLC: (Quote all GT 0.05%) RRT0.69 RRT 1.49 RRT 1.62 (Psilocin) RRT 1.70 Impurity at RRT 1.89 Impurity at RRT 2.45 Impurities LT 0.05% Total Impurities	For information only.	 LT0.05% 0.06% 0.39% 0.05% LT 0.05% LT 0.05% 0.22% 0.72%	 LT 0.05% LT 0.05% 0.07% 0.06% 0.37% LT 0.05% LT 0.05% LT 0.05% 0.34% 0.84%	 LT 0.05% LT 0.05% 0.05% 0.06% 0.37% LT 0.05% LT 0.05% LT 0.05% 0.35% 0.84%
Assay by HPLC (on a dry basis)	For information only	98.65%$^{w}/_{w}$	97.53%$^{w}/_{w}$	98.12%$^{w}/_{w}$
Water content by loss on drying	For information only.	0.32%$^{w}/_{w}$	0.21%$^{w}/_{w}$	0.10%$^{w}/_{w}$

[00417] Table 33: Twelve Month Stability Data for Batch 170231

Test	Specification Limit	T=0	T=12 months	T=12 months
Condition	N/A	N/A	2°C-8°C	25°C/60%RH
Appearance	For information only.	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination	An off white solid. Free from visible contamination
Chemical Purity By HPLC	For information only.	99.28%	99.25%	99.25%
Impurities by HPLC: (Quote all GT 0.05%) RRT0.69 RRT 1.49 RRT 1.62 (Psilocin) RRT 1.70 Impurity at RRT 1.89 Impurity at RRT 2.45 Impurities LT 0.05% Total Impurities	For information only.	 LT0.05% 0.06% 0.39% 0.05% LT 0.05% LT 0.05% 0.22% 0.72%	 LT 0.05% LT 0.05% 0.37% LT 0.05% LT 0.05% LT 0.05% 0.38% 0.75%	 LT 0.05% LT 0.05% 0.37% LT 0.05% ND LT 0.05% 0.38% 0.75%
Assay by HPLC (on a dry basis)	For information only	98.65%$^{w}/_{w}$	99.63%$^{w}/_{w}$	98.97%$^{w}/_{w}$
Water content by loss on drying	For information only.	0.32%$^{w}/_{w}$	0.49% $^{w}/_{w}$	0.61% $^{w}/_{w}$

[00418] Over the first 12 months of the ICH stability study Psilocybin has proven to be chemically stable under low temperature (2-8°C), ambient (25°C/60%RH) and accelerated (40°C/75%RH) conditions. There has been no change in the appearance and HPLC analysis has also remained consistent. The water content has varied in all samples, due to the initial impact and then aging of the desiccant bags used in the study.

Example 9 – Experimental to form Hydrate A

[00419] Psilocybin (200mg) was charged to a crystallisation tube followed by deionised water (4ml). The mixture was equilibrated at 25°C for 2 hours before the solid was isolated by vacuum filtration. The material was split into two equal portions. One portion was not subjected to further drying to give Hydrate A, lot GK2, by XRPD and DSC (diffractogram and thermogram consistent with Fig 7d and Fig 8d respectively).

Example 10– Experimental to form Polymorph B

[00420] Psilocybin Polymorph A (250mg) was charged to a round bottom flask, heated to 173°C using an oil bath and held at temperature for 5 minutes. The solid was cooled to ambient temperature and isolated to give lot GK3 with a recovery of 93%. Analysis by XRPD and DSC revealed lot GK3 to be Polymorph B (diffractogram and thermogram consistent with Fig 7c and Fig 8c respectively).

Example 11 – Solid state investigations

[00421] A number of polymorphism investigations were completed. A summary of the solid forms found is shown in Figure 17. The majority of the forms found were derived from solvent perturbation; in some cases stoichiometric solvates were isolated and in other cases non-stoichiometric solvates.

Slurries of Polymorph A

[00422] Solvent mediated equilibrations of Psilocybin Pattern A were conducted as a primary route into modification of the solid form and to visually assess the solubility of the material in a range of 24 solvents between 25 and 50°C.

[00423] Psilocybin Pattern A (40 mg) was dosed into tubes at room temperature and solvents as listed in Table 34 were added in aliquots of 0.4 ml (10 vol.) to a total volume of 1.2 ml (30 vol.) and observations noted. The mixtures were agitated constantly. Heat cycling was conducted as follows: 50°C for 18 hours, cool over 2 hours to 20°C, mature for 4 hours, heat to 50°C for 4 hours, cool to 20°C over 2 hours, mature for 18 hours. A repeat 50°C – 20°C cycle over 24 hours was conducted and the following applied:

Isolation post heating to 50°C where solids were sufficient = A series

Isolation post cooling to 20°C where solids were sufficient = B series

All isolated solids were dried *in vacuo* at 50°C for 24 hours and analysed by XRPD. The observations are provided in Table 34.

[00424] The API was largely insoluble in the solvents and solvent mixtures tested in 30 volumes at 50°C resulting in heavy suspensions. Water did solubilise Psilocybin at 50°C.

[00425] Table 34 - Tabulated observations for heat cycling slurry maturations and using Pattern A blend as input

Entry	Solvent	Obs. 20°C, 0.4 ml	Obs. 20°C, 0.8 ml	Obs. 20°C, 1.2 ml	Obs. 50°C	XRPD A series	XRPD B series
1	Cyclohexane	Susp.	Susp.	Susp.	Susp.	A	A
2	Chlorobenzene	Susp.	Susp.	Susp.	Susp.	A	A
3	2-Chlorobutane	Susp.	Susp.	Susp.	Susp.	A	A
4	Benzotrifluoride	Susp.	Susp.	Susp.	Susp.	A	A
5	Anisole	Susp.	Susp.	Susp.	Susp.	A	A
6	Nitromethane	Susp.	Susp.	Susp.	Susp.	C	C
7	CPME	Susp.	Susp.	Susp.	Susp.	A	A
8	Heptane	Susp.	Susp.	Susp.	Susp.	A	A
9	TBME	Susp.	Susp.	Susp.	Susp.	C	A
10	MIBK	Susp.	Susp.	Susp.	Susp.	A	A
11	MEK	Susp.	Susp.	Susp.	Susp.	A	A
12	iPrOAc	Susp.	Susp.	Susp.	Susp.	C	C
13	EtOAc	Susp.	Susp.	Susp.	Susp.	A	A
14	Toluene	Susp.	Susp.	Susp.	Susp.	A	A
15	THF	Susp.	Susp.	Susp.	Susp.	A	A
16	$CHCl_3$	Susp.	Susp.	Susp.	Susp.	A	A
17	MeOH	Susp.	Susp.	Susp.	Susp.	D	D
18	EtOH	Susp.	Susp.	Susp.	Susp.	E	E
19	IPA	Susp.	Susp.	Susp.	Susp.	F	F
20	MeCN	Susp.	Susp.	Susp.	Susp.	C	A
21	Water	Susp.	Susp.	Susp.	Solution	n/a	A
22	4:1 EtOH/water	Susp.	Susp.	Susp.	Susp.	A	A
23	4:1 THF/water	Susp.	Susp.	Susp.	Susp.	A	Hydrate A
24	4:1 IPA/water	Susp.	Susp.	Susp.	Susp.	A	C

[00426] Results:

[00427] In the figures (Fig. 18 and Fig. 19), "25C" denotes isolation of the solid at 25°C and "50C" denotes isolation of the solid at 50°C. For example, GM832-20_50_A9 represents GM832 entry 20 (MeCN) isolated at 50°C.

[00428] 50°C Slurries

[00429] Entries 1, 2, 3, 4, 5, 7, 8, 10, 11, 13, 14, 15, 16, 22, 23, 24: XRPD diffractogram broadly consistent with Polymorph A, but with an additional peak of varying intensity at 18°2θ.

[00430] Entries 6, 9, 12, 20: XRPD diffractogram acquired for the isolated solids were broadly consistent (see Fig. 18) with additional peaks at 10°2θ and 13.2°2θ observed for some samples. This XRPD diffractogram was designated Pattern C. There is no chemotype correlation between the solvents (CH_3NO_2, TBME, iPrOAc and CH_3CN).

[00431] Entry 17: XRPD diffractogram acquired had multiple diffraction peaks (Fig. 19). The XRPD diffractogram was designated Pattern D.

[00432] Entry 18: XRPD diffractogram acquired had multiple diffraction peaks (Fig. 19). The XRPD diffractogram was designated Pattern E.

[00433] Entry 19: XRPD diffractogram acquired had multiple diffraction peaks (Fig. 19). The XRPD diffractogram was designated Pattern F.

[00434] 25°C slurries:

[00435] Entries 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 13, 14, 15, 16, 20, 21, 22: XRPD diffractograms are all similar to that acquired for Polymorph A.

[00436] Entries 6, 12, 24: XRPD diffractograms acquired for the isolated solids were broadly consistent (see Fig. 18) with Pattern C.

[00437] Entry 23: XRPD analysis showed Hydrate A had formed.

[00438] Entry 17: XRPD diffractogram acquired had multiple diffraction peaks (Fig. 19). The XRPD diffractogram was designated Pattern D.

[00439] Entry 18: XRPD diffractogram acquired had multiple diffraction peaks (Fig. 19). The XRPD diffractogram was designated Pattern E.

[00440] Entry 19: XRPD diffractogram acquired had multiple diffraction peaks (Fig. 19). The XRPD diffractogram was designated Pattern F.

[00441] Analysis of Results:

[00442] The XRPD diffractograms for the solids isolated at 25°C are broadly the same as for the XRPD diffractograms acquired for the solids isolated at 50°C.

[00443] Patterns D, E and F were derived from alcohols (MeOH, EtOH and IPA). Solvated states were postulated considering an Ethanol Solvate was previously isolated during development. The XRPD diffractograms for the Ethanol Solvate are not identical, however, given that exact solvent level variation may deliver varying states of order within the lattice, the comparison between these XRPD diffractograms provides a strong hypothesis that these more significant phase variations are invoked by solvent entrainment.

[00444] XRPD patterns D, E and F (Fig 19) are all dissimilar to the XRPD diffractogram for Hydrate (Fig. 7d).

[00445] Direct comparison of the XRPD diffractograms acquired for the MeOH, EtOH and IPA derived solids (Patterns D, E and F - Fig. 19) isolated at the two temperatures shows conforming diffractograms; the two MeOH diffractograms are similar while the EtOH and IPA are directly comparable.

[00446] DSC analysis was performed on the isolated solids, and where sufficient sample was available, TGA. The solids that delivered Patterns D, E and F all features endotherms at *ca.* 170-180°C but otherwise proffered distinct thermal profiles. TGA analysis for the MeOH slurry isolated solid showed one protracted mass loss from *ca.* 25 - 190°C (3.1%). A stochiometric methanol solvate would require 10.3% weight solvent. TGA analysis of the EtOH slurry isolated solid showed two distinct mass loss steps. The first one occurred before 100°C (0.3% weight) is considered to be due to water, and the second larger loss (11.5% weight) due to solvent. A stoichiometric ethanol solvate requires 13.9% weight solvent. TGA analysis of the IPA slurry isolated solid featured two distinct mass loss steps. The first mass loss before 100°C (0.4% weight) is considered to be due to water, while the second larger mass loss (13.9% weight) is considered to be due to residual solvent. A stoichiometric IPA solvate requires 17.5% weight solvent.

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[00447] Slurries of amorphous psilocybin

[00448] In order to generate amorphous material a small sample of Psilocybin (0.5g) was dissolved in water (0.5L, 1000vol.), polish filtered and lyophilised. Psilocybin was recovered as an off white fibrous material (lot MC1368A; 412mg, 82%, XRPD amorphous).

[00449] To assess visually solubility of the amorphous API and to induce form modification, a series of slurry maturations were performed as follows:

Psilocybin (15 mg) was charged to tubes. Solvent was then added at ambient temperature (20°C, 0.3ml, 20 vol.) and the suspensions agitated. Observations were made. After 1 hour of stirring, samples were heated to 45°C for 18 hours and observations made. Samples were heated to 50°C for 8 hours and observations were made. The samples were agitated for 72 hours at 25°C and subject to a final heat cycle, prior to isolation. Observations are shown in Table 35.

Table 35 - Observations of amorphous Psilocybin during heat cycling slurry maturation and form fate

Entry	Solvent	Obs. at 20°C	Obs. at 45°C	Obs. at 50°C	XRPD Data
A	Cyclohexane	Susp.	Susp.	Susp.	Semi-crystalline
B	Chlorobenzene	Susp.	Susp.	Susp.	Semi-crystalline

C	Chlorobutane	Susp.	Susp.	Susp.	Pattern B
D	Benzotrifluoride	Susp.	Susp.	Susp.	Semi-crystalline
E	Anisole	Susp.	Susp.	Susp.	Semi-crystalline
F	Nitromethane	Susp.	Susp.	Susp.	Pattern B
G	CPME	Susp.	Susp.	Susp.	Semi-crystalline
H	Heptane	Susp.	Susp.	Susp.	Semi-crystalline
I	TBME	Susp	Susp.	Susp.	Semi-crystalline
J	MIBK	Susp.	Susp.	Susp.	Semi-crystalline
K	MEK	Susp.	Susp.	Susp.	Semi-crystalline
L	iPrOAc	Susp.	Susp.	Susp.	Semi-crystalline
M	EtOAc	Susp.	Susp.	Susp.	Semi-crystalline
N	Toluene	Susp.	Susp.	Susp.	Similar to Solvate A
O	THF	Susp.	Susp.	Susp.	Semi-crystalline
P	Chloroform	Susp.	Susp.	Susp.	Similar to Pattern E
R	MeOH	Susp.	Susp.	Susp.	Semi-crystalline
S	EtOH	Susp.	Susp.	Susp.	Pattern D
T	IPA	Susp.	Susp.	Susp.	Pattern B
U	Acetonitrile	Susp.	Susp.	Susp.	Amorphous
V	Water	Susp.	Susp.	Susp.	Similar to Pattern A
W	4:1 EtOH/water	Susp.	Susp.	Susp.	Similar to Pattern D
X	4:1 THF/water	Susp.	Susp.	Susp.	Similar to Pattern A
Y	4:1 IPA/water	Susp.	Susp.	Susp.	Similar to Pattern A

[00450] Results

[00451] The majority of solvents returned a solid that was considered to be semi-crystalline (predominantly amorphous with a notable reflection at *ca.* 18°2θ).

[00452] Truly amorphous was returned from equilibration in MeCN.

[00453] Polymorph B was returned from chlorobutane, nitromethane and IPA (Fig. 20 and 22).

[00454] Pattern D, which was isolated from MeOH in the Polymorph A slurry experiments discussed above, was returned from the EtOH equilibration whereas MeOH in this study returned a semi-crystalline solid.

[00455] Solids similar to Pattern A were recovered from water, THF:Water and IPA:Water (4:1).

[00456] A solid similar to Pattern D was recovered from EtOH:Water (4:1), supporting the finding of the isolation of Pattern D from EtOH alone.

[00457] A solid similar to Pattern E was recovered from Chloroform.

[00458] From none of the solvents investigated was true Polymorph A or A' isolated following extended equilibration and thermal maturation of amorphous Psilocybin.

CLAIMS

1. Crystalline psilocybin in the form Polymorph A characterised by one or more of:
   a. peaks in an XRPD diffractogram at 11.5, 12.0,14.5 and 17.5 °2θ±0.1°2θ, wherein the peak at 17.5 °2θ±0.1°2θ has a relative intensity compared to the peak at 14.5 °2θ±0.1°2θ of at least 5%, and which is substantially absent of a peak in an XRPD diffractogram at 10.1 °2θ±0.1°2θ; and
   b. an endothermic event in a DSC thermogram having a first onset temperature of between 145°C and 155°C and a second onset temperature of between 210 and 220°C; and
   having a chemical purity of greater than 97% by HPLC, and no single impurity of greater than 1% including phosphoric acid as measured by 31P NMR, and psilocin as measured by HPLC.

2. Crystalline psilocybin in the form Polymorph A, according to claim 1 wherein the endothermic event in the DSC thermogram has a second onset temperature of between 210 and 215°C.

3. Crystalline psilocybin in the form Polymorph A, according to claim 1 or 2 further characterised by one or more peaks in an XRPD diffractogram at 19.7, 20.4, 22.2, 24.3 or 25.7 °2θ±0.1°2θ.

4. Crystalline psilocybin in the form Polymorph A, according to claim 3 having peaks in an XRPD diffractogram at each of 19.7, 20.4, 22.2, 24.3 or 25.7 °2θ±0.1°2θ.

5. Crystalline psilocybin in the form Polymorph A, according to any of the preceding claims having peaks in an XRPD diffractogram at each of 5.6, 11.5, 12.0, 14.5, 17.5, 19.7, 20.4, 22.2, 23.2, 24.3, 25.7, 26.8, 27.8, 29.7, 31.2, 32.6 and 33.7 °2θ±0.1°2θ

6. Crystalline psilocybin in the form Polymorph A, according to any of claims 1 to 5 having an XRPD diffractogram substantially as illustrated in Figure 7a.

7. Crystalline psilocybin in the form Polymorph A, according to any of claims 1 to 6 having a DSC thermogram substantially as illustrated in Figure 8a.

8. Crystalline psilocybin in the form Polymorph A, according to any of the preceding claims further characterised by having either:

   i) a water content of <0.5% w/w; or

   ii) <0.5% w/w loss in the TGA thermogram between 25°C and 200 °C.

9. Crystalline psilocybin in the form Polymorph A according to any preceding claims wherein the crystalline psilocybin is a white to off white solid comprising rod shaped crystals with a size range of 50 to 200 microns.

10. Crystalline psilocybin according to any preceding claim comprising the acceptance criteria of one or more quality attributes of:

   i) a loss on drying of no more than 2% w/w, as measured by European Pharmacopoeia 2.2.32;

   ii) residue on ignition of no more than 0.5% w/w, as measured by US Pharmacopoeia <281>;

   iii) no single drug related impurities of more than 1.0 area %, as measured by HPLC;

   iv) assay (on a dry basis), 95-103 weight %, as measured by HPLC

   v) residual solvent content - methanol no more than 3000ppm, ethanol no more than 5000ppm, tetrahydrofuran (THF) no more than 720ppm, and Toluene no more than 890ppm, as measured by HRGC; and

   vi) elemental analysis: Cd no more than 1.5ppm, Pb no more than 1.5ppm, As no more than 4.5ppm, Hg no more than 9.0ppm, Co no more than 15ppm, V no more than 30ppm, Ni no more than 60ppm, Li no more than 165ppm, and Pd no more than 30ppm, as measured by ICP-MS US Pharmacopoeia <233>

11. A batch of at least 100g of crystalline psilocybin in the form Polymorph A, as claimed in any of the preceding claims.

12. A pharmaceutical formulation comprising crystalline psilocybin Polymorph A as claimed any of claims 1 to 10 together with one or more excipients.

13. A pharmaceutical formulation as claimed in claim 12 which is an oral dosage form.

14. A pharmaceutical formulation as claimed in claim 12 or 13 wherein the psilocybin is present in an amount providing a dose of from 0.01mg/kg to 1mg/kg.

15. A pharmaceutical formulation as claimed in claim 14 wherein a unit dose is 10mg or 25mg.

16. A pharmaceutical formulation as claimed in any of claims 12 to 15 wherein the one or more excipients comprise microcrystalline cellulose or starch.

17. Crystalline psilocybin Polymorph A as claimed in any of claims 1 to 10 for use in medicine.

18. Crystalline psilocybin as claimed in any of claims 1 to 10 for use in treating a central nervous system disorder.

19. Crystalline psilocybin as claimed in any of claims 1 to 10 for use in treating drug resistant depression.

20. A method for large scale manufacture of psilocybin in the form Polymorph A (12) having a chemical purity of greater than 97% by HPLC, and no single impurity of greater than 1% including phosphoric acid as measured by 31P NMR, and psilocin as measured by HPLC characterised in that the method comprises subjecting psilocybin (12) to a water crystallization step, with controlled drying, to produce a crystalline psilocybin in the form Polymorph A as claimed in of any of claims 1 to 10.