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(54) Title: 2,2,2-TRIFLUOROACETIC ACID 1-(2,4-DIMETHYLPHENYL)-2-[(3-METHOXYPHENYL) METHYLENE] HYDRAZIDE POLYMORPHS AND METHOD OF MAKING THE SAME

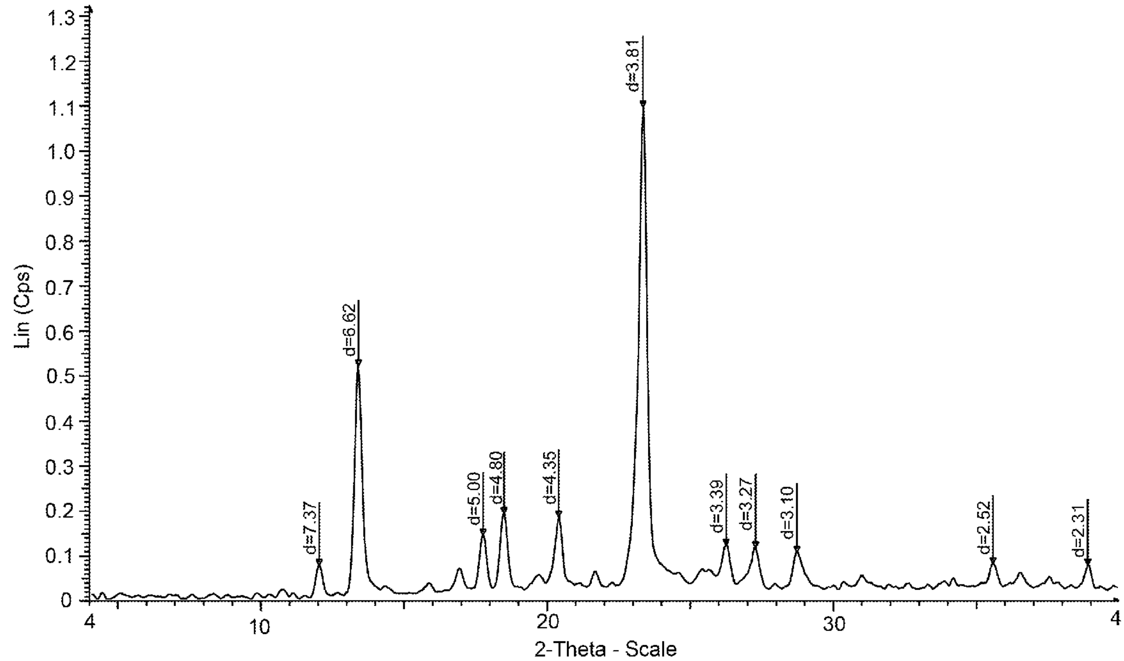

FIG 13

(57) Abstract: Crystalline polymorph forms of neurotrophic agent 2,2,2-trifluoroacetic acid 1-(2,4- Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), and process for producing the crystalline polymorphic form are provided.

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[Continued on next page]

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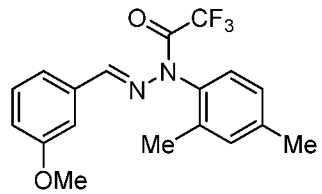
2,2,2-TRIFLUOROACETIC ACID 1-(2,4-DIMETHYLPHENYL)-2-[(3-METHOXYPHENYL)METHYLENE] HYDRAZIDE POLYMORPHS AND METHOD OF MAKING THE SAME

BACKGROUND

[0001] The present disclosure relates to polymorph forms of a pharmaceutical active agent. In particular, the present disclosure relates to polymorph forms of neuroprotective agent 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene] hydrazide (J147).

[0002] 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene] hydrazide (J147) is a potent orally active neurotrophic agent discovered during screening for efficacy in cellular models of age-associated pathologies and has a structure given by Formula I:



I

[0003] J147 is broadly neuroprotective, and exhibited activity in assays indicating distinct neurotoxicity pathways related to aging and neurodegenerative diseases, with EC_{50} between 10 and 200 nM. It has been indicated to improve memory in normal rodents, and prevent the loss of synaptic proteins and cognitive decline in a transgenic AD mouse model. Furthermore, it has displayed neuroprotective, neuroanti-inflammatory, and LTP-enhancing activity.

[0004] The neurotrophic and nootropic effects have been associated with increases in BDNF levels and BDNF responsive proteins. Interestingly, despite this mechanism of action, J147's neuroprotective effects have been observed to be independent of TrkB receptor activation.

J147 has been indicated to reduce soluble Aβ40 and Aβ42 levels, and it is currently being researched for potential applications in treating ALS.

SUMMARY

[0005] The present disclosure is directed to a crystalline Form II of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), and methods of making thereof.

[0006] Certain embodiments of the present disclosure provide a method of making crystalline Form II of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene] hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta, the method comprising: providing a slurry comprising saturated amorphous or crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene] hydrazide (J147) in a solvent/anti-solvent mixture; and mixing the slurry to provide crystalline Form II.

[0007] Certain embodiments of the present disclosure provide an isolated crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta.

BRIEF DESCRIPTION OF DRAWINGS

[0008] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0009] Various embodiments of the present disclosure will be described herein below with reference to the figures wherein:

[0010] FIG. 1 shows the X-ray Diffraction (XRD) diffractogram of crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene] hydrazide (J147).

[0011] FIG. 2A shows a differential scanning calorimetry (DSC) thermogram of crystalline Form I of J147.

[0012] FIG. 2B shows a thermogravimetric (TGA) thermogram of crystalline Form I of J147.

[0013] FIG. 3A shows dynamic vapor sorption isotherms of crystalline Form I of J147 using TGA.

[0014] FIG. 3B shows kinetic plots of crystalline Form I of J147 using TGA.

[0015] FIG. 4 shows the Fourier transform infrared (FTIR) spectrum of crystalline Form I of J147.

[0016] FIG. 5 shows the Raman spectrum of crystalline Form I of J147.

[0017] FIG. 6 shows proton nuclear magnetic resonance (NMR) spectrum of crystalline Form I of J147.

[0018] FIG. 7 shows graphical output using Debye cone integration using a two dimensional detector during XRD analysis of a sample of J147.

[0019] FIG. 8 shows a dendrogram of all XRD results in screening samples of J147.

[0020] FIG. 9 shows a cell plot of all XRD results in screening samples of J147.

[0021] FIG. 10 shows a cluster plot of all XRD results in screening samples of J147.

[0022] FIG. 11A shows a DSC thermogram of crystalline Form I of J147.

[0023] FIG. 11B shows the XRD spectrum of crystalline Form I J147.

[0024] FIG. 12A shows a representative photomicrograph of crystalline Form I J147.

[0025] FIG. 12B shows another representative photomicrograph of crystalline Form I J147.

[0026] FIG. 13 shows the XRD spectrum of crystalline Form II J147.

[0027] FIG. 14A shows a DSC thermogram of crystalline Form II of J147.

[0028] FIG. 14B shows a TGA thermogram of crystalline Form II of J147.

[0029] FIG. 15 shows a representative photomicrograph of crystalline Form II J147.

[0030] FIG. 16A shows the moisture sorption-desorption isotherm of crystalline Form II J147 using dynamic vapor sorption (DVS) analysis.

[0031] FIG. 16B shows kinetic plots of crystalline Form II J147 using DVS analysis.

[0032] FIG. 17 shows a computer predicted powder XRD pattern of a single crystal of Form II of J147. The predicted pattern matched the pattern of experimental pattern.

DETAILED DESCRIPTION

[0033] Embodiments herein are directed to various polymorph forms of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147) and methods of preparing such forms. Discovery of polymorph forms of active pharmaceutical ingredients is recognized as an important practice in product development. Polymorph forms of J147 disclosed herein may affect various physicochemical properties of J147 including, without limitation, hardness, stability, filterability, solubility, hygroscopicity, melting point, solid density and flowability. Altering one or more physicochemical properties of J147 may lead to downstream improvements in both manufacture as well as having an impact on pharmacokinetics and other aspects of administering the API.

[0034] In some embodiments, there is provided an isolated crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta.

[0035] As used herein, “isolated” refers to the separation of a polymorph form, specifically of J147, to the substantial exclusion of other forms as well as impurities and, if so desired, solvent. For example, an isolated polymorph may have a purity of at least about 95%, or about 98%, or about 99%, or about 99.5%, including up to the limits of detection of impurities, or nominally 100% pure.

[0036] In some embodiments, isolated crystalline Form I may further comprise X-ray diffraction peaks located at 8.64, 13.36, 19.25, 21.64, and 26.81 +/- 0.2 degrees 2-theta.

[0037] In some embodiments, there is provided isolated crystalline Form II of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta.

[0038] In some embodiments, isolated crystalline Form II may further comprise X-ray diffraction peaks located at 17.74, 20.39, 26.25, and 28.74 +/- 0.2 degrees 2-theta.

[0039] Other XRD minor peaks in the spectra of Form I and Form II may be present, as disclosed herein below in the Examples.

[0040] In some embodiments, there are provided methods of making crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, the method comprising recrystallizing 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide from an organic solvent selected from the group consisting of nitromethane, methylethylketone, tetrahydrofuran, acetone, acetonitrile, heptane, isopropyl ether, isopropyl acetate, and chloroform.

[0041] In general, recrystallization techniques familiar to those skilled in the art apply in the practice of the methods disclosed herein. For example, a sample of J147 may be dissolved in a minimal amount solvent including dissolution at elevated temperatures for a given solvent. J147 has been found to be reasonably soluble across an array of solvent types, including polar protic and polar aprotic solvents. J147 generally has lower solubility in highly hydrophobic hydrocarbon solvents such as heptane, or at the opposite end, lower solubility in water. Thus, such solvents can serve as co-solvents or anti-solvents during recrystallization. Recrystallization may be performed with or without stirring, mixing, agitation, or the like.

[0042] In some embodiments, the methods of preparing Form I may further employ water as an anti-solvent. In some embodiments, a ratio of organic solvent to anti-solvent water is in a range from about 4:1 to about 1:4.

[0043] In accordance with the methods disclosed herein and as exemplified below, a yield of Form I is obtainable in some embodiments in a range from about 50% to about 100%, or about 90% to about 100%. In some embodiments, the yield may be at least about 95% based on the amount of J147 being recrystallized, or at least about 98%, or at least about 99%, or quantitative recovery.

[0044] In some embodiments, there are provided methods of making crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, the method comprising recrystallizing 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide from a solvent-anti-solvent mixture comprising an alcohol as the solvent. In some such embodiments, a ratio of solvent to the anti-solvent is in a range from about 4:1 to about 1:4. In some embodiments, the solvent alcohol is a C_1-C_4 alcohol. Thus, for example, the alcohol may be selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol, trifluoroethanol, 1-butanol and 2-butanol. In some embodiments the anti-solvent is water or heptane.

[0045] In accordance with the methods disclosed herein and as exemplified below, a yield of Form I is obtainable in some embodiments in a range from about 50% to about 100%. In some embodiments, the yield may be at least about 95% based on the amount of J147 being recrystallized, or at least about 98%, or at least about 99%, or quantitative recovery.

[0046] In some embodiments, there are provided method of making crystalline Form II of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta, the method comprising providing a slurry comprising a saturated crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147) in a solvent/anti-solvent mixture comprising water as anti-solvent and mixing the slurry at ambient temperature to provide crystalline Form II.

[0047] As used herein, “mixing” is broadly intended to include mixing, stirring, agitation and the like.

[0048] In some embodiments, Form II can be accessed via amorphous J147.

[0049] In some embodiments, the methods to access Form II of J147 may employ a solvent which is selected from the group consisting of an alcohol, dimethylformamide (DMF), and dimethylacetamide (DMA).

[0050] In some embodiments, the methods to access Form II of J147 involve the use of a solvent alcohol which is selected from the group consisting of methanol, ethanol, trifluoroethanol, 1-propanol, and 2-propanol.

[0051] In some embodiments, the methods to access Form II of J147 a ratio of the solvent/anti-solvent mixture is in a range from about 1:2 to about 1:1.

[0052] In some embodiments, the methods to access Form II of J147 includes mixing the slurry of saturated J147 over several days such as about 6 days. In some embodiments, mixing may optionally be accompanied by heating. However, ambient laboratory conditions, i.e., about 25 °C are generally sufficient. In some embodiments, Form II may be accessible in reasonable purity and quantities after about 3 days, or about 4 days, or about 5 days. Naturally, Form II may also be isolated at intervals longer than 6 days if so desired, including about 7 days or about 8 days.

[0053] In some embodiments, methods for forming Form II from the slurry includes the use of an apparatus comprising a vessel connected to a re-circulation system. In some embodiments, the recirculation can be conducted through a homogenization apparatus in which a shear force is applied. The homogenization apparatus may comprise a stator and a rotatable rotor, and the high-shear mixing force is applied by rotating the rotor at a speed of more than 250 rpm. The rotor can also be rotated as speeds of more than 500 rpm and more than 1,000 rpm.

[0054] Re-circulating the slurry may comprise regulating the flow of slurry through the outlet and the inlet of the vessel. The energy for the re-circulation can be provided by a pump. Conventional flow regulation mechanisms such as metering pumps, valves, and the like may be used for this purpose. The process can also be conducted in a continuous mode.

[0055] In embodiments, Form I or amorphous J147 may be in a supersaturated solution dissolved in a solvent. This solution may be mixed with an anti-solvent solution. The anti-solvent refers to any solvent in which the chemical material has a poor solubility. It may be a mixture of anti-solvents and solvents. For example, the anti-solvent may comprise water or heptane. Mixing the solutions reduces the solubility of the material in the solvent mixture, causing it to crystallize out.

[0056] In embodiments, methods may also include the step of introducing seed crystals into the vessel to facilitate crystallization. The seed crystals may be placed into the supersaturated solution or into an anti-solvent. These seed crystals are selected to be insoluble in the individual solvents and in the solvent mixture.

[0057] Mixing may comprise regulating the flow of the solution into the vessel. Conventional flow regulation mechanisms such as metering pumps, valves, and the like may be used for this purpose.

[0058] The temperature may be adjusted prior to their introduction into the vessel. This may be achieved by any conventional temperature adjusting equipment, such as a heater or a cooling bath associated with the solution source.

[0059] In embodiments, the re-circulation system may comprise a homogenization apparatus; outlet means for transferring the slurry from the vessel to the homogenization apparatus; and inlet means for receiving the slurry from the homogenization apparatus into the vessel. The homogenization apparatus may comprise a stator and a rotatable rotor, and means for applying a high-shear mixing force by rotating the rotor. The high-shear mixing force can be applied by rotating the rotor at a speed of more than 250 rpm. The rotor can also be rotated as speeds of more than 500 rpm and more than 1,000 rpm.

[0060] The apparatus may include a means for regulating the flow of slurry through the homogenization apparatus. Conventional flow regulation mechanisms such as metering pumps, valves, and the like may be used for this purpose. The apparatus may also include a means for adjusting the temperature of the slurry in the vessel. This may be achieved by any conventional temperature adjusting equipment, such as a heater or a cooling bath associated with the solution source or the vessel.

[0061] In accordance with the methods disclosed herein and as exemplified below, in some embodiments, a yield of Form II is obtainable in a range from about 50% to about 100%, or about 90% to about 100%. In some embodiments, the yield may be at least about 95% based on the amount of J147 being recrystallized, or at least about 98%, or at least about 99%, or quantitative recovery.

[0062] The following Examples are being submitted to illustrate embodiments of the present disclosure. These Examples are intended to be illustrative only and are not intended to limit the scope of the present disclosure. Also, parts and percentages are by weight unless otherwise indicated. As used herein, "room temperature" refers to a temperature of from about 20 ° C to about 25° C.

EXAMPLES

[0063] This example describes the screening of J147 for polymorphic behavior. The screen was performed using solvent recrystallization, hydration experiments, competitive and non-competitive slurry experiments, and grinding to manipulate the solid state form of the test material. Samples generated were characterized using differential scanning calorimetry (DSC), polarized light microscopy, thermogravimetric analysis (TGA), Fourier transform nuclear magnetic resonance (NMR) spectroscopy, powder X-ray diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR), Raman Spectroscopy and dynamic vapor sorption/desorption (DVS). The polymorph screen revealed that the J147 is polymorphic, and several solid state forms were identified and characterized. The thermodynamically stable form was identified.

EXPERIMENTAL METHODS

[0064] Microscopy: A Zeiss Universal and/or Olympus BX53 microscope configured with a polarized visible light source and polarizable analyzer were used to evaluate the optical properties of the samples. The specimens were typically mounted on a microscope slide with a cover glass. Observations of particle/crystal size and shape and birefringence were recorded.

[0065] Hot Stage Microscopy (Hsm): A Linkam hot stage accessory was used in tandem with the microscope. The specimens were mounted on a microscope slide with a cover glass. The samples were heated from room temperature through melting using a Linkam TMS 94 temperature control and Linksys 32 data capture software system. Observations of possible phase change, melting, recrystallization, decomposition, etc. were recorded.

[0066] Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR): The samples were prepared by dissolving 1 to 10 mg of the API in deuterated chloroform with 0.05% (v/v) tetramethylsilane (TMS). The spectra were collected at ambient temperature on a Bruker 400 MHz NMR spectrometer.

[0067] Differential Scanning Calorimetry (DSC): Differential Scanning Calorimetry (DSC) is a technique used to measure characteristic heat flux of a test article as it is scanned through a temperature gradient under a controlled atmosphere. Thermal phase transitions such as endothermic melting and exothermic decomposition were recorded. The DSC data were collected on a TA Instruments DSC. In general, samples in the mass range of 1 to 10 mg were crimped in aluminum sample pans and scanned from 25 to approximately 150 °C at heating rates of 2, 10, 20, and 50 °C/min using a nitrogen purge of 50 mL/min.

[0068] Thermogravimetric Analysis (TGA): Thermogravimetric Analysis involves the determination of the mass of a specimen as a function of temperature. The TGA data were collected on a TA Instruments Q500 TGA. In general, samples in the mass range of 2 to 10 mg were placed in an open, pre-tared platinum sample pan and attached by fine wire to a microbalance. The sample was suspended in a furnace, which was heated from 25 to about 250 °C at 10 °C/min using a nitrogen purge at 100 mL/min. The sample weight change as a function of temperature was observed.

[0069] X-Ray Powder Diffraction (XRD): X-ray diffraction is an analytical technique used to study the crystalline nature of solid materials. X-rays incident on crystalline material are scattered in all directions. In certain directions, the scattered X-rays are constructively reinforced to form diffracted beams. The conditions for constructive diffraction are described by Bragg's Law and depend on the unique composition and spatial arrangements of the crystal structure. As such, each molecular solid diffracts X-rays in different directions and at different intensities resulting in a unique X-ray diffraction pattern. A variable temperature hot stage was used to manipulate sample temperature for some experiments.

[0070] The X-ray powder diffraction patterns were obtained using a Bruker D8 Discovery diffractometer equipped with an XYZ stage, laser video microscope for positioning, and a two dimensional HiStar area Detector or a scintillation detector. A Cu K a radiation 1.5406 angstrom source operating at 40 kV and 40 mA was used to irradiate samples. The X-ray optics consists of a Gobel mirror coupled with a pinhole collimator of 0.5 mm. Theta-theta continuous scans were employed with a sample-detector distance of approximately 30 cm, which gives an effective 2 0 range of 4-40. The samples were mounted in low background quartz plates.

[0071] Hygroscopicity–Dynamic Vapor Sorption (DVS): DVS is a gravimetric screening technique that measures how quickly and how much of a solvent (water) is adsorbed by a sample. The relative humidity or vapor concentration surrounding the sample is varied while the change in mass of the sample is measured. A vapor sorption isotherm shows the equilibrium amount of vapor sorbed as a function of relativity humidity. The mass values at each relative humidity step are used to generate the isotherm. Isotherms are divided in two components: sorption for increasing humidity steps and desorption for decreasing humidity steps. A plot of kinetic data is also supplied which shows the change in mass and humidity as a function of time.

[0072] The samples were analyzed using a TA Q2000 automated dynamic vapor sorption analyzer. The samples were dried at 40 °C for 5 hours and then cooled to 25 °C with a dry nitrogen purge over them until they no longer lost mass at 0% RH. The samples were then subjected to 0 to 95% RH, back to 0% RH at 25 °C in 5% RH steps.

[0073] Fourier Transform Infrared Spectroscopy (FTIR): The Infrared spectra were obtained using a Nicolet 510 M-O Fourier transform infrared spectrometer, equipped with a Harrick Splitpea™ attenuated total reflectance device. The spectra were acquired from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}; 128 scans were collected for each analysis.

[0074] Raman Spectroscopy: The Raman spectra were obtained with a Thermo DXR dispersive Raman spectrometer using laser excitation at 780 nm. The spectra were acquired from 3300 to 100 cm^{-1}. The samples were analyzed as bulk powders.

[0075] Screening of J147 samples: Initial testing was performed by XRD, DSC, TGA, proton NMR, FTIR, and Raman spectrometry. X-ray powder diffraction was used to examine the material to determine if it was crystalline. Figure 1 shows the XRD pattern of this material which was crystalline and designated as Form I. The corresponding peaks and their abundance are show in Table A below.

Table A

Angle	d value	Intensity	Intensity %
2-Theta °	Angstrom	Cps	%
8.64	10.23	0.07	35.4
11.85	7.46	0.09	42.2
12.84	6.89	0.04	20.4
13.36	6.62	0.08	36.2
16.04	5.52	0.05	23.8
17.11	5.18	0.09	44.7
17.79	4.98	0.21	100.0
19.25	4.61	0.08	38.4
21.64	4.10	0.08	37.6
23.40	3.80	0.09	42.0

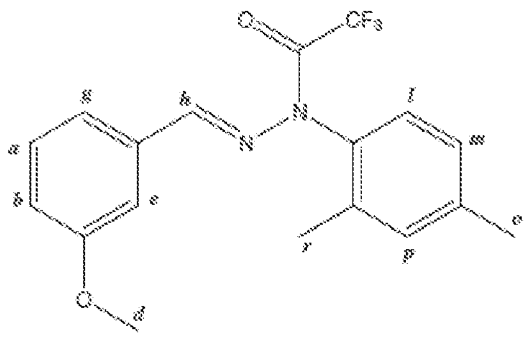
26.81	3.32	0.07	35.3
29.76	3.00	0.06	28.7

[0076] The thermal behavior of Form I was determined by DSC and TGA. The DSC thermogram exhibited a melting endotherm with an onset of 62.6 °C. The endotherm was split with peak maxima at 66.5 °C and 74.7 °C. The heat of fusion was 123.8 J/g. This split endotherm proved to be due to a mixture of two polymorphic forms as observed by hot stage microscopy as described further below. The scanning TGA thermogram indicated the sample was free of volatiles with a weight loss of less than 0.4 weight % from 25 °C to 104.7 °C. Figures 2A and 2B show the DSC and TGA thermograms, respectively.

[0077] Dynamic vapor sorption isotherms and the kinetic plots are shown in Figure 3A and 3B, respectively. The material proved very hydrophobic and did not appear to be prone to hydrate formation. A total weight *loss* of approximately 0.5 % was observed at 95% RH. This unusual event (weight loss with high humidity) may be due to differences in the adsorption characteristics of the sample and reference pans and not the J147 sample.

[0078] The Fourier transform infrared (FTIR) spectrum is shown in Figure 4. Based on visual inspection the spectrum is consistent with structure. The Raman spectrum is in agreement with the FTIR spectrum and is shown in Figure 5. The proton NMR data is consistent with the structure of J147 and is shown in Figure 6. The proton NMR data is also shown in tabulated form in Table B below.

Table B



Chemical Shift (ppm)	Peak Type	Peak Area Integral	Tentative Proton Assignment
7.298, 7.279 ~ 7.26	doublet doublet	4.94	a
7.256	singlet		chloroform
~7.26 to ~ 7.23	overlapping multiplets		p, e, h
~ 7.22 to ~ 7.19	broad multiplet	1.02	m
7.134 to 7.110	multiplet	1.04	g
7.059, 7.039	doublet	1.02	l
6.694 to 6.935	doublet doublet doublet	1.00	b
3.828	singlet	3.06	d
2.419	singlet	3.05	o, r
2.085	singlet	3.08	
1.547	broad peak	0.58	water
1.416	broad peak	0.13	impurities
1.255	singlet	1.20	
1.110	broad peak	0.38	
0897 to 0.862	overlapping multiplets	0.17	
0.000	singlet	NA	TMS

[0079] Polymorph Screening: The screen was performed using solvent-based recrystallization followed by X-ray diffraction analysis of the solids. Suspension slurry experiments and grinding were also employed to search for additional solid state forms.

[0080] Visual Solubility Measurement: Approximately 80 mg of J147 was placed into each of 25 vials. Solvent was added and vials were stirred for several minutes at ambient temperature, followed by visual observation for remaining solids. The solvent was incrementally added until the solids were dissolved, or until a maximum volume of solvent was added and the experiment was terminated. These stock solutions were used to set up further panels of experiments. J147 was found to be quite soluble in all solvents surveyed except for water. The visual solubility was determined and shown in Table 1.

Table 1

Solvent	**Approximate Solubility mg/mL**
methanol	> 80
ethanol	> 80
trifluoroethanol	> 80
1-propanol	> 80
2-propanol	40
1-butanol	40
2-butanol	40
water	< 1.6
dimethyl formamide (DMF)	> 80
dimethylacetamide (DMA)	> 80
butyl amine	> 80
pyridine	> 80
nitromethane	> 80

acetone	> 80
methyl ethyl ketone (MEK)	> 80
isopropyl ether	> 80
ethyl acetate	> 80
methyl tert butyl ether (MTBE)	> 80
isopropyl acetate	> 80
tetrahydrofuran (THF)	> 80
acetonitrile (ACN)	> 80
dichloromethane (DCM)	> 80
chloroform	> 80
toluene	> 80
heptane	8.0

[0081] Solvent Recrystallization: To perform the solvent-based portion of the polymorph screen, the test material was recrystallized using various solvents under approximately 150 different crystal growth conditions. The scale of the recrystallization experiments was from approximately 6 mL to 15 ml. The crystal growth conditions were changed by using binary gradient arrays of solvent mixtures. Saturated solutions were prepared by agitating excess (as possible) test material in contact with the various solvent systems at the saturation temperature. If solids did not completely dissolve in the solvent, the mother liquor was separated from the residual solids by filtration. The mother liquor was then heated above the saturation temperature (overheated) to dissolve any remaining solids. The temperature of each solution was then adjusted to the growth temperature and a controlled nitrogen shear flow was introduced to begin solvent evaporation. Due to the high solubility of J147 in the majority of solvents, an ambient growth temperature was used in all experiments. The recrystallization conditions for the six solvent based panels used are summarized in Table 2.

Each recrystallization panel contained from 15 to 27 wells. The wells within each panel contained different solvent compositions. Because of the different solvent composition in each well, each well acted as a different crystal growth experiment. Based on the XRD analysis carried out, a new polymorph of J147 was discovered. The first polymorph was designated as Form I while the second polymorph was designated as Form II. Table 2 summarizes the recrystallization panels for solvent-based polymorph screening. The compositional solvent matrices for the six recrystallization panels used during the solvent-based portion of the polymorph screen are shown in Tables 3 to 8.

Table 2

Panel	No. of Wells	Scale (mL)	Solvent	Saturation Temp. (°C)	Overheat Temp. (°C)	Growth Temp. (°C)	N_2 Flow Rate (psi)
1	25	15	Single	25	40	25	1
2	27	15	Binary	25	40	25	1
4	27	6	Binary	25	40	25	1
5	27	6	Binary	25	40	25	1
6	15	6	Binary	25	40	25	1

Table 3

Well	**Solvent**	**XRD Form**	**Appearance**
1	methanol	amorphous	orange glass
2	ethanol	amorphous	clear glass
3	trifluoroethanol	amorphous	clear glass
4	1-propanol	amorphous	clear glass
5	2-propanol	amorphous	clear glass
6	1-butanol	amorphous	clear glass

7	2-butanol	no sample	no sample
8	water	no sample	no sample
9	dimethyl formamide (DMF)	amorphous	clear glass
10	dimethylacetamide (DMA)	amorphous	clear glass
11	butyl amine	amorphous	orange glass
12	pyridine	amorphous	clear glass
13	nitromethane	I	yellow solid
14	acetone	amorphous	clear glass
15	methyl ethyl ketone (MEK)	I	yellow solid
16	isopropyl ether	amorphous	clear glass
17	ethyl acetate	amorphous	clear glass
18	methyl tert butyl ether (MTBE)	amorphous	clear glass
19	isopropyl acetate	amorphous	clear glass
20	tetrahydrofuran (THF)	I	white solid
21	acetonitrile (ACN)	amorphous	clear glass
22	dichloromethane (DCM)	amorphous	clear glass
23	chloroform	I	white solid
24	toluene	amorphous	clear glass
25	heptane	amorphous	clear glass

Table 4

Solvent Matrix and XRD Result for Recrystallization Panel 2					
		Ratio of Solvents			
Solvent	Sample ID	1	2	3	Co/Anti-Solvent
methanol	A	12:3	7.5:7.5	3:12	water
ethanol	B	12:3	7.5:7.5	3:12	water
trifluoroethanol	C	12:3	7.5:7.5	3:12	water
1-propanol	D	12:3	7.5:7.5	3:12	water
2-propanol	E	12:3	7.5:7.5	3:12	water
1-butanol	F	12:3	7.5:7.5	3:12	water
2-butanol	G	12:3	7.5:7.5	3:12	water
DMF	H	12:3	7.5:7.5	3:12	water
DMA	I	12:3	7.5:7.5	3:12	water
Solvent	Sample ID	XRD Form			Co/Anti-Solvent
		1	2	3	
methanol	A	I	amorphous	I	water
ethanol	B	I	I	I	water
trifluoroethanol	C	amorphous	I	I	water
1-propanol	D	I	amorphous	I	water
2-propanol	E	amorphous	I	I	water
1-butanol	F	amorphous	amorphous	amorphous	water
2-butanol	G	amorphous	I	amorphous	water
DMF	H	amorphous	amorphous	amorphous	water
DMA	I	amorphous	amorphous	amorphous	water

Table 5

Solvent Matrix and XRD Result for Recrystallization Panel 3					
		Ratio of Solvents			
Solvent	Sample ID	1	2	3	Co/Anti-Solvent
butyl amine	A	12:3	7.5:7.5	3:12	water
pyridine	B	12:3	7.5:7.5	3:12	water
nitromethane	C	12:3	7.5:7.5	3:12	water
acetone	D	12:3	7.5:7.5	3:12	water
isopropyl acetate	E	12:3	7.5:75	3:12	water
THF	F	12:3	7.5:7.5	3:12	water
ACN	G	12:3	7.5:7.5	3:12	water
heptane	H	12:3	7.5:7.5	3:12	water
isopropyl ether	I	12:3	7.5:7.5	3:12	water
Solvent	Sample ID		XRD Form		Co/Anti-Solvent
		1	2	3	
butyl amine	A	amorphous	amorphous	amorphous	water
pyridine	B	amorphous	amorphous	amorphous	water
nitromethane	C	amorphous	amorphous	I	water
acetone	D	I	amorphous	amorphous	water
isopropyl acetate	E	amorphous	amorphous	I	water
THF	F	amorphous	amorphous	I	water
ACN	G	amorphous	amorphous	I	water
heptane	H	I	I	amorphous	water
isopropyl ether	I	I	amorphous	amorphous	water

Table 6

Solvent Matrix and XRD Result for Recrystallization Panel 4					
		Ratio of Solvents			
Solvent	Sample ID	1	2	3	Co/Anti-Solvent
methanol	A	12:3	7.5:7.5	3:12	heptane
ethanol	B	12:3	7.5:7.5	3:12	heptane
trifluoroethanol	C	12:3	7.5:7.5	3:12	heptane
1-propanol	D	12:3	75:7.5	3:12	heptane
2-propanol	E	12:3	7.5:7.5	3:12	heptane
1-butanol	F	12:3	7.5:7.5	3:12	heptane
2-butanol	G	12:3	7.5:7.5	3:12	heptane
DMF	H	12:3	7.5:7.5	3:12	heptane
DMA	I	12:3	7.5:7.5	3:12	heptane
Solvent	Sample ID		XRD Form		Co/Anti-Solvent
		1	2	3	
methanol	A	amorphous	amorphous	amorphous	heptane
ethanol	B	amorphous	amorphous	amorphous	heptane
trifluoroethanol	C	amorphous	I	amorphous	heptane
1-propanol	D	amorphous	amorphous	amorphous	heptane
2-propanol	E	amorphous	amorphous	amorphous	heptane
1-butanol	F	amorphous	amorphous	not enough material	heptane
2-butanol	G	I	I	not enough material	heptane
DMF	H	amorphous	not enough material	amorphous	heptane
DMA	I	amorphous	amorphous	amorphous	heptane

Table 7

Solvent Matrix and XRD Result for Recrystallization Panel 5					
		Ratio of Solvents			
Solvent	Sample ID	1	2	3	Co/Anti-Solvent
butyl amine	A	12:3	7.5:7.5	3:12	heptane
pyridine	B	12:3	7.5:7.5	3:12	heptane
nitromethane	C	12:3	7.5:7.5	3:12	heptane
acetone	D	12:3	7.5:7.5	3:12	heptane
MEK	E	12:3	7.5:7.5	3:12	heptane
isopropyl ether	F	12:3	75:7.5	3:12	heptane
ethyl acetate	G	12:3	7.5:7.5	3:12	heptane
MTBE	H	12:3	7.5:7.5	3:12	heptane
isopropyl acetate	I	12:3	7.5:7.5	3:12	heptane
Solvent	Sample ID		XRD Form		Co/Anti-Solvent
		1	2	3	
butyl amine	A	amorphous	amorphous	amorphous	heptane
pyridine	B	amorphous	amorphous	not enough material	heptane
nitromethane	C	amorphous	amorphous	not enough material	heptane
acetone	D	amorphous	amorphous	amorphous	heptane
MEK	E	amorphous	amorphous	amorphous	heptane
isopropyl ether	F	amorphous	amorphous	amorphous	heptane
ethyl acetate	G	amorphous	amorphous	amorphous	heptane
MTBE	H	not enough material	amorphous	amorphous	heptane
isopropyl acetate	I	amorphous	amorphous	amorphous	heptane

Table 8

Solvent Matrix and XRD Result for Recrystallization Panel 6					
		Ratio of Solvents			
Solvent	Sample ID	1	2	3	Co/Anti-Solvent
THF	A	12:3	7.5:7.5	3:12	heptane
ACN	B	12:3	7.5:7.5	3:12	heptane
DCM	C	12:3	7.5:7.5	3:12	heptane
chloroform	D	12:3	7.5:7.5	3:12	heptane
toluene	E	12:3	7.5:7.5	3:12	heptane
Solvent	Sample ID		XRD Form		Co/Anti-Solvent
		1	2	3	
THF	A	amorphous	amorphous	amorphous	heptane
ACN	B	amorphous	amorphous	amorphous	heptane
DCM	C	amorphous	not enough material	amorphous	heptane
chloroform	D	not enough material	amorphous	amorphous	heptane
toluene	E	amorphous	amorphous	amorphous	heptane

[0082] Non-Competitive Slurry Experiments: In addition to the solvent recrystallization experiments, non-competitive slurry experiments were performed to search for new solid-state forms. These experiments rely on solubility differences of different polymorphic forms (if the compound exists in different polymorphic forms). As such, only polymorphs having a lower solubility (that is, are more stable) than the original crystalline form can result from a noncompetitive slurry experiment.

[0083] When a solid was mixed with solvent to create slurry, a saturated solution eventually resulted. The solution was saturated with respect to the polymorphic form dissolved.

However, the solution was supersaturated with respect to any polymorphic form that is more stable (more stable forms have lower solubility) than the polymorphic form initially dissolved. Therefore, any of the more stable polymorphic forms can nucleate and precipitate from solution. In addition, noncompetitive slurry experiments are often useful in identifying solvents that form solvates with the compound.

[0084] The slurry experiments were performed by exposing excess supplied material to solvents and agitating the resulting suspensions for several days at ambient temperature. The solids were filtered (Whatman Grade 1, 11 µm pore size) and analyzed by XRD to determine the resulting form(s). To avoid possible desolvation or physical change after isolation, the samples were not dried before X-ray analysis. A summary of non-competitive slurry experiments is shown in Table 9.

[0085]

Table 9

Vehicle	Initial Form	Duration	Final Form
Methanol/water (1:1)	I	6 days	II
Ethanol/water (1:1)	I	6 days	II
Trifluoroethanol/water (1:1)	I	6 days	II
1-propanol/water (1:1)	I	6 days	II
2-proponal/water (1:1)	I	6 days	II
DMF/water (1:1)	I	6 days	II
DMA/water (1:1)	I	6 days	II

[0086] Based on their X-ray scattering behavior, all of the slurry experiments of Form I resulted in Form II after 6 days of slurring. These data indicate that Form II is more stable than Form I at ambient temperature and pressure. No new polymorphs, solvates, or hydrates were isolated in these experiments.

[0087] Solids generated from the solvent based recrystallization panels and synthesized were analyzed by powder XRD. To mitigate preferred grain effects, a two dimensional detection system was used to collect all the XRD screening data. The two dimensional detector integrates along the concentric Debye cones which helps reduce pattern variation. An example of the Debye cone integration using a two dimensional detector is shown in Figure 7. If bright spots appear in the conical rings, it indicates strong preferred grain effects that can lead to considerable variability in the observed diffraction patterns including changes in peak intensities. Some samples of J147 exhibited preferred grain effects based on the appearance of the scattering behavior.

[0088] The results of this analysis revealed the material exists as two different polymorphs. The polymorphs were designated as Forms I and II. The initially supplied material was designated as Form I. The resulting form designation for each individual (solvent-based) recrystallization experiment is shown in Tables 3 through 8 above. The XRD data collected during the study was evaluated using a full profile chemometric treatment to determine if the crystalline form of the samples had changed during the experiment. To simplify the assessment, X-ray amorphous samples were not included in the chemometric treatment. The analysis entailed cluster analysis and multivariate statistics to group together any patterns that were determined to be statistically the same. The results of this analysis are summarized in the dendrogram in Figure 8, cell plot in Figure 9, and cluster plot in Figure 10. These figures provide a pictorial cluster analysis of product similarity.

[0089] The chemometric analysis of the diffraction data categorized the samples into 3 different groups (or clusters) labeled A through C. A summary showing the number of members in each group (A through C) is shown in Table 10.

Table 10

Group	Designated Form	Members
A	II	30
B	I	38
C	Low crystallinity, no form identified	1

[0090] The characteristics of each group/form are summarized as follows. After classifying the data into different forms based on diffraction behavior, each form was studied to determine if other properties of the forms could be differentiated. The characterization of each form began by comparing the diffraction data representative of each form with that from the other forms. This was generally followed by DSC, TGA, DVS analysis and microscopy.

[0091] Form I (Group B): The characteristic diffraction behavior of this form is shown in Figure 1. The XRD patterns of the Form I samples were all crystalline and very similar. This form was obtained from a variety of solvents in approximately 50% of the experiments. The DSC profile of the Form I samples exhibited a melting endotherm with an onset of approximately 63 °C. In all but one of the Form I samples analyzed, the endotherm was split with peak maximums ranging from approximately 67 °C to 75 °C. This split endotherm is believed to be due to a mixture of two polymorphic forms as observed by hot stage microscopy. A representative DSC thermogram is shown in Figure 2A. A sample of Form I exhibited a single endotherm in the DSC profile with an onset of melting at approximately 63° C. The XRD pattern matched that of the other Form I samples. Figures 11A and 11B show the DSC and XRD profiles, respectively, of this sample. The scanning TGA thermogram of Form I (Figure 2B) showed it to be free from volatiles with a weight loss of less than 0.4 weight % from 25 °C to 104.7 °C.

[0092] Microscopic evaluation of Form I showed a mixture of columnar and needle shaped particles ranging from approximately 10 to 300 microns in length. Upon heating this sample, the larger particles appear to melt at approximately 67 °C, with the smaller needles unmelted. Figures 12A and 12B show representative photomicrographs at room temperature and 67 °C. This along with the multiple endotherms in the DSC profile suggests the majority of the Form I samples may actually be a mixture of Forms I and II.

[0093] The dynamic vapor sorption isotherms and the kinetic plots for a representative sample of Form I are shown in Figures 3A and 3B, respectively. The material is very hydrophobic and does not appear to be prone to hydrate formation. A total weight *loss* of approximately 0.5 % was observed at 95% RH. This unusual event (weight loss with high humidity) is most likely due to differences in the adsorption characteristics of the sample and reference pans and not the J147 sample. A sample of Form I was placed in a 40 °C in oven over weekend. XRD data showed a conversion to Form II. A variable temperature (VT) XRD experiment was performed on Form I. A sample of Form I was held in the XRD at 35°C over a weekend with no form conversion observed. Overall, Form I was attributed to a dry crystalline, polymorphic form of the compound.

[0094] Form II (Group A): The Form II polymorph was not obtained from the recrystallization screening experiments but was observed in approximately 50% of all experiments. The XRD patterns representative of Form II samples indicate that the samples were crystalline and very similar. Figure 13 shows a characteristic XRD pattern of Form II. The peaks are summarized in Table C below.

Table C

Angle 2-Theta °	d value Angstrom	Intensity Cps	Intensity % %
12.00	7.37	0.08	6.9
13.37	6.62	0.52	47.4
17.74	5.00	0.14	13.1
18.47	4.80	0.19	17.4
20.39	4.35	0.18	16.7
23.34	3.81	1.10	100.0
26.25	3.39	0.12	11.0
27.28	3.27	0.12	10.5
28.74	3.10	0.11	9.6
35.60	2.52	0.08	7.3
38.91	2.31	0.08	7.0

[0095] The DSC thermograms of this form exhibit a well-defined melting endotherm with an extrapolated onset of approximately 74 °C, a peak maximum of approximately 75 °C and an enthalpy of fusion of approximately 68 J/g. Figure 14A shows the DSC profile of Form II. The TGA thermogram for Form II showed it to be free from volatiles with a weight loss less than 0.1% from 25 °C to 75 °C. Figure 14B shows the TGA profile. Microscopic evaluation of Form II showed birefringent needle shaped particles ranging from approximately 10 to 50 microns in length. Figure 15 shows a representative photomicrograph of Form II crystals at room temperature. Upon heating, one melting event was observed at approximately 75°C. Figures 16A and 16B show the moisture sorption-desorption isotherm and the kinetic plots, respectively, for Form II. As seen with Form I, This form is also very hydrophobic and does not appear to be prone to hydrate formation A total weight loss of approximately 0.5 % was observed at 95% RH. This unusual event (weight loss with high humidity) is most likely due to differences in the adsorption characteristics of the sample and reference pans and not the J147 sample. The single crystal predicted powder XRD pattern matched the pattern of experimental Form II as shown in Figure 17. A sample of Form II was placed in a 40 °C in oven over weekend. XRD data showed no form conversion. Overall, Form II was attributed to a dry crystalline polymorphic form of the compound.

[0096] A number J147 samples were produced in this Example. The polymorph screen recrystallization experiments produced either Form I or an amorphous form of J147 (as shown in Table 11). The Form II polymorph was produced by creating a saturated slurry of Form 1 and agitating/stirring for several days at ambient temperature. Many of Form I samples also contained some amount of Form II indicating that the two polymorphs have a tendency to nucleate and grow concomitantly. Form II appears to be the thermodynamically stable form under ambient conditions based on the results of the competitive slurry experiments.

Table 11

Panel No.	No. of Experiments	Form I	Amorphous
Panel 1	25	4	21
Panel 2	27	12	15
Panel 3	27	8	19
Panel 4	27	3	24
Panel 5	27	None	26
Panel 6	15	None	13
Total	148	27	118
% of total	100%	18%	80%

[0097] Competitive Slurry Experiments: In addition to the solvent recrystallization experiments, two competitive slurry experiments were also performed to determine the most stable form. These experiments rely on the solubility differences of different polymorphic forms. As such, only polymorphic forms (and solvates) having a lower solubility (more stable) than the form initially dissolved can result from a competitive slurry experiment.

[0098] The slurry experiments were performed by exposing excess material of Forms I and II to a small volume of solvent/water and agitating the resulting suspensions for several days at ambient temperature. The solids were filtered and analyzed by XRD and DSC to determine the resulting form. To avoid possible desolvation or physical change after isolation, the sample was not dried before x-ray analysis. Table 12 shows the results of the competitive slurry experiment.

Table 12

Initial Forms (XRD)	Solvent	Slurry Duration	Final Form (XRD/DSC)
I & II	1:1 ratio ethanol:water	4 days	II
I & II	1:1 ratio methanol:water	8 days	II

[0099] The competitive slurry experiments resulted in transformation of the sample to Form II. These results suggest that Form II is a less soluble, more thermodynamically stable polymorph relative to Form I at ambient temperature and pressure.

[00100] Grinding: Form I was ground using a Crescent Wig-L-Bug ball mill for 1 minute at 4800 oscillations per minute (3.2 m/s). XRD analysis showed no transformation under these conditions.

[00101] Recrystallization Using Heat: Amorphous/glass samples from Panel 1 were placed in a vacuum oven at 60 °C for 6 days. XRD analysis of these solids exhibited the XRD

pattern of Form II. A sample of Form I was placed in a 40°C in oven over a weekend. XRD data showed a conversion to Form II. A variable temperature (VT) XRD experiment was performed on Form I. A sample of Form I was held in the XRD at 35°C over a weekend with no form conversion observed.

[00102] Thermodynamic Relationship: DSC experiments were performed in order to obtain heat of fusion and melting data. This data can often be used to determine if polymorphs exist in an enantiotropic or monotropic relationship. The heat of fusion rule states that, if the higher melting polymorph has the lower heat of fusion, the two forms are enantiotropes. Conversely, if the higher melting polymorph has a higher heat of fusion, the two forms are monotropes. For a monotropic system, any transition from one polymorph to another is irreversible. For an enantiotropic system, it may be possible to convert reversibly between the two polymorphs on heating and cooling.

[00103] A sample of pure Form I and two samples of Form II were analyzed by DSC at a slow heating rate of 2° C/minute with similar sample sizes. The average melting temperatures and heat of fusion data, from 10 runs, is shown in Table 13. These data indicate that Form I has a lower melting temperature and that Form II has a higher melting temperature. The heat of fusion values are very close with a high standard deviation for the Form I sample.

Table 13

Sample ID	Onset (°C)	Heat of Fusion (J/g)
Form I	63.7±0.4	71.5 ±7.6
Form II	73.9±0.1	70.3±0.9

[00104] Static Vapor Sorption Study: A dynamic vapor sorption study was done in a sealed humidity chamber with an automatic moisture sorption balance. Data collected during dynamic vapor sorption studies often are not at thermodynamic equilibrium. To determine if

the Form I and Form II material form a hydrate over time, samples were monitored in a 75% static humidity chamber.

[00105] In these studies, samples were stored in open Petri dishes in a chamber containing a saturated salt solution to maintain the relative vapor pressure. A solution of saturated sodium chloride (75% RH) salt at ambient temperature was used.

[00106] The samples were examined gravimetrically after 5 and 12 days. After both time periods, neither Form I nor II showed significant weight change, indicating no hydrate formation.

[00107] The raw diffraction data generated from the polymorph screening experiments were categorized into two polymorphic forms. Samples of these different forms were used to perform additional experiments (DSC, TGA, HSM, etc.) to refine the forms. A brief description of the discovered polymorphic forms is summarized in Table 14.

Table 14

Form Designation	Description	Comments
Form I	Metastable	Pure Form I difficult to obtain. Tendency to nucleate and grow concomitantly with Form II.
Form II	Thermodynamically Stable	Good crystallinity and thermal properties. Form II readily obtained from solvent slurry and heat treatment of Form I.

[00108] Table 14 summarizes the different solid state forms of J147 observed during the study. The main result is the discovery of two anhydrous polymorphic forms of J147. The polymorphic forms isolated in this Example were designated I and II. At room temperature and pressure Form II is the thermodynamically form of J147. Form I is the metastable form at room temperature and pressure.

[00109] Various experimental results have confirmed Form I is the metastable form at room temperature and pressure. Evidence of a transformation of Form I to Form II was

observed after approximately 3 days of storage at 40°C. A competitive slurry of a 50/50 mix of Forms I and II in 2 different solvent systems (at ambient temperature) showed conversion of Form I to Form II after 4 and 8 days. Non-competitive slurry experiments of Form I in 7 different solvents systems showed conversion to Form II after 6 days.

[00110] The screen entailed subjecting the material to solvent crystallization, heating, grinding, sorption experiments, and competitive and noncompetitive slurry experiments. Overall, the J147 was recrystallized under more than 150 different crystal growth conditions and analyzed using powder x-ray diffraction. The x-ray data was used to categorize the samples into different groups. These groups were studied using thermal, optical, spectroscopic, and other tools to elucidate the unique solid state forms of the API. In general, the J147 exhibits two different polymorphic forms designated as Forms I and II in addition to the amorphous form. No solvates or hydrates were uncovered in this Example. Of the two polymorphic forms, the thermodynamically stable polymorph under ambient conditions was Form II.

[00111] EXAMPLE OF PREPARATION OF FORM II OF J147

Batch Process: About 100 kg of crude J147 from its synthetic preparation was evaporated twice from about 80 kg of ethanol. The crude product was taken up in about 48 kg of ethanol and the batch temperature was adjusted to 28 °C. About 37 kg of water was added gradually to the batch. The batch was held at about 30 °C for about 1.7 hours. A sample of the batch was pulled from the reactor and solids precipitated by addition of 45 mL of water. The solids obtained were added back to the batch as seed crystals and the mixture stirred for 40 minutes at 30 °C. An additional about 34 kg of water was added. The batch was held at about 18 °C for about 58 hours and then cooled to about 10 °C for another about 5.5 hours. Analysis of the resultant solids indicated the presence of Form I. Form I was converted to Form II by heating the slurry to about 45 °C for about 16 hours and then cooling back to about 10 °C and holding the batch at this temperature for about 3 hours. about 17.7 kg of solid Form II of J147 were recovered by filtration after washing and drying.

WHAT IS CLAIMED IS:

1. A method of making crystalline Form II of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta, the method comprising:

providing a slurry comprising saturated amorphous or crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene] hydrazide (J147) in a solvent/anti-solvent mixture; and

mixing the slurry to provide crystalline Form II.

2. The method of claim 1, wherein the slurry comprises saturated J147 in crystalline Form I.

3. The method of claims 1-2, wherein the mixing step is carried out from about 25 °C to about 50 °C.

4. The method of claims 1-2, wherein the mixing step is carried out from about 40 °C to about 50 °C.

5. The method of claims 1-4, wherein the anti-solvent is water.

6. The method of claims 1-5, wherein the solvent is an alcohol.

7. The method of claim 6, wherein the alcohol is selected from the group consisting of methanol, ethanol, trifluoroethanol, 1-propanol, and 2-propanol.

8. The method of claim 6, wherein the alcohol is ethanol.

9. The method of claims 1-5, wherein the solvent is dimethylformamide (DMF), or dimethylacetamide (DMA).

10. The method of claims 1-4, wherein the solvent/anti-solvent mixture is ethanol-water.

11. The method of claims 1-10, wherein a ratio of the solvent/anti-solvent mixture is in a range from about 4:1 to about 1:4.

12. The method of claims 1-11, wherein a ratio of the solvent/anti-solvent mixture is about 1:2.

13. The method of claims 1-12, wherein mixing occurs over about 6 hours to about 6 days.

14. The method of claims 1-13, wherein a yield of Form II is in a range from about 50% to about 100%.

15. The method of claims 1-14, wherein a purity of Form II is at least 98%.

16. The method of claims 1-15, wherein crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147) having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, is obtained by:

recrystallizing 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide from an organic solvent selected from the group consisting of nitromethane, methylethylketone, tetrahydrofuran, acetone, acetonitrile, heptane, isopropyl ether, isopropyl acetate, and chloroform, and optionally in the presence of water as anti-solvent.

17. A method of making crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, the method comprising:

recrystallizing 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide from an organic solvent selected from the group consisting of nitromethane, methylethylketone, tetrahydrofuran, acetone, acetonitrile, heptane, isopropyl ether, isopropyl acetate, and chloroform, and optionally further comprising water as anti-solvent.

18. The method of claim 17, wherein the recrystallizing is performed with water as the anti-solvent and the ratio of organic solvent to anti-solvent is in a range from about 4:1 to about 1:4.

19. A method of making crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, the method comprising:

recrystallizing 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide from a solvent-anti-solvent mixture comprising an alcohol as the solvent and water or heptane as the anti-solvent.

20. The method of claim 19, wherein the ratio of solvent to the anti-solvent is in a range from about 4:1 to about 1:4.

21. The method of claims 19-20, wherein the alcohol of the alcohol is a C_1-C_4 alcohol.

22. The method of claim 21, wherein the alcohol is selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol, trifluoroethanol, 1-butanol and 2-butanol.

23. The method of claims 19-22, wherein the anti-solvent is water.

24. Isolated crystalline Form I of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta.

25. Isolated crystalline Form I of claim 24, further comprising X-ray diffraction peaks located at 8.64, 13.36, 19.25, 21.64, and 26.81 +/- 0.2 degrees 2-theta.

26. Isolated crystalline Form II of 2,2,2-trifluoroacetic acid 1-(2,4-Dimethylphenyl)-2-[(3-methoxyphenyl)methylene]hydrazide (J147), having a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta.

27. Isolated crystalline Form II of claim 26, further comprising X-ray diffraction peaks located at 17.74, 20.39, 26.25, 21.64, and 28.74 +/- 0.2 degrees 2-theta.

J147 starting material

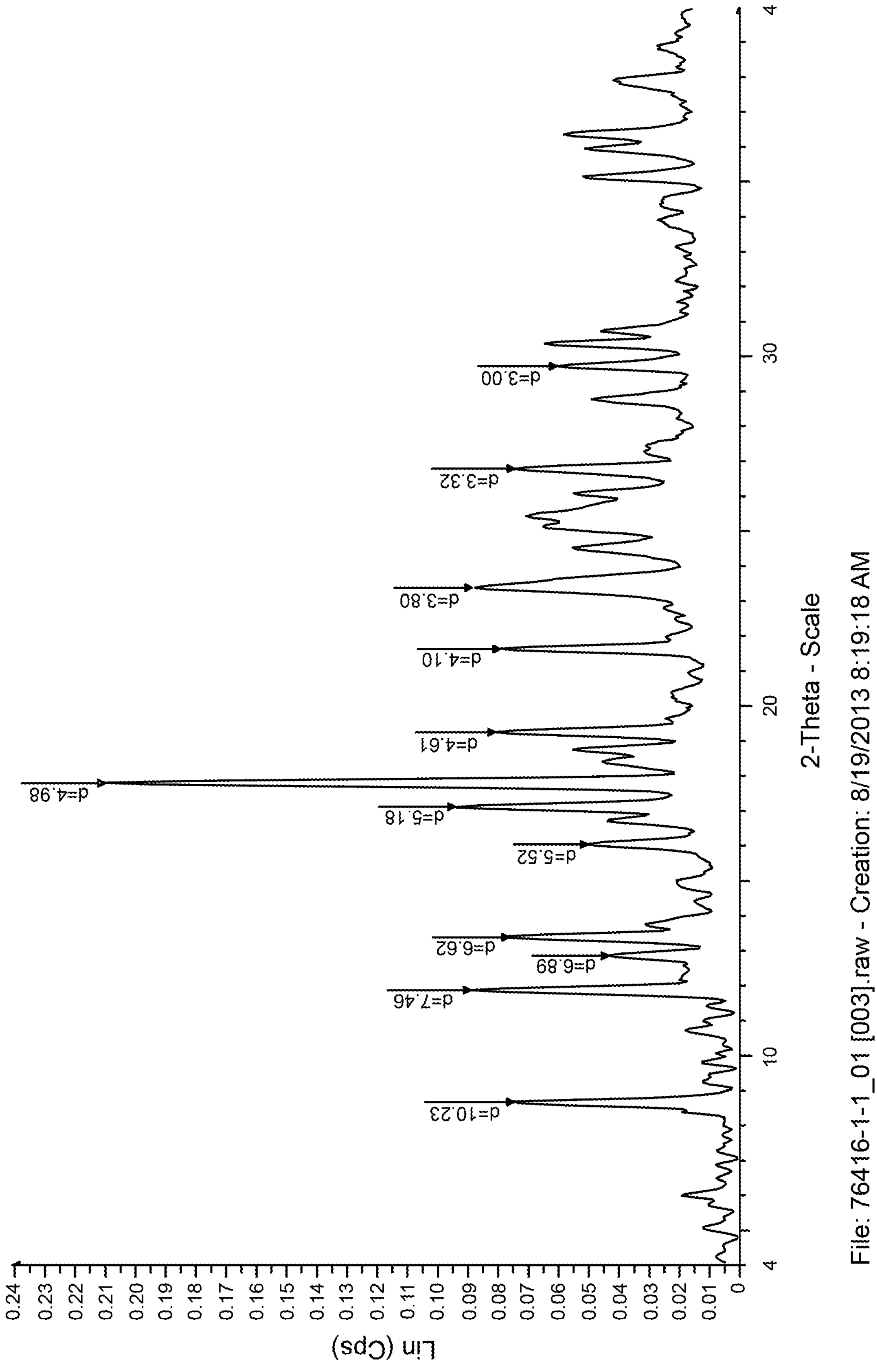


FIG 1

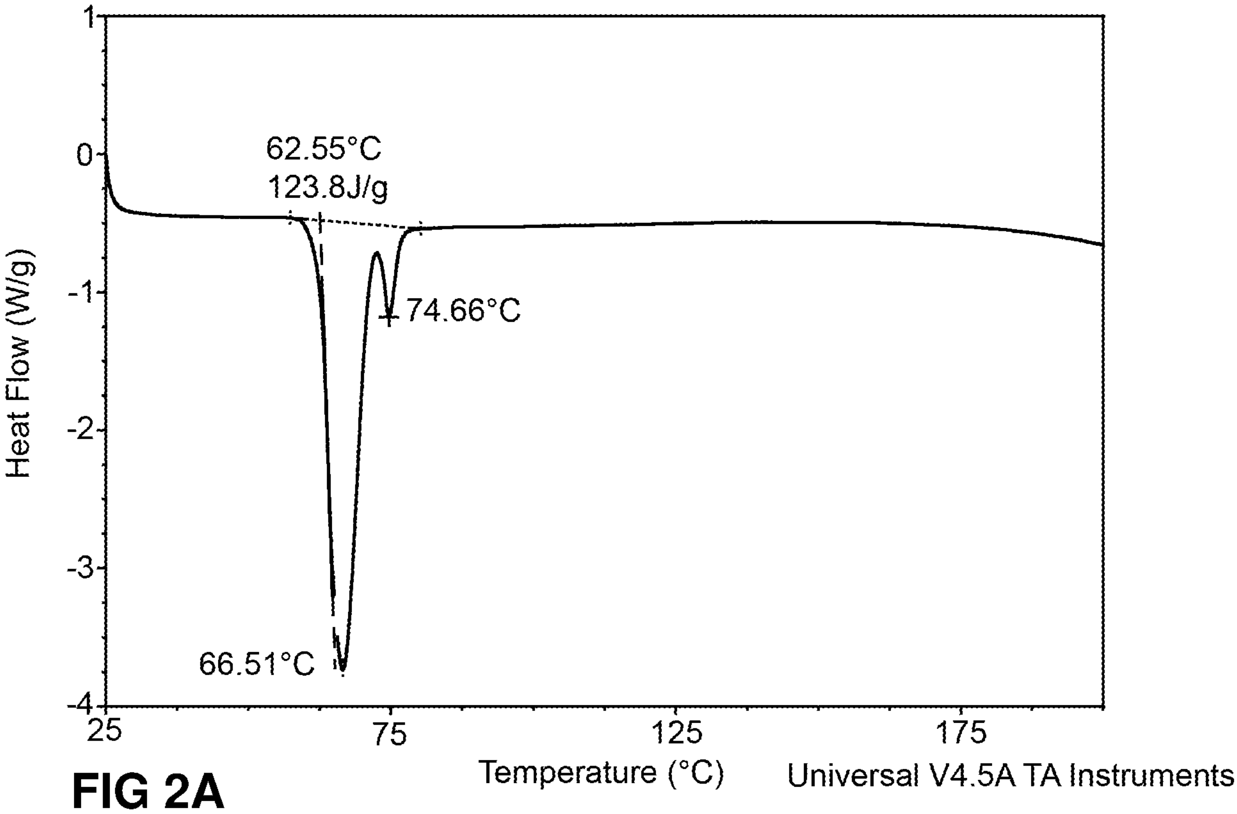

62.55°C
123.8J/g
74.66°C
66.51°C
Heat Flow (W/g)
Temperature (°C)
Universal V4.5A TA Instruments

FIG 2A

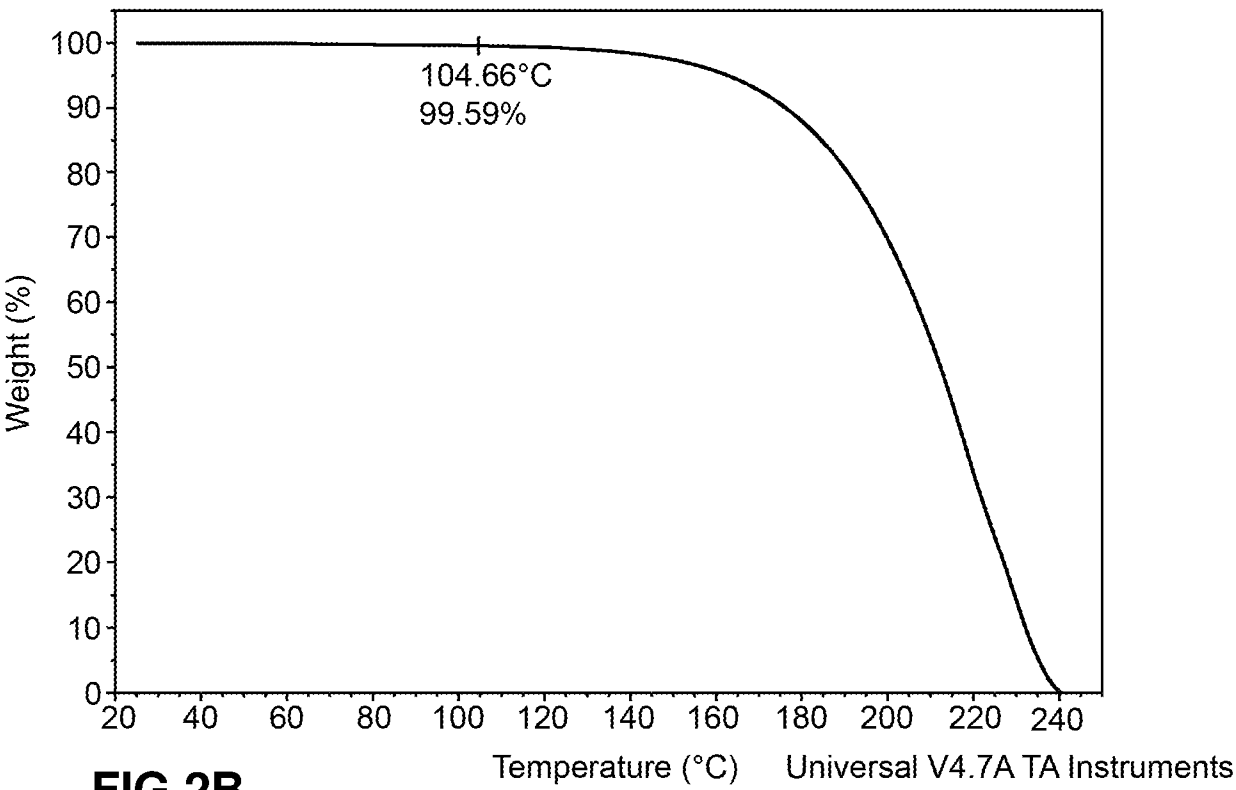

104.66°C
99.59%
Weight (%)
Temperature (°C)
Universal V4.7A TA Instruments

FIG 2B

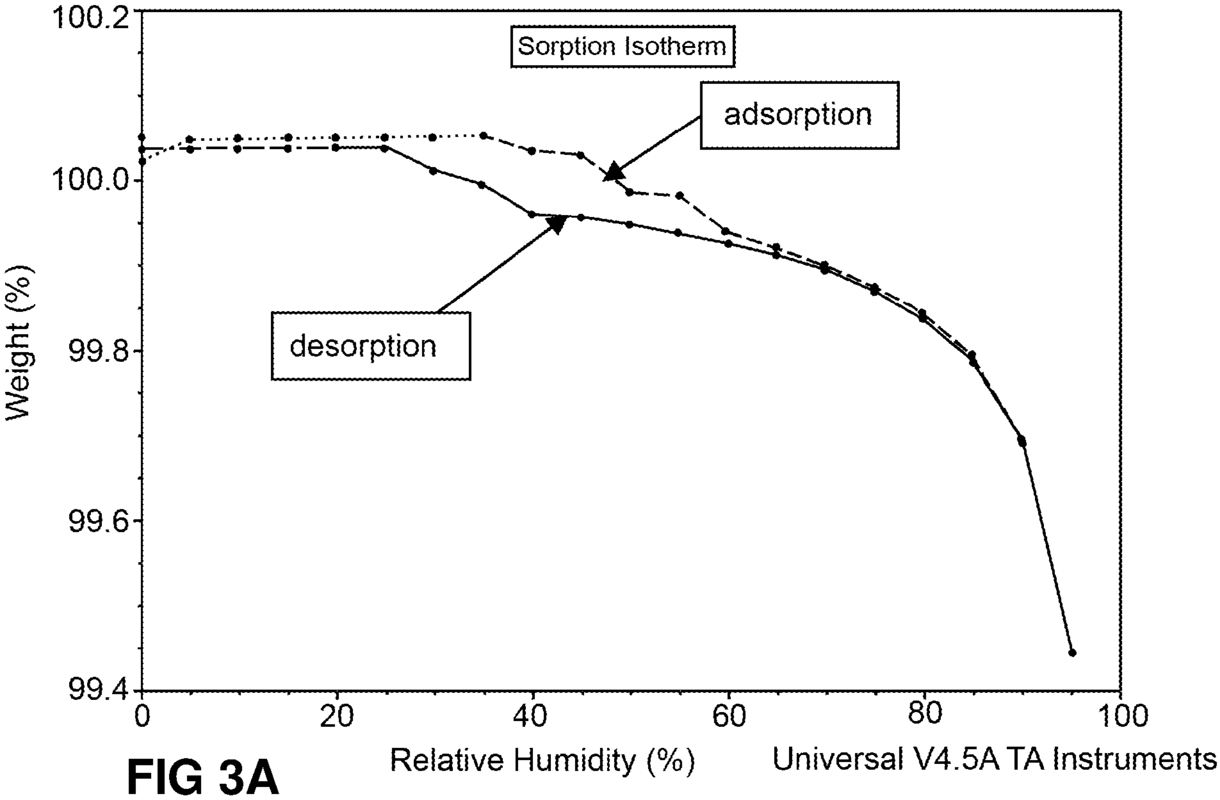

100.2
Sorption Isotherm
adsorption
100.0
desorption
99.8
99.6
99.4
Weight (%)
0
20
40
60
80
100
Relative Humidity (%)
Universal V4.5A TA Instruments

FIG 3A

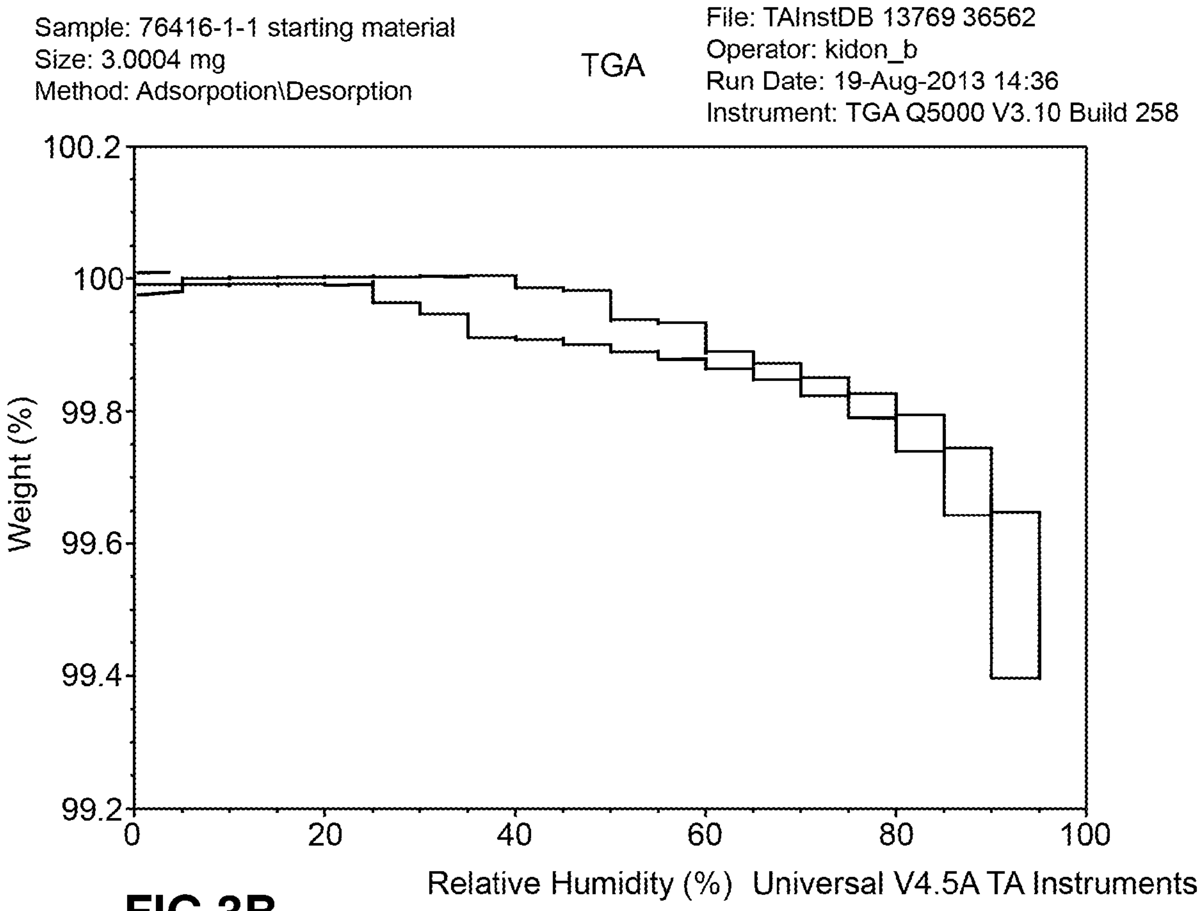

Sample: 76416-1-1 starting material
Size: 3.0004 mg
Method: Adsorpotion\Desorption
TGA
File: TAInstDB 13769 36562
Operator: kidon_b
Run Date: 19-Aug-2013 14:36
Instrument: TGA Q5000 V3.10 Build 258
100.2
100
99.8
99.6
99.4
99.2
Weight (%)
0
20
40
60
80
100
Relative Humidity (%)
Universal V4.5A TA Instruments

FIG 3B

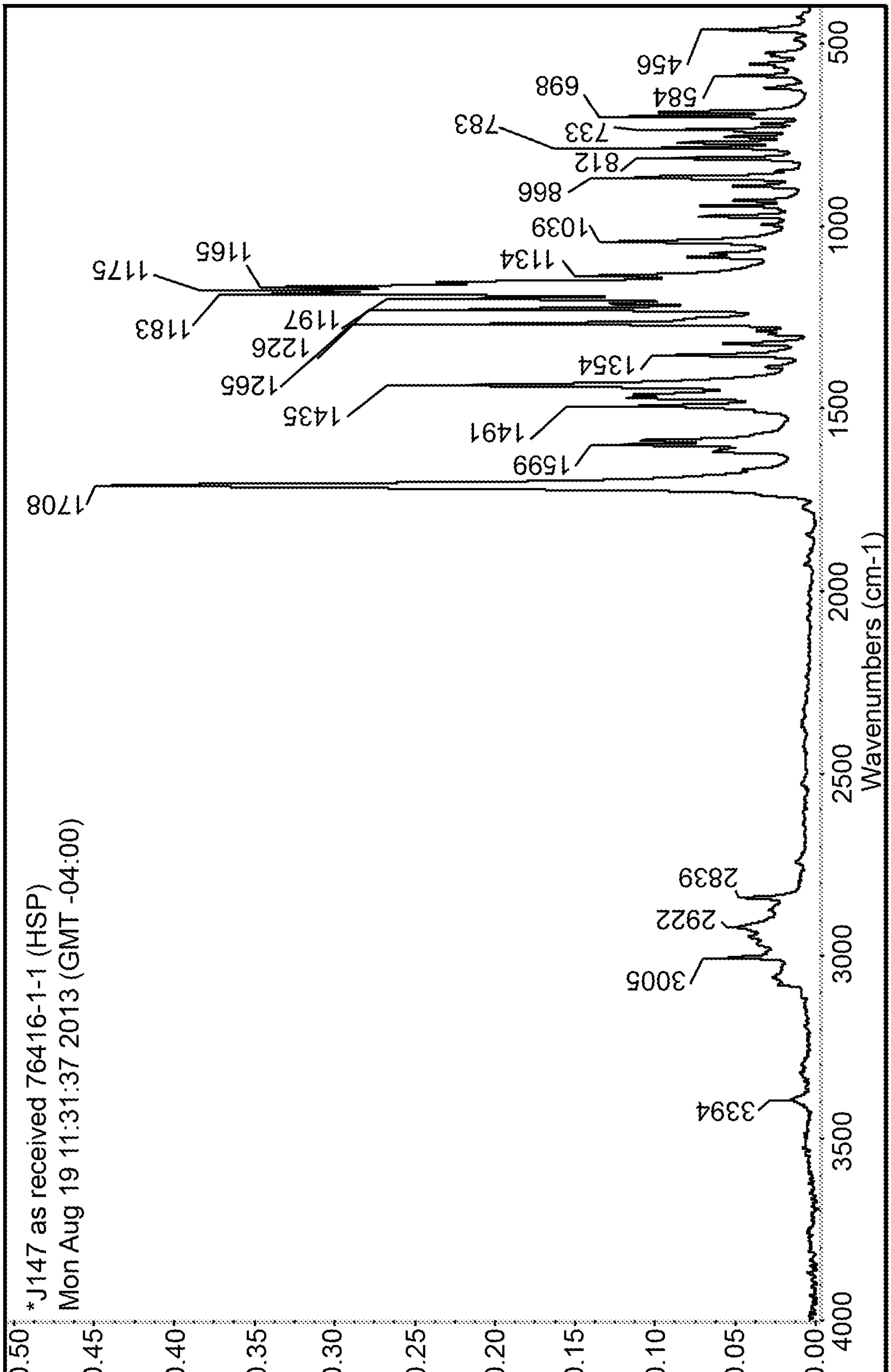

*J147 as received 76416-1-1 (HSP)
Mon Aug 19 11:31:37 2013 (GMT -04:00)
Absorbance
Wavenumbers (cm-1)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
4000
3500
3000
2500
2000
1500
1000
500
3394
3005
2922
2839
1708
1599
1491
1435
1354
1265
1226
1197
1183
1175
1165
1134
1039
866
812
783
733
698
584
456

FIG 4

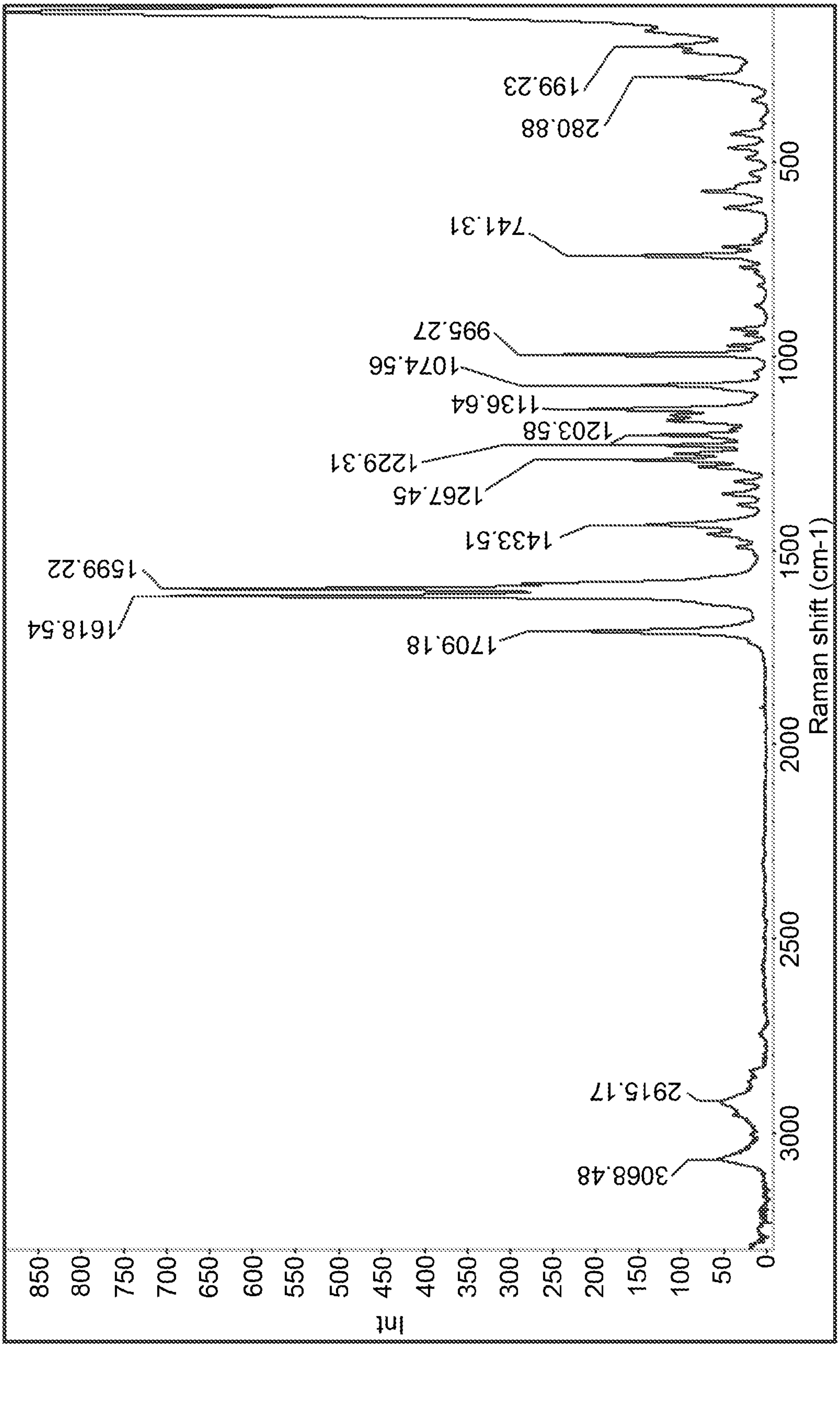

Int
Raman shift (cm-1)
199.23
280.88
741.31
995.27
1074.56
1136.64
1203.58
1229.31
1267.45
1433.51
1599.22
1618.54
1709.18
2915.17
3068.48

FIG 5

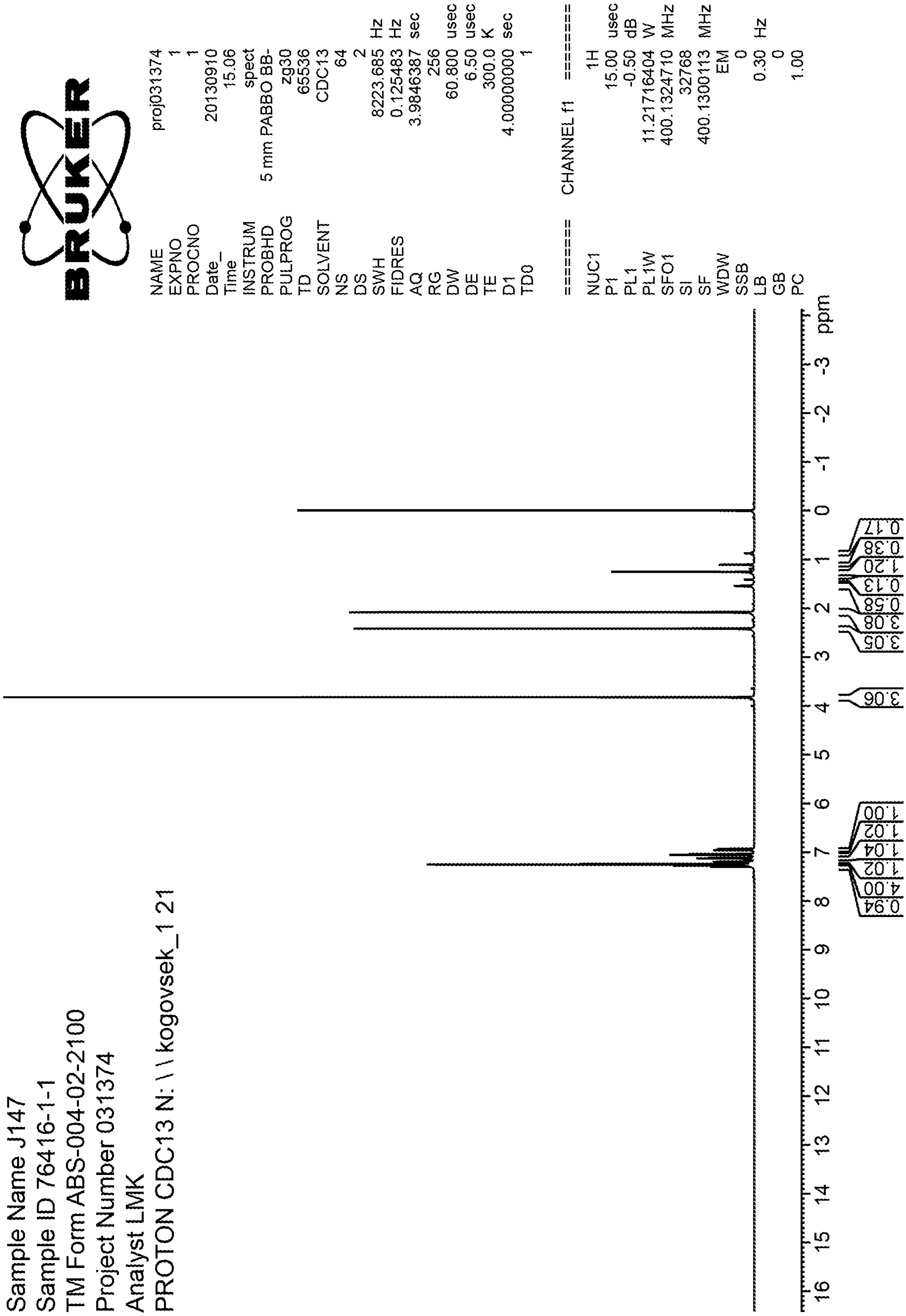

Sample Name J147
Sample ID 76416-1-1
TM Form ABS-004-02-2100
Project Number 031374
Analyst LMK
PROTON CDC13 N: \ \ kogovsek_1 21
NAME proj031374
EXPNO 1
PROCNO 1
Date_ 20130910
Time 15.06
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 64
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 256
DW 60.800 usec
DE 6.50 usec
TE 300.0 K
D1 4.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 -0.50 dB
PL1W 11.21716404 W
SFO1 400.1324710 MHz
SI 32768
SF 400.1300113 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.17
0.38
1.20
0.13
0.58
3.08
3.05
3.06
1.00
1.02
1.04
1.02
4.00
0.94
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 ppm

FIG 6

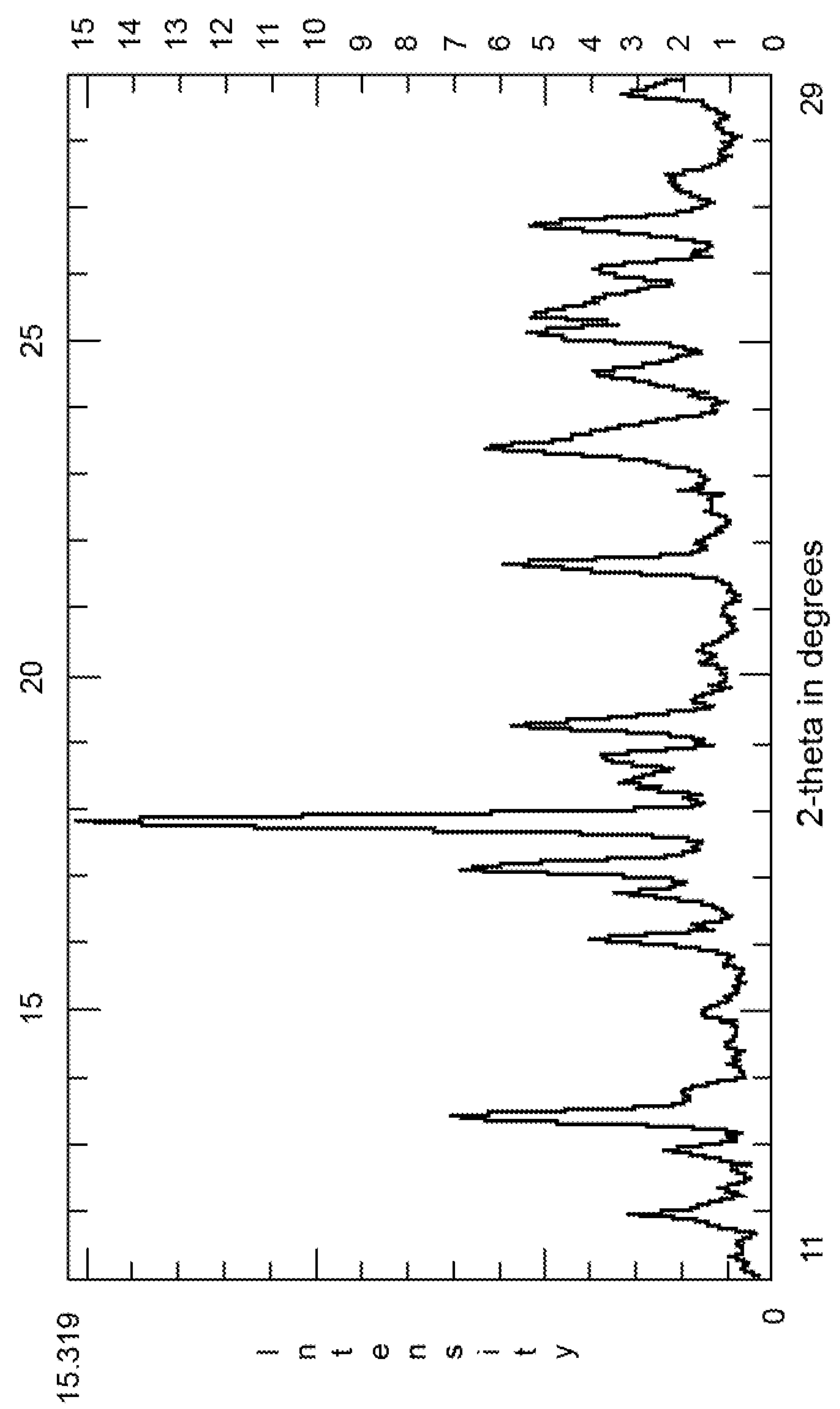

15.319
Intensity
0
2-theta in degrees
11
15
20
25
29
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

FIG 7

s

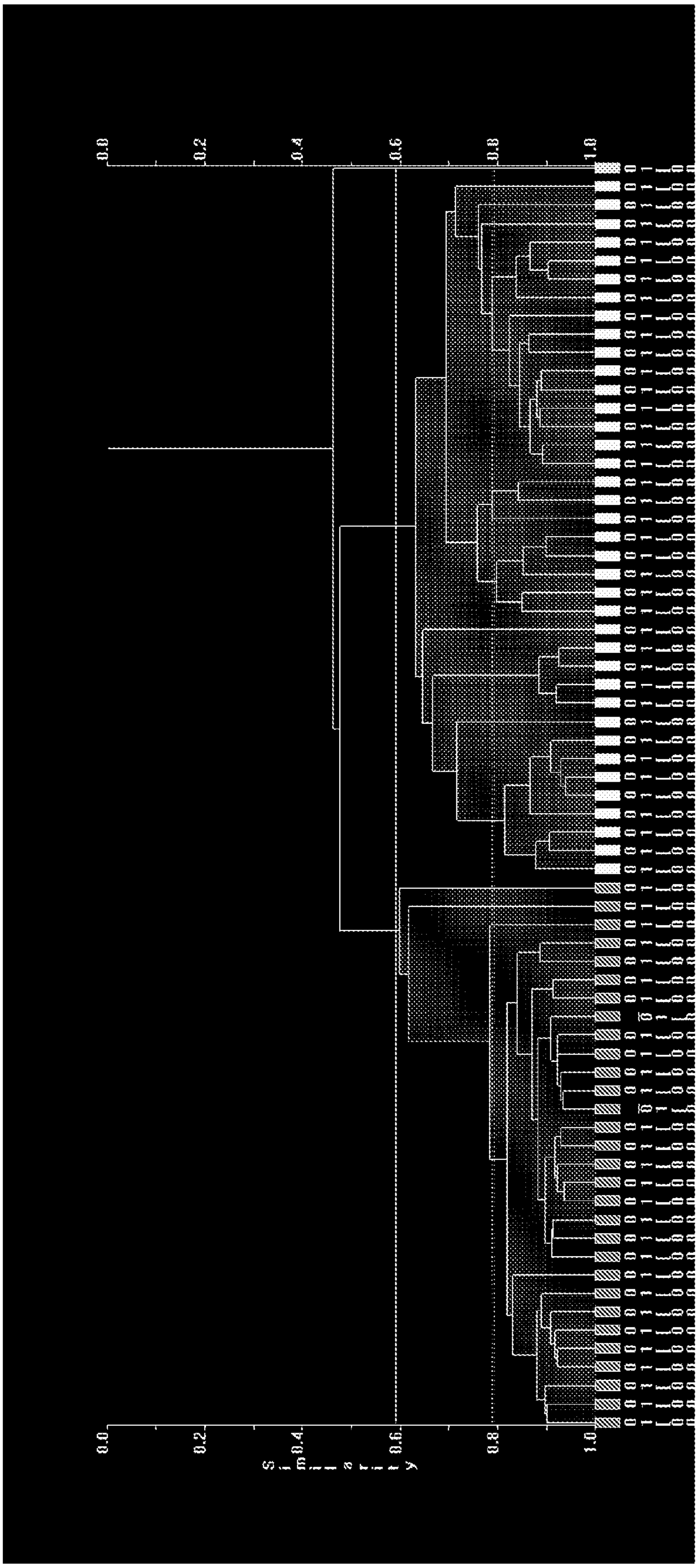


FIG 8

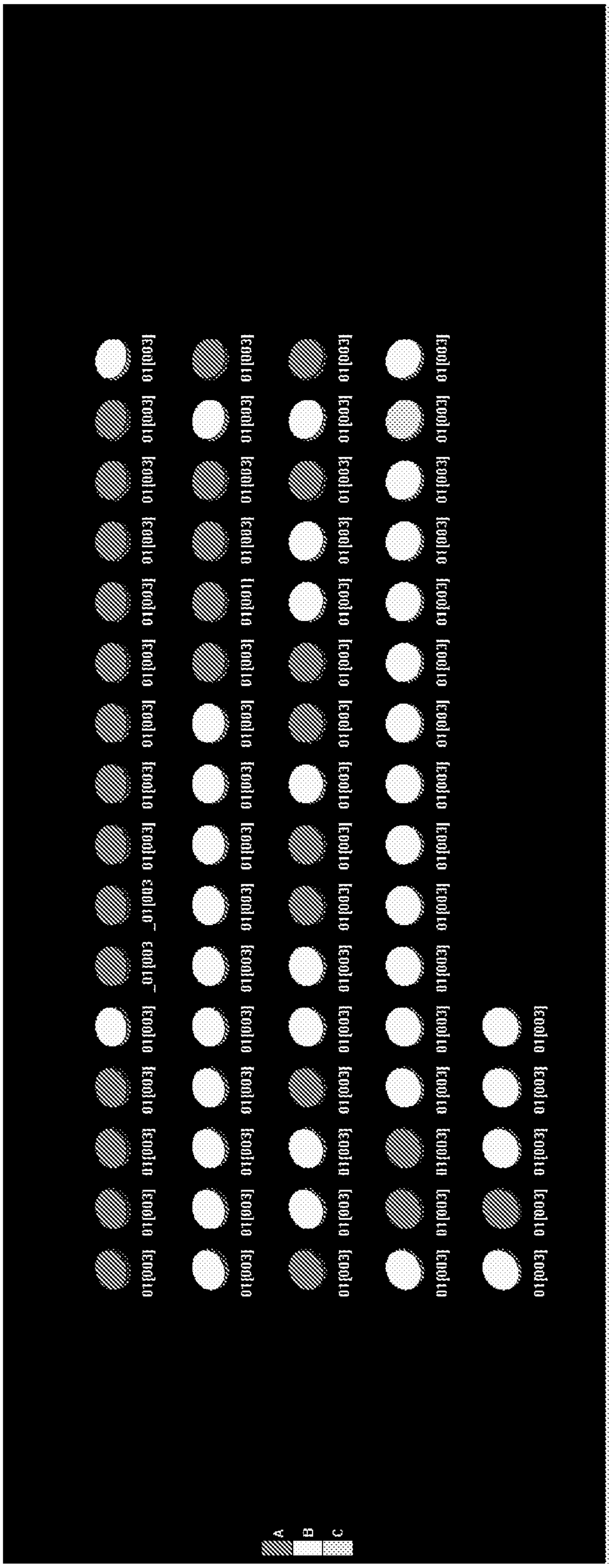


FIG 9

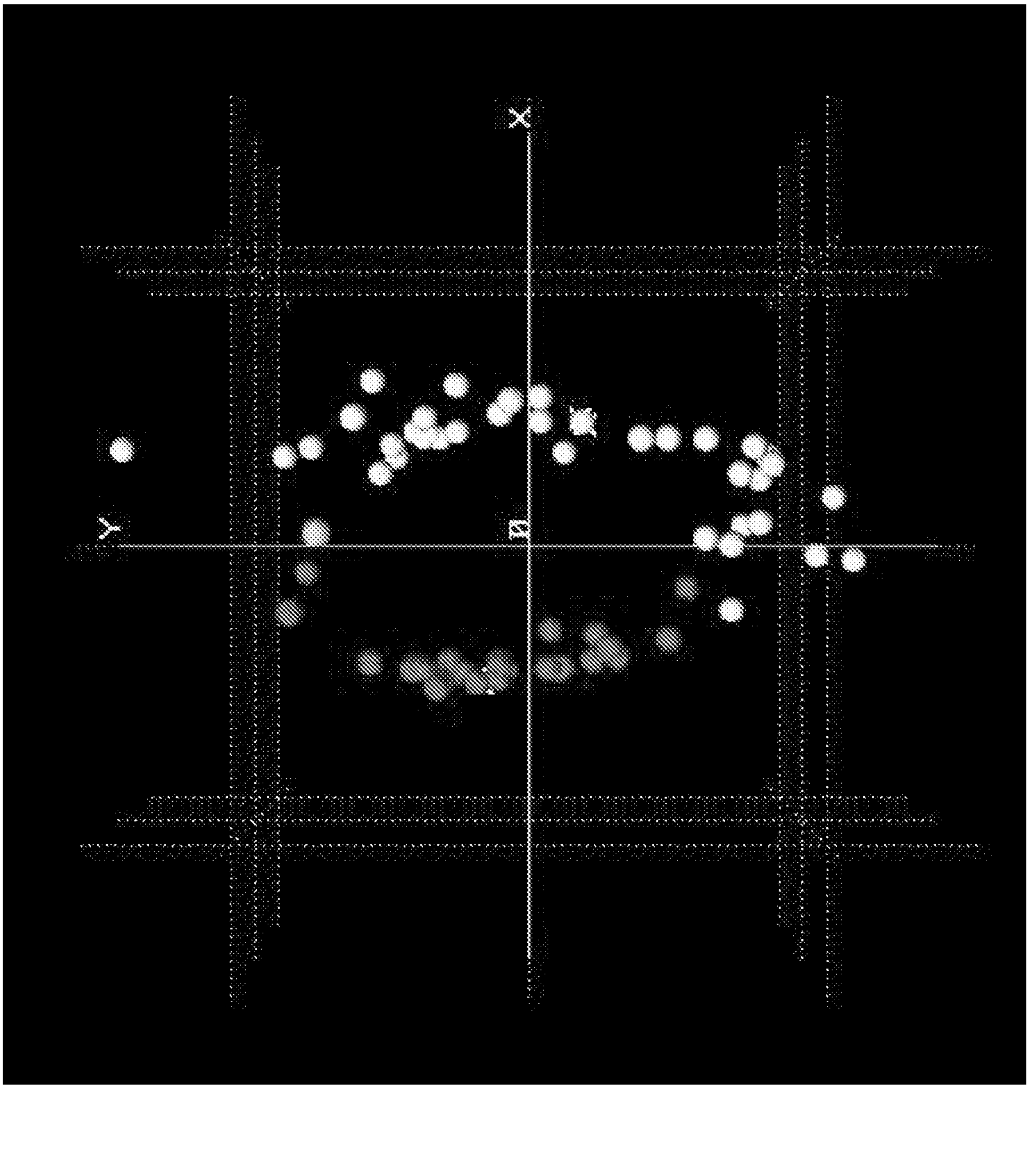


FIG 10

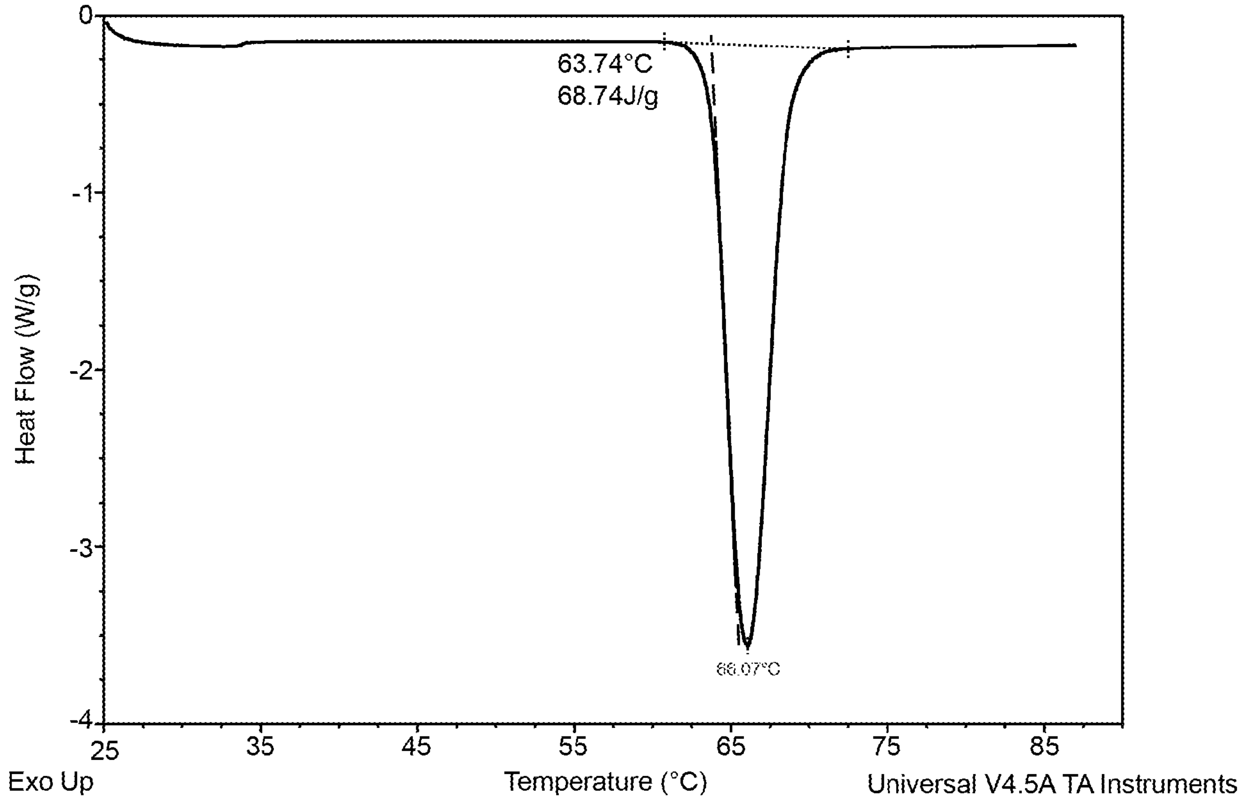


FIG 11A

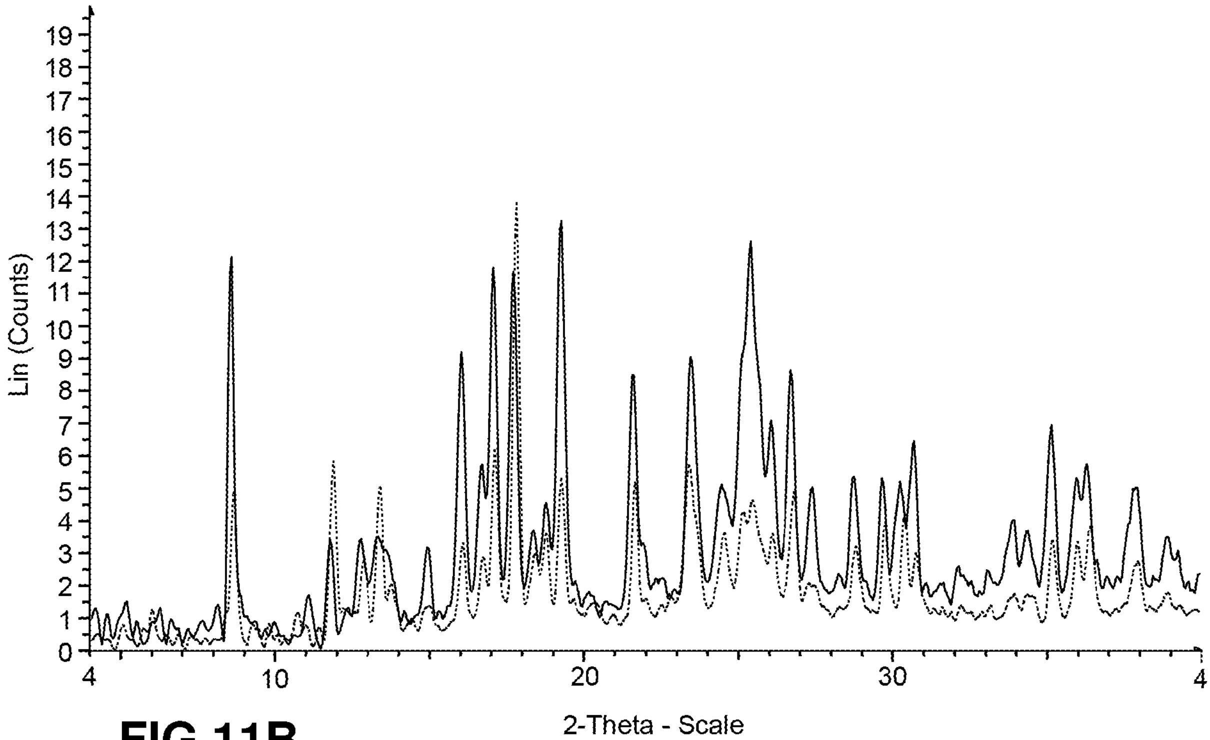


FIG 11B

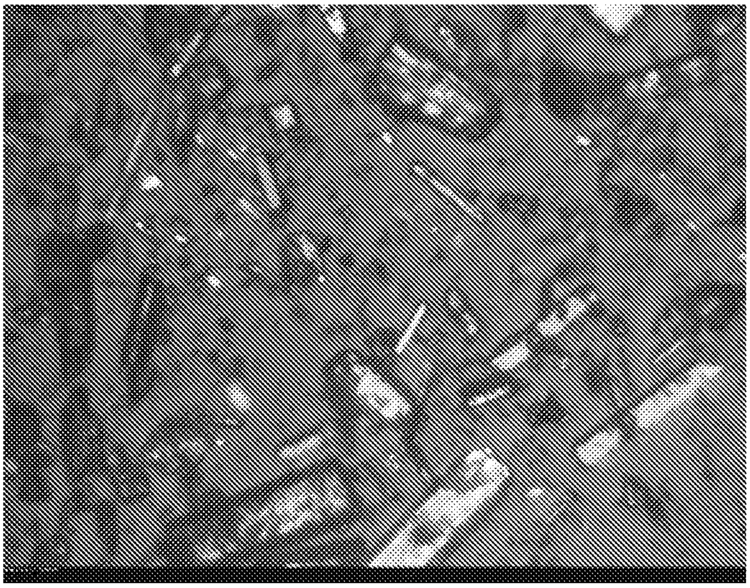


Figure 12A

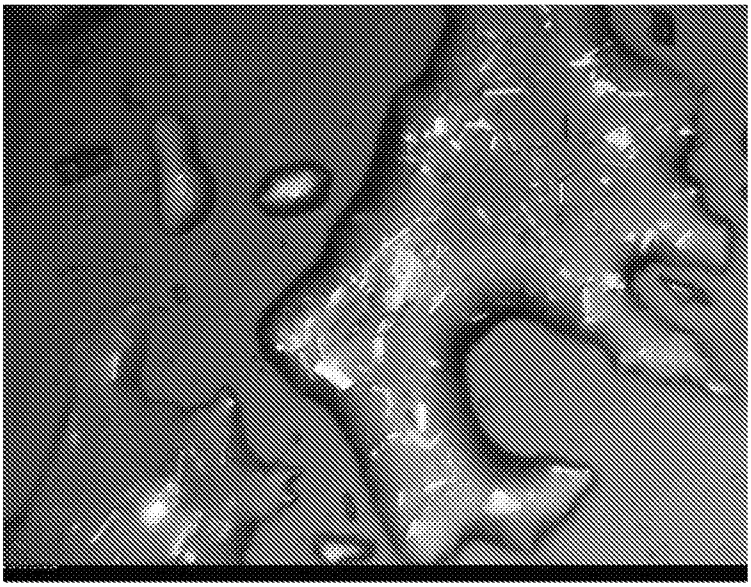


Figure 12B

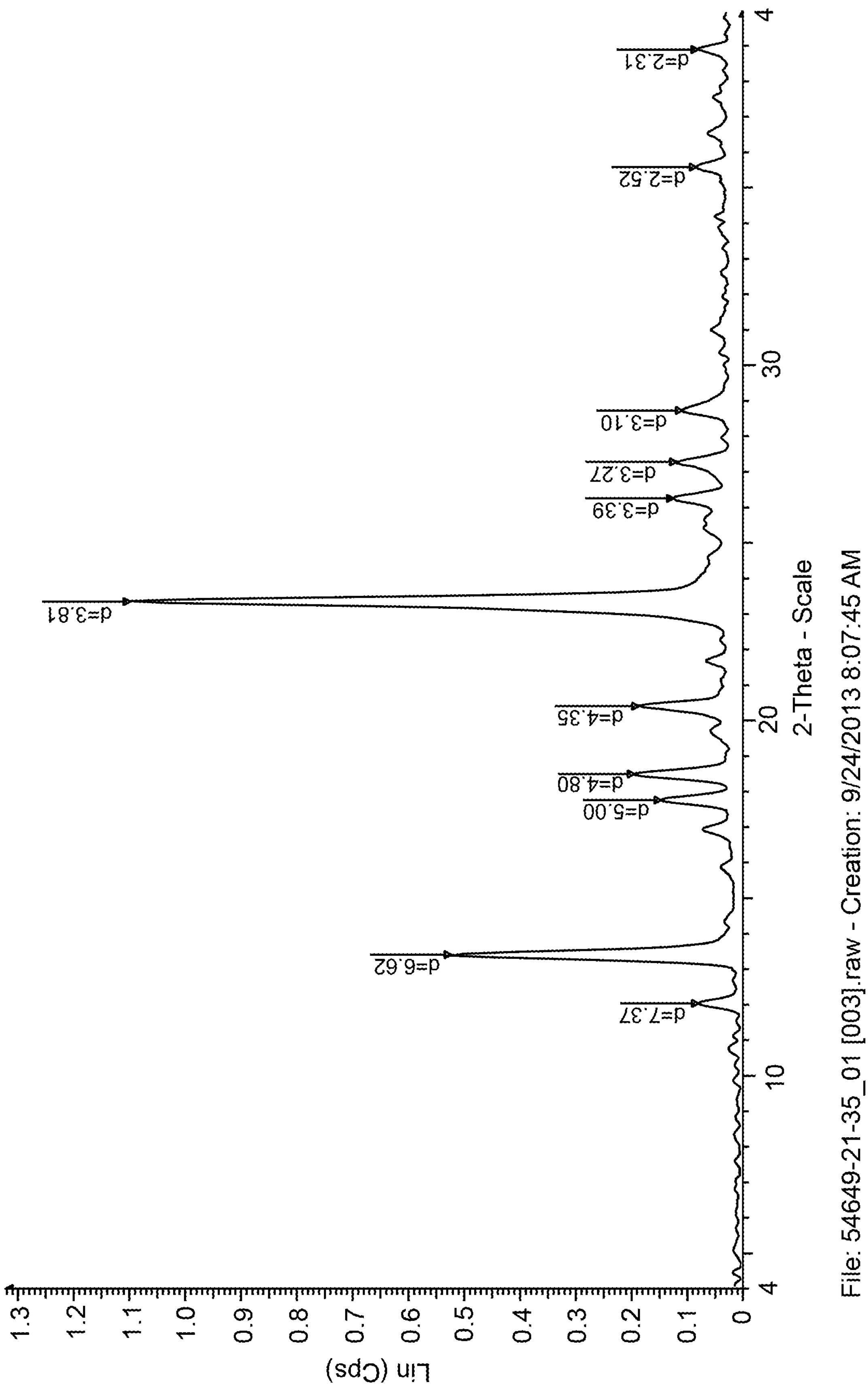

Lin (Cps)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
4
10
20
30
2-Theta - Scale
d=7.37
d=6.62
d=5.00
d=4.80
d=4.35
d=3.81
d=3.39
d=3.27
d=3.10
d=2.52
d=2.31
File: 54649-21-35_01 [003].raw - Creation: 9/24/2013 8:07:45 AM

FIG 13

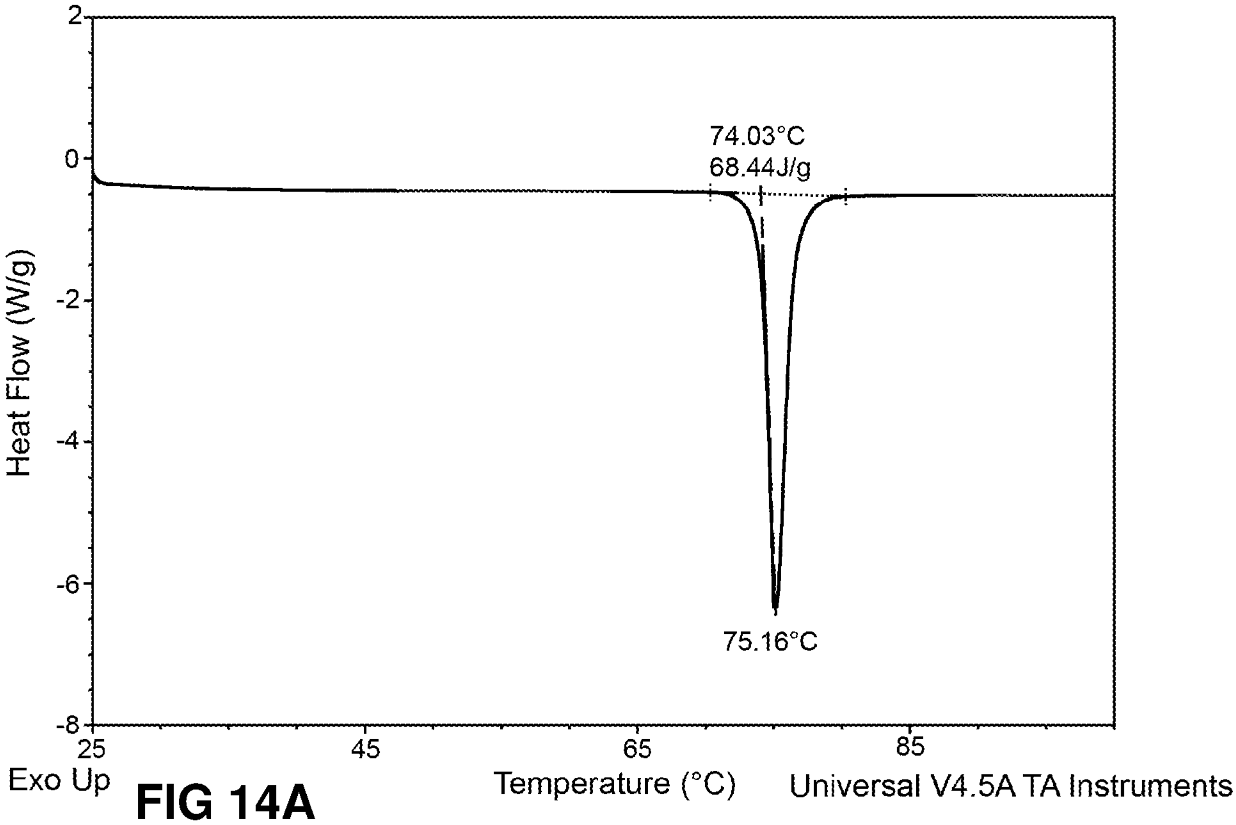


FIG 14A

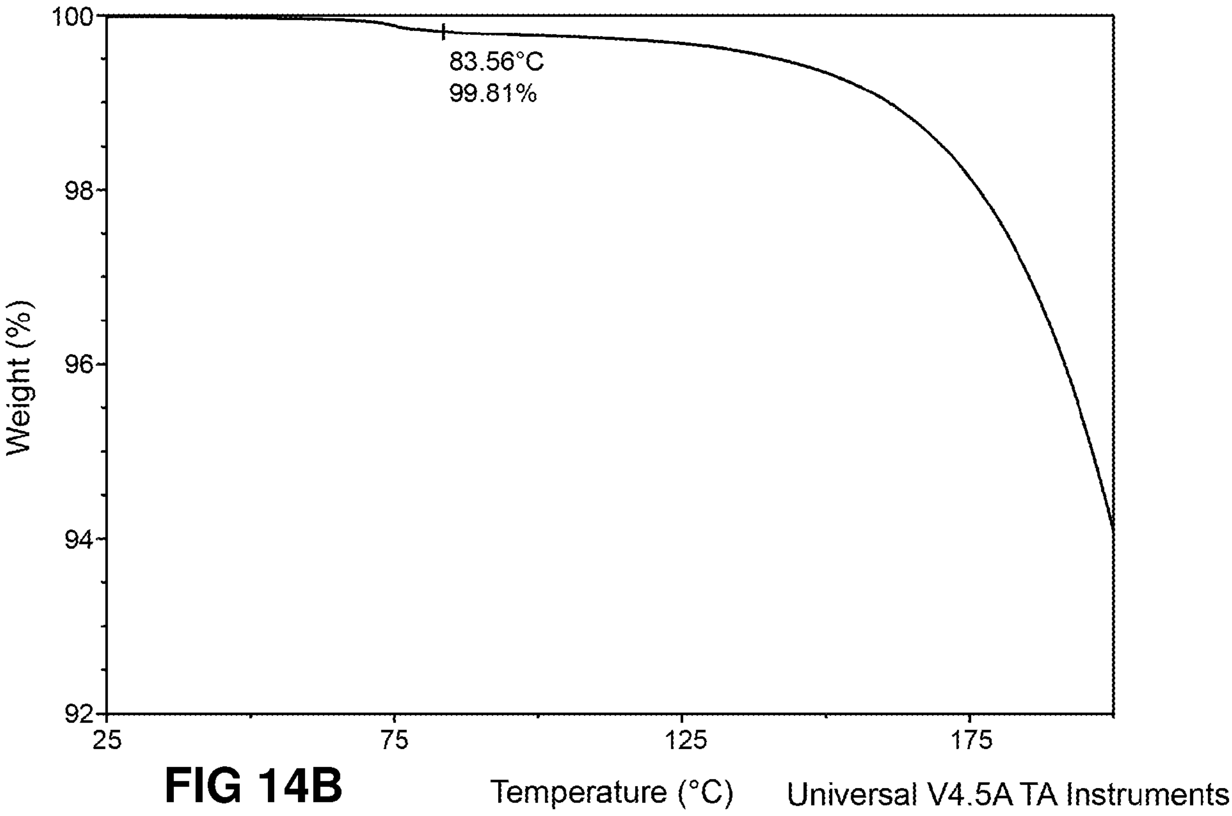


FIG 14B

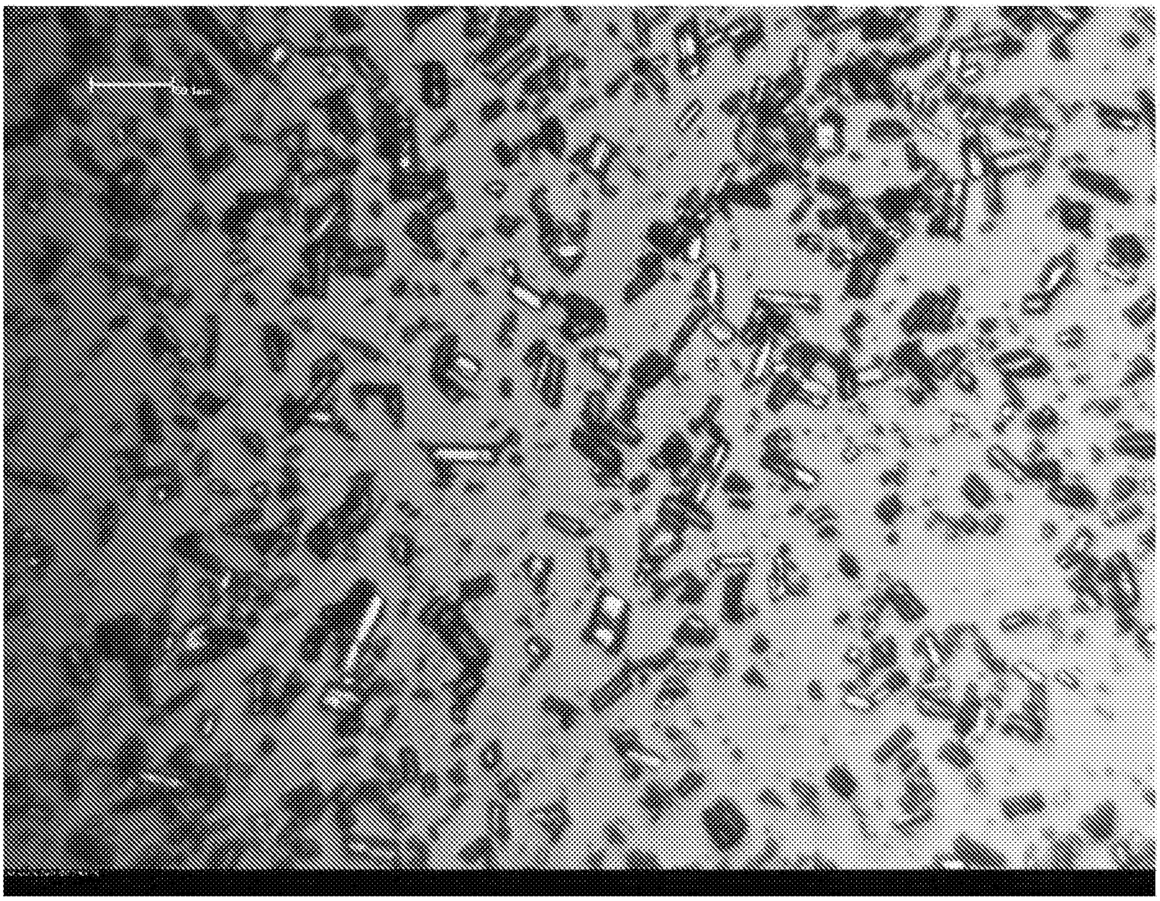


Figure 15

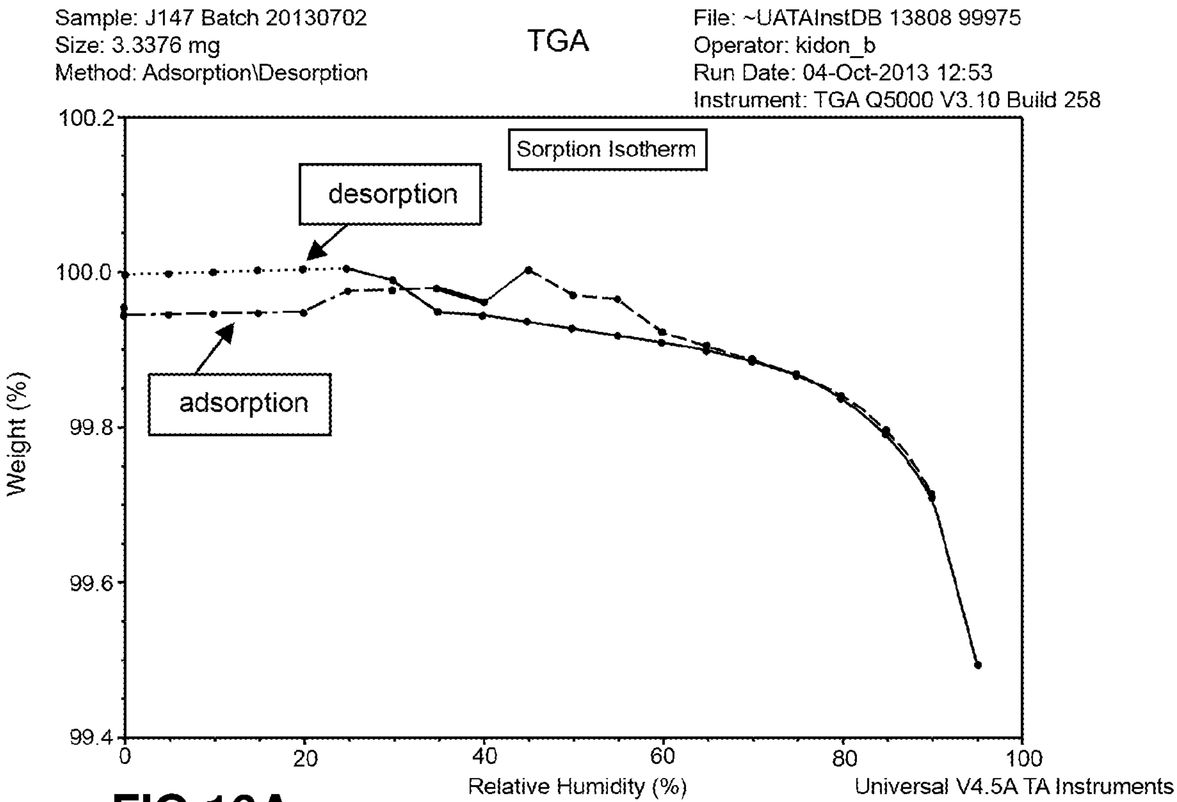


FIG 16A

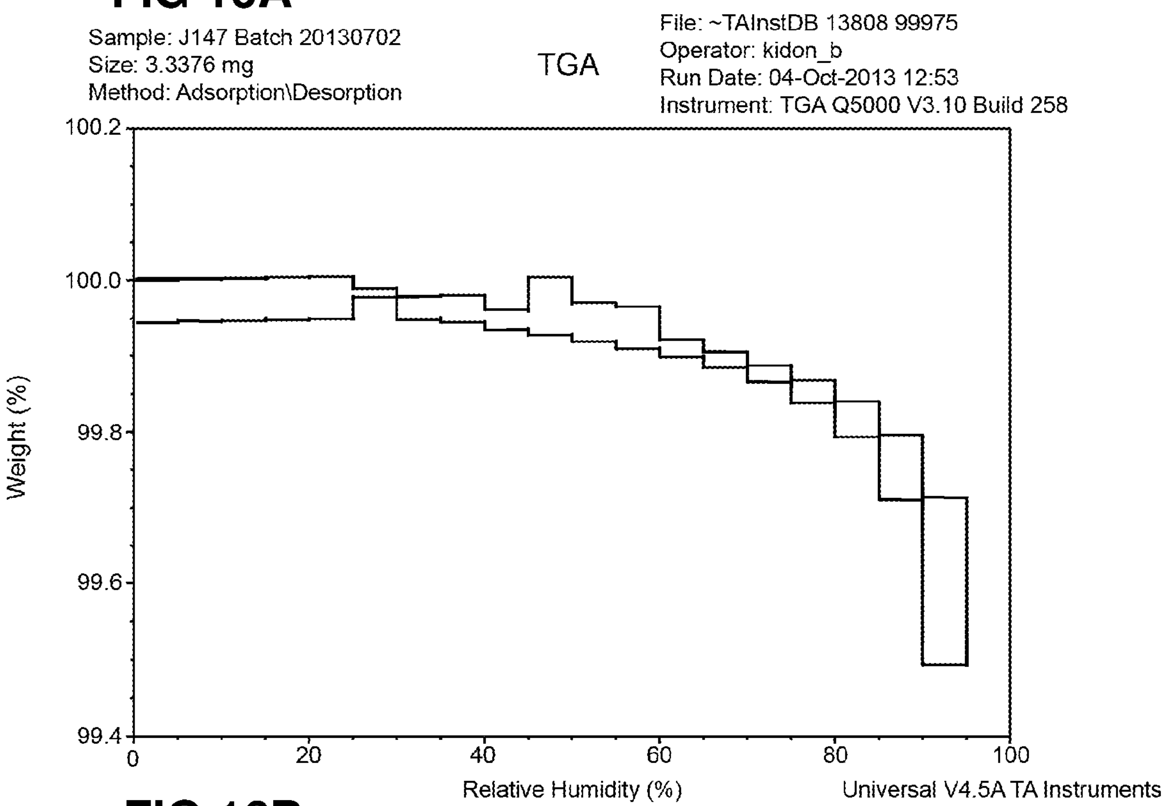


FIG 16B

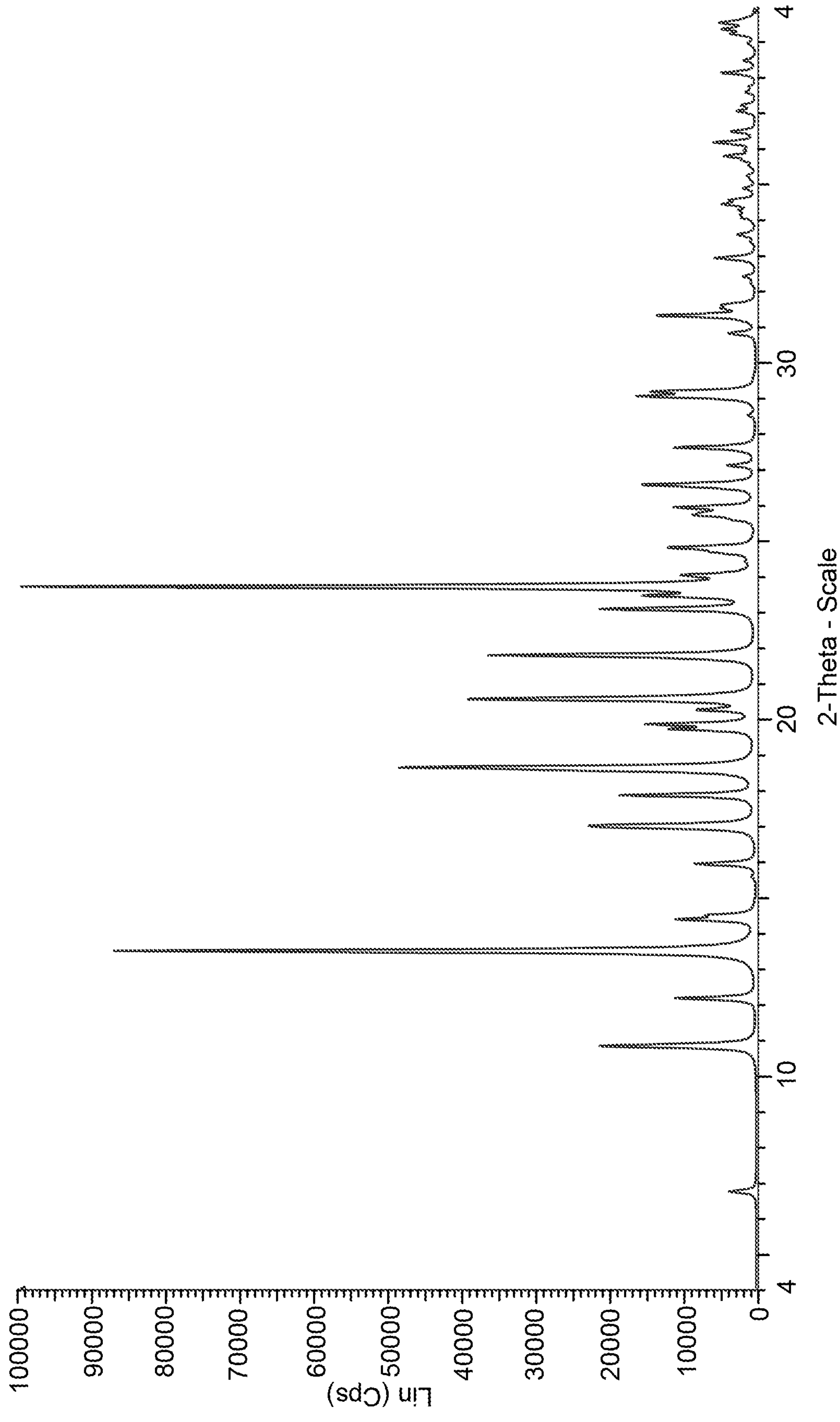

Lin (Cps)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
4
10
20
30
4
2-Theta - Scale

FIG 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/18835

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-16, 23
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Continued Within the Next Supplemental Box-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 26-27

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/18835

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 31/31, 31/175; A61P 25/28 (2019.01)
CPC - A61K 31/31, 31/175; A61P 25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	(PRIOR, M et al.) The neurotrophic compound J147 reverses cognitive impairment in aged Alzheimer's disease mice. Alzheimer's Research & Therapy. 2013, Vol. 5, No. 25, pp. 1-19; page 6, heading synthesis of J147	1-4, 26-27
A	(CHEN, Q et al.) A Novel Neurotrophic Drug for Cognitive Enhancement and Alzheimer's Disease. PLOS ONE. December 2011, Vol. 6, No. 12, pp. 1-17; page 15, heading 1-2,4-dimethylphenyl-2-3-Methoxybenzylidene hydrazine hydrochloride; figure 10	1-4, 26-27
A	(DAUGHERTY, D et al.) A novel Alzheimer's disease drug candidate targeting inflammation and fatty acid metabolism. Alzheimer's Research & Therapy. 2017, Vol. 9, No. 50, pp. 1-17; figure 1	1-4, 26-27
A	WO 2009/052116 A1 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 23 April 2009; paragraph [0068]	1-4, 26-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2019 (30.03.2019)	19 JUN 2019

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

-***-Continued from Box No. III Observations where unity of invention is lacking -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.
Group I: Claims 1-4, 26-27 are directed toward a method of making crystalline Form II of 2,2,2-trifluoroacetic acid 1-2,4-Dimethylphenyl-2-3-methoxyphenyl methylene hydrazide J147, a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta, a solvent.

Group II: Claims 17-22, 24-25 are directed toward a method of making and isolating crystalline Form I of 2,2,2-trifluoroacetic acid 1-2,4-Dimethylphenyl-2- 3-methoxyphenyl methylene hydrazide J147, having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, a solvent.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
the special technical feature of Group I include Form II of 2,2,2-trifluoroacetic acid 1-2,4-Dimethylphenyl-2-3-methoxyphenyl methylene hydrazide J147, a powder X-ray diffraction pattern comprising peaks located at 13.37, 18.47, and 23.34 +/- 0.2 degrees 2-theta, which is not present in Group II.
the special technical feature of Group II include Form I of 2,2,2-trifluoroacetic acid 1-2,4-Dimethylphenyl-2- 3-methoxyphenyl methylene hydrazide J147, having a powder X-ray diffraction pattern comprising peaks located at 11.85, 17.11, 17.79, and 23.40 +/- 0.2 degrees 2-theta, which is not present in Group I.

The common technical features of Groups I-II are J147 and a solvent.
The common technical features of Groups I-II are disclosed by "The neurotrophic compound J147 reverses cognitive impairment in aged Alzheimer's disease mice" by Prior, et al. (hereinafter 'Prior'). Prior discloses J147 and a solvent (J147 was prepared from ethanol (solvent); page 6, heading synthesis of J147).

Since the common technical features are previously disclosed by Prior, the common features are not special and so Groups I-II lack unity.